

A *Clostridium perfringens* outbreak traced to temperature-abused beef stew, Norway, 2012

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On 21 January 2012, the Norwegian Food Safety Authority was informed about gastrointestinal illness among 111 swimming club members, who were staying at a hotel in Trondheim. A hotel dinner on 20 January was their only common meal. Kitchen staff were interviewed, and food leftovers and kitchen environment were sampled. A case was defined as a swimming team member staying at the hotel from 20 to 22 January, who fell ill with diarrhoea, abdominal pain or nausea during this period. A total of 43 cases were identified, with median duration of symptoms of 35 hours. *cpe*-positive *Clostridium perfringens* (3.8×10^8 CFU), but not *Bacillus cereus*, was isolated from beef stew eaten by cases. *cpe*-negative *C. perfringens* was detected in a sample from the kitchen floor. SDS-PAGE showed indistinguishable protein profiles among *C. perfringens* cultures isolated from the beef stew, but slightly different profiles from the culture isolated from the kitchen floor. Cohort analysis showed that eating beef stew and rice was significantly associated with illness. No pathogens were detected in the rice. The temperature control of the stew, but not of the rice, was poor. Our results strongly indicate that cases were infected by *Clostridium perfringens* in beef stew that had inadequate temperature control during preparation.

Outbreak description

On the evening of 21 January 2012, the Norwegian Food Safety Authority, district office of Trondheim and Orkdal, was alerted to an outbreak of gastrointestinal disease among participants from several swimming clubs attending a swimming competition in Trondheim. Initial information was passed on in a private conversation between a swimming coach and an employee of the Food Safety Authority, who happened to be present at the swimming competition. Symptoms had started early that day. In response, the Food Safety Authority conducted a few enquires the same evening, which showed that the sick swimmers had stayed at the same hotel and that dinner at this hotel the previous evening (20 January) was the only known meal common to all the swimming teams, clearly indicating a probable food-borne outbreak associated with this meal.

The Food Safety Authority launched an investigation, according to standard procedures, aimed at preventing possible continuation of the outbreak, describing the outbreak, identifying the source and causal agent, and if possible advise on preventive measures. Initial information allowed further investigations to focus on factors specifically associated with the suspected hotel dinner. We describe here the investigation and results of what turned out to be among the largest *Clostridium perfringens* outbreaks reported in Norway, adding to the current evidence base of *C. perfringens* outbreaks.

Outbreak population data

A total of 111 people from six swimming clubs from different parts of Norway, including swimmers, coaches and tour leaders, aged 12–55 years, stayed at the hotel from Friday 20 to Sunday 22 January, while they attended a swimming competition in Trondheim that weekend. In addition to the swimming team members, approximately 50 other guests stayed at the hotel that weekend. For those other guests, information on food consumption and possible illness was not available, and they were therefore not included in the outbreak investigation. Consequently, the outbreak population was defined as all members of the six swimming teams staying at the hotel from 20 to 22 January.

Members from all six swimming teams had dinner at the hotel on 20 January at approximately 9 p.m., whereas members from only two of the swimming teams had lunch at the hotel that day. Except for these meals at the hotel, the members from the six teams had no other known common meal or other common contacts, neither during 20 January, nor during the preceding month.

Case definition

An outbreak case was defined as a member of a swimming team staying at the hotel from 20 to 22 January, who fell ill with diarrhoea, abdominal pain or nausea during this period.

Kitchen inspections and guidance

Food control officers inspected the hotel kitchen several times. On 23 January, the number and group affiliations of guests and list of hotel meals eaten were collected, and food control officers observed the basic hygiene status of the kitchen. Kitchen staff were asked to describe all dishes served at the dinner on 20 January and all relevant procedures for preparing and handling of foods served at this meal. The investigation specifically addressed factors associated with the growth of spore-forming pathogens in foods: *C. perfringens* and *Bacillus cereus* – such as time and temperature aspects of chilling and reheating, and temperature during serving – because preliminary descriptions of the symptoms and outbreak setting provided reasons to suspect one of these pathogens as the causative agent. On this inspection, available food leftovers from the dinner – beef stew and boiled rice – were sampled. During a second inspection of the kitchen on 26 January, more detailed data on food handling were collected, and the kitchen was swabbed. On meeting with the kitchen manager on 31 January, preliminary microbial results were presented and control officers gave guidance on hygiene, food safety and preventive measures.

Background

C. perfringens is a spore-forming bacterium widely distributed throughout the environment, which may cause food-borne disease [1]. *C. perfringens* enterotoxin (CPE) encoded by the *cpe* gene is the major virulence factor, causing tissue damage of intestinal epithelial cells in an infected person and leading to self-limiting diarrhoea and abdominal pain as main symptoms. The incubation period is 6–24 hours (usually 10–12 hours) [2]. The duration of illness is mostly reported to be a maximum of 24 hours. Longer duration has been reported from at least one outbreak (mean: 2.3 days; range: 1–10) [3]. The infective dose is estimated to be 10^6 – 10^7 cells [1,4].

Vegetative cells of *C. perfringens* grow between 15 °C and 50 °C, with optimal growth around 43–45 °C [1]. The generation time may be as low as 7–8 minutes under optimal growth conditions [5]. *C. perfringens* spores in food will survive boiling. If food handling includes long time intervals at temperatures permitting rapid growth, the content of *C. perfringens* may rise to a level that causes risk of food-borne infection.

The majority of environmental *C. perfringens* strains are *cpe*-negative [6]. *cpe*-positive *C. perfringens* strains are shown to produce spores with a higher heat resistance [1] and will therefore be selected for in kitchen environments. For these reasons, food-borne outbreaks due to *C. perfringens* are most often associated with foods subjected to poor temperature control, produced in commercial kitchens [1,7], as demonstrated by reported outbreaks [8,9]. Direct or indirect person-to-person faecal–oral transmission is not considered to be an important transmission route [1,2].

C. perfringens is traditionally considered to be a frequent cause of food-borne infection in Norway [4] and other industrialised countries [1], but as the symptoms are usually mild, outbreaks are often not reported. Among 242 food-borne outbreaks with a recognised causal microbial agent reported in Norway during 2005 to 2011, eight outbreaks (3%) were caused by *C. perfringens*, of which the largest included 45 cases (personal communication, B. Heier, Norwegian Institute of Public Health, 4 May 2012).

Methods

Sampling and microbiological analysis

When the hotel kitchen was inspected on 23 January, the only available leftovers from the dinner on 20 January were beef stew and boiled rice: these were sampled. After the meal, these leftovers had been stored at room temperature for a few hours (information on the exact time was not available) and then placed in a refrigeration room at 4 °C. Samples of leftovers were analysed for *C. perfringens* and *B. cereus*, for the reasons stated above. Analysis for detection of *C. perfringens* was performed as described by the Nordic Committee on Food Analysis (NMKL) (anaerobic cultivation at 37 °C for 24 hours on membrane *C. perfringens* (m-Cp) agar and blood agar) [10]. Analysis for detection of *B. cereus* was performed as described by NMKL (aerobic cultivation on blood agar at 37 °C for 24 hours) [11].

In order to assess pathogenic potential of the *C. perfringens* flora, four isolated *C. perfringens* cultures were selected arbitrarily from primary culture plates and further analysed by PCR, for detection of *cpe*. Colonies were isolated using AVDAMAX beads (Edge Bio, Gaithersburg, United States), according to the manufacturer's protocol. DNA was suspended in 10 mM Tris-HCl at pH 7.5–7.8. Two primers: CAAGTCAAATTCTTAATCCT and CATCACCTAAGGACTGTCT were used. Amplification was carried out with initial denaturation at 95 °C (3 min.) and then 30 cycles with 1 min. at 92 °C, 1 min. at 50 °C, and 1 min. at 72 °C, and finally 7 min. at 72 °C.

For the purpose of source tracing and characterisation of the general load of *C. perfringens* in the kitchen environment, 20 points on the surfaces of walls, floors, working desks and equipment in the kitchen were sampled by swabbing on 26 January. Some of the sampling points had been washed before sampling. The swabs were smeared on blood agar plates. Protocols for detection of *C. perfringens* by incubation on blood agar and for PCR detection of *cpe* were identical to those described above.

The protein profile of four selected cultures of *C. perfringens* isolated from the beef stew and one culture isolated by swabbing the kitchen were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [12]. After incubation of the cultures in lactose broth at 37 °C in 12 hours and centrifugation at 13,000 rpm at 5 min., supernatant was precipitated

with 80% (NH₄)₂SO₄. The profiles were compared by visual inspection.

Epidemiological investigation

A self-administered questionnaire was prepared, including questions on demography, consumption on food items eaten during dinner at the hotel 20 January, as well as illness and symptoms with onset between 20 and 22 January. Food items included in the questionnaire were based on a list supplied by the hotel kitchen manager. The questionnaire was distributed by email on 24 January, via contact persons for each swimming team, to all the 111 members who had stayed at the hotel during the competition in Trondheim. For children aged under 16 years, the questionnaires were addressed to their parents, and they were asked to assist in answering the questions. One week later, the contact persons were asked to remind those who had not yet responded. From the returned questionnaires, basic descriptive epidemiological parameters were investigated and a cohort analysis was performed.

Results

Details of hotel dinner and food storage

The hotel dinner on 20 January was served from 6 p.m. to 9.30 p.m. as a buffet with six cold dishes (cheese, ham, cured meat sausage, liver paste ('leverpostei'), green salad and bread) and two hot dishes (beef stew and rice). The rice had been boiled immediately before the meal, whereas the beef stew had been prepared the day before, cooled to room temperature and placed in a refrigerated room at 4 °C overnight and reheated before serving. During cooking, chilling, storage, reheating and service, the stew was kept in trays containing approximately 15 L. The duration of storage and temperature of the food during cooling, reheating and serving of the stew had not been recorded. The kitchen had no clear procedures for control of these aspects of food handling, and could not provide data on relevant temperature tests to validate general procedures.

Microbial analysis

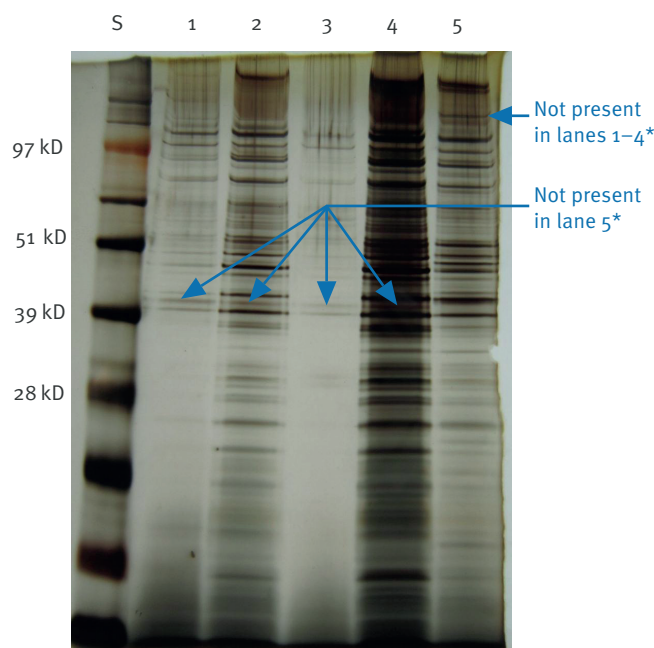
From the beef stew, 3.8 x 10⁸ colony-forming units (CFU) of *C. perfringens* per gramme of food was found; *B. cereus* was not detected. Further investigation of cultures from four selected *C. perfringens* colonies isolated from beef stew showed the presence of *cpe* in all these cultures. Neither *B. cereus* nor *C. perfringens* was detected in the rice.

Among 20 swab samples taken from different parts of the kitchen, *cpe*-negative *C. perfringens* was detected in one culture from the floor; the remaining 19 samples were negative. *cpe*-positive *C. perfringens* was not detected in any of the samples from the kitchen environment.

From four *cpe*-positive *C. perfringens* cultures isolated from the stew, SDS PAGE analysis showed an indistinguishable protein profile among these cultures,

FIGURE

SDS-PAGE protein profiles from *Clostridium perfringens* cultures isolated from beef stew and kitchen environment, Trondheim, Norway, 2012



SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis.

* Protein bands representing differences between lanes 1-4 versus lane 5.

Gel from SDS-PAGE protein profile analysis of four *Clostridium perfringens* colonies isolated from beef stew with indistinguishable profiles (lanes 1-4) and one *C. perfringens* isolated from the kitchen environment with a different profile (lane 5). Lane S shows the reference marker: SeeBlue Plus2 Pre-Stained Standard, Invitrogen.

whereas the profile of the single *cpe*-negative *C. perfringens* culture isolated from the sample from the kitchen floor showed several protein bands not present in the profile from the cultures isolated from the stew (Figure).

Descriptive epidemiological results

Of the outbreak population comprising 111 individuals, 61 (55%) responded and returned the questionnaires. The median age of the respondents was 16 years (range:12-55). A total of 43 respondents met the case definition, giving an attack rate of 70% (43/61) among the respondents.

The median age of the cases was 16 years (range: 12-47). The median incubation period (counting from the start of the dinner on 20 January, at 9 p.m.) was 10 hours (range: 3-28 hours). The most frequent

TABLE

Relative risk of gastrointestinal disease among questionnaire respondents (n=61), by food exposure at hotel dinner, outbreak of *Clostridium perfringens* infection, Trondheim, Norway, 2012

Food item	Exposed		Unexposed		Relative risk	95% CI
	Number of cases who ate food item/total	Attack rate (%)	Number of cases who did not eat food item/total	Attack rate (%)		
Beef stew	42/47	89	1/14	7	12.51	1.89–82.91
Rice	41/49	84	2/12	17	5.02	1.41–17.90
Green salad	28/40	70	15/21	71	0.98	0.70–1.37
Bread	30/46	65	13/15	87	0.75	0.56–1.01
Cured meat sausage	3/6	50	40/55	73	0.69	0.30–1.55
Ham	5/17	29	38/44	86	0.34	0.16–0.72
Cheese	6/21	29	37/40	93	0.31	0.16–0.61
Liver paste	1/5	20	42/56	75	0.27	0.05–1.55

symptoms were diarrhoea and abdominal pain: both were reported from 39 of the 43 cases, followed by nausea (n=31), headache (n=15) and vomiting (n=5). The median duration of symptoms was 35 hours (range: 8–96). No cases reported having consulted a physician. A total of 10 cases reported to have withdrawn from scheduled participation in swimming events because of the illness, and 10 other cases reported having participated in all the swimming events as scheduled, but with lower performance than expected, due to the illness.

Cohort analysis

Cohort analysis of the 61 respondents showed that both hot dishes served at the hotel dinner on 20 January – beef stew (relative risk (RR): 12.51; 95% CI: 1.89–82.91) and rice (RR: 5.02; 95% CI: 1.41–17.90) were significantly associated with illness. One case did not eat beef stew and two cases did not eat rice. Among 47 persons who ate the stew, all but one also ate rice. For all six cold dishes served at the dinner, the RR was <1 (Table).

Food safety and public health action

In accordance with legislation and normal procedures of the Norwegian Food Safety Authority, the hotel kitchen management was advised to implement routines to prevent similar incidents, including procedures for control of time and temperature during food handling, cleaning procedures, and to ensure that these routines were understood and followed by all staff.

Discussion

This outbreak of 43 cases of gastrointestinal disease, occurring within a period of 25 hours among the swimming team members at the hotel, strongly suggested a common source and possible food-borne outbreak. The setting – a communal meal prepared in a commercial

kitchen [1] – clinical symptoms and incubation period [2] were typical of *C. perfringens* infection. *C. perfringens* in high numbers, exceeding the assumed infection dose, was isolated from beef stew eaten by all cases but one. The presence of *cpe* and indistinguishable protein profiles among all four of the isolated *C. perfringens* cultures that were tested indicated that the microbial flora of the stew was dominated by a single *cpe*-positive *C. perfringens* strain. These findings together strongly indicated *C. perfringens* as the causative agent. Conditions in which the leftovers were stored after the dinner and before sampling (three days after consumption) would have allowed further growth of *C. perfringens*. Therefore, the concentration and heterogeneity of *C. perfringens* in the stew at the time of consumption is unknown, but considering the short generation time under optimal conditions, the concentration probably increased substantially during preparation and handling before consumption.

Since none of the cases visited a physician, no stool samples were taken. Detection of *C. perfringens* in stool samples from one or more cases might have supported the identification of the causative agent.

The univariate cohort analysis demonstrated significant association between disease and eating beef stew. The only exposure other than consumption of stew showing a RR >1 was consumption of rice. Preparation and handling of the stew before serving was not satisfactory, providing conditions (time and temperature) that permitted rapid growth of *C. perfringens*, whereas for the rice, this was not the case. Reheating before serving would not have killed the bacterial spores. The pathogenic bacteria were found in high concentrations in the stew but not in the rice. These findings strongly suggest that the beef stew was the source of the pathogens. A RR >1 for the rice in the univariate analysis can

be explained as a confounding effect, attributed to the fact that all persons but one who ate the stew also ate the rice, and is therefore compatible with a hypothesis of the stew as source of the pathogens. Had a multivariate analysis been performed, confounding effects might have been better clarified. Due to lack of resources, however, this was not performed.

The case definition was chosen not to include vomiting. Only five of those who were ill reported vomiting, which is consistent with reports of other authors, thus we regard this as a variable rare symptom of *C. perfringens* infection [1]. Inclusion of vomiting in the case definition would not have altered the epidemiological results and conclusions substantially.

The symptoms experienced by the cases were mild (mainly diarrhoea, abdominal pain and nausea) and the median incubation period was 10 hours. These clinical observations correspond well with the typical characteristics of *C. perfringens* infection [2]. The median duration of symptoms (35 hours; range 8–96), however, deviates from the typical duration of symptoms for *C. perfringens* infection – generally assumed to be less than 24 hours [1,2]. Duration of symptoms exceeding 24 hours has been described in one other outbreak of *C. perfringens* infection, in which the median duration was reported to be two days [3]. Elderly or immunosuppressed persons may experience longer duration of symptoms [13]. Among the cases in the outbreak described here, there was, however, no indication of immunosuppression – on the contrary, most cases were young athletes, assumed to be in good health condition. We cannot envisage other specific factors that could explain the unusually long duration of symptoms among the cases in this outbreak. The clinical data in our study were self reported, subject to individual judgment and possible recall bias, and should therefore be considered with caution. We consider it however unlikely, that the long duration of symptoms observed in our study can be attributed exclusively to bias. Thus our findings and the report from Eriksen et al. [3] suggest that the normal range for duration of symptoms for *C. perfringens* infection among the general population should be considered to exceed 24 hours, possibly up to two or three days.

No samples from the kitchen environment contained *cpe*-positive *C. perfringens* and only one contained *cpe*-negative *C. perfringens*. Thus the sampling did not reveal any substantial reservoir of *C. perfringens* spores in the kitchen. Furthermore, as the kitchen had been washed before sampling, the absence of *cpe*-positive *C. perfringens* in the samples cannot be considered fully representative of the status of the kitchen environment during preparation of the meal. The sampling from kitchen environment gave therefore no indication of the mode or source of transmission for the contamination of the beef stew.

When inferring epidemiological data from respondents to the whole outbreak population, representativeness should be considered. We did not have data on age and sex of non-responders, but if we assume that the attack rate among all 111 swimming team members was the same as that among the 61 respondents (70%), the total number of cases would have been as high as 77. However, as those affected by food poisoning are more likely to respond to a questionnaire than those who are healthy, such assumption may lead to an overestimation. The response rate of 55% may be modest, but we consider that it did not substantially undermine the clear conclusions derived from the descriptive data and cohort analysis. And if all non-respondents were healthy, the attack rate among all swim team members would still have been high (at 39%). Counting only the 43 reported cases, this outbreak still ranks among the largest outbreaks of *C. perfringens* infection ever reported in Norway.

A large proportion of outbreaks of *C. perfringens* infection are probably never recognised or reported. Several factors contributed to the recognition and elucidation of different aspects of this outbreak. Firstly, initial information about a possible outbreak was reported to the Food Safety Authority by coincidence. Since none of the cases consulted a physician, it is doubtful whether authorities would have been informed about the outbreak in any other way. Had the Food Safety Authority not been alerted, this outbreak would have been undetected. Secondly, the outbreak occurred among swimming teams, facilitating case finding and collection of information from those staying at the hotel. Thirdly, food leftovers were available and sampling led to detection of the presumed causative agent.

The strengths of this investigation are: (i) findings of a homogenous strain of *cpe*-positive *C. perfringens* in high concentration in beef stew eaten by most cases; (ii) high and significant association between disease and consumption of beef stew in the cohort analysis; and (iii) reports of suboptimal handling of beef stew permitting growth of *C. perfringens* before serving. Several limitations must however also be acknowledged – these include: (i) a modest response rate in the epidemiological investigation among swimming team members and possible recall bias; (ii) only univariate analysis was carried out in the cohort study and therefore no adjustment could be made for possible confounding factors; (iii) food leftovers were not sampled until three days after consumption; (iv) lack of detection of the outbreak *C. perfringens* strain in the kitchen environment; and (v) there were no stool samples from cases.

The food safety and public health action carried out was in accordance with the procedures of the Norwegian Food safety Authority for dealing with food-borne outbreaks. Due to the high infective dose, person-to-person transmission is assumed to be only a theoretical possibility for *C. perfringens*, compared with that

for many other important food-borne pathogens. There was therefore no need for specific measures to prevent secondary infections among cases' close contacts.

In conclusion, the outbreak described is one of the largest *C. perfringens* outbreaks reported in Norway. Although the incubation period and symptoms were typical for *C. perfringens* infection, the duration of the symptoms was markedly longer in this outbreak compared with that described in most reports, suggesting that the range for duration of symptoms for *C. perfringens* infection should be reconsidered.

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