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A FIVE SITE CLOSTRIDIUM PERFRINGENS FOOD-BORNE OUTBREAK: A RETROSPECTIVE COHORT STUDY

IZBRUH OKUŽB S CLOSTRIDIUM PERFRINGENS, PRENESENIH S HRANO, NA PETIH LOKACIJAH: RETROSPEKTIVNA KOHORTNA RAZISKAVA

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ABSTRACT

Introduction. In May of 2012, we investigated a food-borne *Clostridium perfringens* outbreak in Slovenia involving a single kitchen and five venues, with 477 exposed persons.

Methods. In order to identify the causative agent, vehicle of infection and source of contamination, we conducted microbiological and environmental investigations and an analytical cohort study (n = 138).

Results. The case definition in the outbreak was met by 104 persons. Predominant symptoms were diarrhoea, nausea and abdominal cramps. Median incubation time and duration of illness were 12 and 22.5 hours respectively. Stool samples were collected from 18 persons and in 13 *C. perfringens* spores were present; enterotoxin was detected in 9 persons. PCR and PFGE analysis of isolates from a cook with earlier onset time, who did not consume the implicated food, and cases from four venues showed the same strain of *C. perfringens* type A (with *cpe*-gene), indistinguishable by PFGE analysis. No food samples could be obtained. An analytical study showed that one food item (French salad) was the most likely vehicle of infection (RR: 6.35; 95% CI: 1.62-24.90).

Conclusions. This was the largest *C. perfringens* outbreak in Slovenia to date. Proper analytical study in combination with detailed laboratory investigation with genotypisation enabled us to identify a causative agent, vehicle of infection and possible source of contamination. Fast response and interdisciplinary collaboration led to timely implementation of control measures. These have led to the kitchen acquiring new equipment and improving staff knowledge of risks and processes, thus reducing the likelihood of future reoccurrences.

Keywords:
infectious diseases outbreaks, epidemiology, *Clostridium perfringens*, cohort studies

IZVLEČEK

***Uvod**. V maju 2012 smo preiskovali izbruh okužb s Clostridium perfringens, prenesenih s hrano, v katerega so bile vpletene ena kuhinja in pet lokaciji s 477 izpostavljenimi osebami.*

***Metode**. Da bi ugotovili povzročitelja, pot prenosa in vir kontaminacije, smo izvedli laboratorijsko in okoljsko preiskavo ter analitično kohortno raziskavo (n = 138).*

***Rezultati**. Definiciji primera v izbruhu so ustrezale 104 osebe. Prevladujoči simptomi so bili: driska, slabost in trebušni krči. Mediana časa inkubacije in trajanja bolezni je bila 12 ur in 22,5 ure. Vzorci blata so bili odvzeti 18 osebam; pri 13 so bile prisotne spore C. perfringens, enterotoksin je bil zaznan pri 9 osebah. PCR- in PFGE-analiza izolatov kuharja z zgodnejšim časom pojava obolenja, ki ni užival impliciranih živil, in primerov s štirih lokacij sta pokazali enak sev C. perfringens tipa A (s cpe genom), ki se ni razlikoval pri analizi PFGE. Vzorcev živil ni bilo mogoče pridobiti. Analitična študija je pokazala, da je bila najverjetnejša pot prenosa okužbe eno izmed živil (francoska solata) (RT: 6,35; 95% IZ: 1,62-24,90).*

***Zaključek**. To je bil največji izbruh s C. perfringens v Sloveniji do zdaj. Ustrezna analitična raziskava v kombinaciji s podrobno laboratorijsko preiskavo z genotipizacijo nam je omogočila identifikacijo povzročitelja, pot prenosa okužbe in mogoč vir kontaminacije. Hiter odziv in interdisciplinarno sodelovanje je pripeljalo do pravočasne implementacije nadzornih ukrepov. Ti so privedli do nabave nove opreme v kuhinji, izboljšanja znanja osebja o tveganjih in procesih ter s tem zmanjšali verjetnost za ponovitve v prihodnje.*

***Ključne besede**:*
izbruhi nalezljivih bolezni, epidemiologija, Clostridium perfringens, kohortne raziskave

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1 INTRODUCTION

C. perfringens is a Gram-positive, rod-shaped, spore forming anaerobic bacterium whose association with food-borne outbreaks is well documented (1, 2). It is also a common inhabitant of normal human gastrointestinal microbiota, making its confirmation as the causative agent of a food-borne outbreak more complicated (3).

C. perfringens is classified into five types (A–E) on the basis of its ability to produce one or more of the major exotoxins α, ß, ε and ι (4). *C. perfringens* enterotoxin (cpe) is encoded by *cpe*-gene and it is produced by less than 5% of *C. perfringens* type A strains (5). Enterotoxin producing *C. perfringens* type A is reported as one of the most common food poisoning agents worldwide (3, 6–8). Incubation time ranges between 6-25 hours, usually 10-12 hours (1). The most common clinical manifestations of food poisoning caused by this microorganism are acute diarrhoea and abdominal cramps. Symptoms generally last from 12 to 24 hours (1, 2). Elderly and immunocompromised people are more likely to have prolonged or severe symptoms (2). The more severe form of the disease may cause necrosis of the small intestine, peritonitis and septicaemia (2). Case fatalities are very rare, occurring in <0.03% of cases (9). The most common vehicles for the pathogen are meat and poultry, although it has been also found on vegetable products, including spices and herbs, and in raw and processed foods. Settings where large quantities of food are prepared several hours before serving (hospitals, nursing homes, cafeterias, schools, prisons, etc.) are the most common locations where *C. perfringens* poisoning occurs (2). Essential measures to prevent food-borne diseases caused by *C. perfringens* are: appropriate cooking, cooling rapidly through the temperature range between 55-15 °C, storing foods at temperatures <10-12 °C and re-heating the food to an internal temperature of above 70 °C before consumption (10, 11).

In the Unites States of America, estimates made in 2011, based on active and passive surveillance, place *C. perfringens* as the third most common cause of food-borne illness, accounting for 10% of cases (9). In the European context, during the last ten years (up to 2011) England and Wales reported 81 outbreaks, affecting almost 3000 persons (12).

In Slovenia, notifications of infectious diarrhoea comprise a quarter of all infectious diseases notifications, remaining an important public health issue (13). Food-borne illness and outbreaks caused by *C. perfringens* are reportable. In the last ten years, two such outbreaks were notified: one in 2003 (31 cases) and one in 2004 (55 cases) (14).

On the afternoon of Thursday, 17 May 2012, the regional Health Inspectorate of Nova Gorica was informed, by an anonymous phone call, about several cases of a gastrointestinal illness affecting the guests of a cafeteria at a local food factory. There was a kitchen at the same location providing food for the cafeteria and another four venues, exposing 477 adults. The outbreak investigation team from the regional Institute of Public Health of Nova Gorica, in collaboration with health inspectors, went on site.

The objective of our comprehensive investigation of the event (epidemiological study, microbiological investigation and environmental risk assessment) was to confirm the outbreak and identify the causative agent, the vehicle of infection and the source of contamination in order to implement appropriate control measures.

2 METHODS

2.1 Epidemiological study

We conducted a retrospective cohort study and used it to conduct case findings for confirmation of the outbreak and for descriptive and analytical analysis. The study population included all attendees and kitchen staff present at one of the venues served by the kitchen under scrutiny on Wednesday, 16 May 2012. We excluded any persons who reported a symptom onset time before the defined date. If the excluded person was considered a possible index case/source of food contamination, we performed a face-to-face interview. The study population was identified on the basis of presence lists at each venue. Local staff members distributed questionnaires during a five day period to encompass all the persons who might be absent due to illness or personal motives. A phone reporting system for regional doctors of family practice and regional emergency departments was established to include persons who might be too ill to be present during questionnaire distribution. The questionnaire was self-administered and included questions on demography, clinical symptoms, location and food consumption. To facilitate recall of consumed items, we included a copy of the menu that was available.

A case definition was established for probable and confirmed case. A probable case was defined as any attendee or kitchen staff with one or more of the following symptoms: diarrhoea, nausea, vomiting or abdominal pain within 48 hours after attending one of the implicated venues on Wednesday, 16 May 2012.

A confirmed case was defined as any attendee or kitchen staff who satisfied the criteria for probable case and had laboratory confirmation. In our analysis, we used both probable and confirmed cases.

Data from compiled questionnaires were digitalised with Epidata Entry (Epidata DK. Denmark, EpiData Association, 2000-2012. Available from: http://www.epidata.dk). Data quality was assured by double data entry (15). The software used for analysis was STATA 12 (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP). We described the study population. We compared exposed and non-exposed to various food items in terms of disease occurrence. We tested the association between eating various food items and the risk of becoming subsequently ill with Fisher's exact test. To analyse the possible differences in groups regarding continuous variables, the Student t-test was used. Multivariable analysis was done by fitting a robust Poisson regression model. The level of statistical significance was set at $p<0.05$.

2.2 Microbiological investigation

Recommended laboratory criteria used in association with clinical presentation and epidemiological evidence to implicate *C. perfringens* in food-borne outbreaks are: (I.) isolation of 10^5 organisms/g from epidemiologically implicated food, (II.) isolation of 10^6 spores/g from stool of two or more ill persons, (III.) identification of the same serotype of *C. perfringens* in stools from different patients, (IV.) identification of the same serotype in both food and stool isolates and (V.) demonstration of cpe in the stool of two or more ill persons (1, 16–18). We defined a laboratory confirmed case as: isolation of 10^6 spores/g of stool from a symptomatic person or detection of cpe in the stool. We collected stool samples within two days of illness onset and tested them for *C. perfringens*, *Bacillus cereus*, *Salmonella* spp., *Campylobacter* spp., *Shigella* spp., *Yersinia enterocolitica*, enteric E. *coli*, *Aeromonas* spp., enterotoxic *Staphylococcus aureus, noroviruses* and *rotaviruses*. When enough samples were available, we tested for the presence of cpe (Techlab ELISA *C. perfringens* enterotoxin test).

We analysed selected *C. perfringens* isolates using multiplex polymerase chain reaction (PCR) for toxynotypisation and *cpe*-gene detection. We used pulsed field gel electrophoresis (PFGE) for molecular genotypisation. We collected hand swabs from kitchen staff that could be in contact with food or had access to clean kitchen areas and tested them for: coagulase-positive staphylococci, *Escherichia coli,* faecal streptococci, *Pseudomonas aeruginosa* and *Proteus* spp. Collection of nasopharyngeal swabs of kitchen staff was planned but not executed, since microbiological evidence of *C. perfringens* among guests was already available.

2.3 Environmental risk assessment

The outbreak investigation team visited and assessed the kitchen and the distribution point on site. All kitchen records, including previous inspections and hazard analysis and critical control points (HACCP) documentation, were reviewed. Temperatures and processes of preparation, storage and transportation of food items prepared on Wednesday, 16 May were reviewed. Staff practices on food preparation, hygiene, cooking, storage, handling and distribution, with focused attention on hygiene, temperature control and transport, were assessed. We did not collect environmental samples before confirmation of the outbreak (18 May). At that time, kitchen cleaning was already being performed. Environmental samples were collected after disinfection and tested for coagulase-positive staphylococci, *Escherichia coli,* enterococci, *Pseudomonas aeruginosa*, *Proteus* spp. and aerobic mesophilic bacteria. Food leftovers or ingredients used for preparation were not available for sampling.

3 RESULTS

3.1 Epidemiological study

We received responses from 139 of 477 (29%) overall, of whom 129 of 467 (28%) were attendees and 10 of 10 (100%) were staff. One cook was excluded on the basis of established criteria. No cases were reported by regional doctors of family practice or regional emergency departments.

According to our definitions, we identified 104 cases, with an overall attack rate of 75% (104/138). Attack rate among attendees was 78% (101/129) and 33% (3/9) among kitchen staff. Males and females represented 56% and 44% of the cohort respectively. The median age was 43 years (range: 22-58 years). The symptoms and signs reported among the 104 cases were diarrhoea (88%), nausea (74%), abdominal cramps (38%), vomiting (13%) and fever defined as body temperature above 37.5 °C (8%). The incidence of abdominal cramps was significantly different among genders (18.5% in males versus 46.6% in females; p = 0.001).

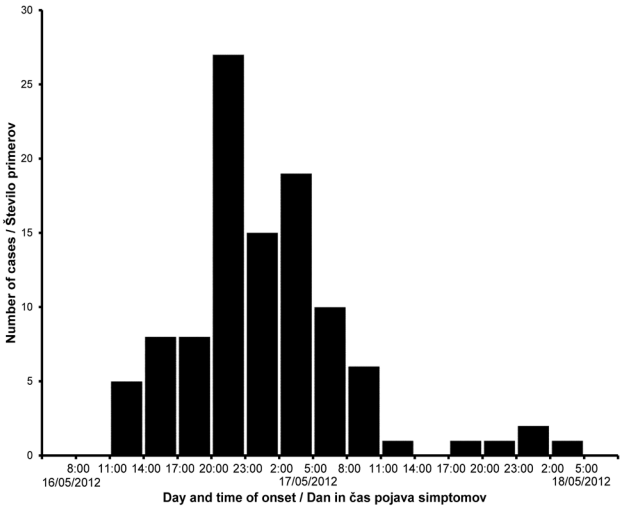

Figure 1. Epidemic curve, *Clostridium perfringens* gastroenteritis outbreak cases, Nova Gorica-Ajdovščina-Sežana, May 2012 (n = 104).

Food was served in three menus. Variations of the single food items ordered were possible. Serving started at 10:30 until 12:00 (n = 124). At one venue, food was served again at 17:00 (n = 12) and 19:30 (n = 2). The epidemic curve is shown in Figure 1. The median incubation time was 12 hours (n = 104; range: 0.5-37.2 hours). The median duration of illness was 23 hours (n = 85; range: 4-59 hours); in 42% of the population symptoms lasted for more than 24 hours. The epidemic curve is consistent with a point-source outbreak. Table 1 shows the results of univariate analysis of food items served. Two items present in the first menu seemed significantly associated with disease occurrence. To control for confounding, these food items were included in a robust Poisson regression model. In the multivariable analysis, the only significant risk that remained was for French salad, with those eating it having 6.4 times the risk of being ill compared to those not eating it, taking into account the other variable (Table 2). The interview with the excluded cook disclosed a clinical picture compatible with *C. perfringens* poisoning with symptoms onset time on Tuesday, 15 March 2012. In later stages, this statement was changed to Wednesday, 16 March 2012. The cook did not consume any food prepared at the implicated kitchen on the day of the outbreak.

Table 1. Relative risk of illness (and 95% confidence intervals) for food items served on 16 May, *Clostridium perfringens* outbreak, Nova Gorica-Ajdovščina-Sežana, May 2012 (n = 138).

	Exposed			Non Exposed					
	Cases	Non Cases	AR%	Cases	Non Cases	AR%	RR	95% CI	p value
French salad	102	18	85.0	2	16	11.1	7.65	2.07--28.32	0.000
Chicken pane	94	17	84.7	10	17	37.0	2.29	1.39--3.76	0.000
Vale stake	4	6	40.0	100	28	78.1	0.51	0.24--1.10	0.015
Roasted potatoes	1	7	12.5	103	27	79.2	0.16	0.03--0.99	<0.001
Green salad	0	7	0.0	104	27	79.4	0.0	n.c./n.i.	0.000
Vegetable soup	0	3	0.0	104	31	77.0	0.0	n.c./n.i.	0.014
Cheese dumplings	0	6	0.0	104	28	78.8	0.0	n.c./n.i.	<0.001

AR: attack rate; RR: risk ratio; CI: confidence interval; n.c.: non computable

Table 2. Multivariable analysis showing final model and relative risk of illness for food items served on 16 May, *Clostridium perfringens* outbreak, Nova Gorica-Ajdovščina-Sežana, May 2012 (n = 138).

Food item	RR	95% CI	p value
French salad	6.35	1.62-24.90	0.008
Chicken pane	1.33	0.88-2.00	0.173

RR: risk ratio; CI: confidence interval; n.c.: non computable

3.2 Microbiological investigation

Twelve cases and 5 non cases provided a stool sample. Among cases, 9 samples tested positive for *C. perfringens* with a spore count higher than 10^6/g; 8 tested positive for cpe (8/9). Out of 4 tested samples, all of them were identified as *C. perfringens* Type A positive for *cpe*-gene and were indistinguishable by PFGE analysis. Each of the four samples was collected from a different venue. Among non-cases, 3 samples tested positive for *C. perfringens* with a spore count higher than 10^6/g; none tested positive for cpe (0/3). Additionally, we collected a stool sample from the excluded cook with an earlier symptom onset time. The sample tested positive for *C. perfringens* with a spore count higher than 10^6/g; it also tested positive for cpe. It was identified as a *C. perfringens* Type A strain positive for the presence of *cpe*-gene and indistinguishable by PFGE analysis from the other tested samples. None of the 5 hand swabs collected tested positive (one sample was from the excluded cook).

3.3 Environmental risk assessment

The kitchen provided food for five venues in a 40 km radius: a food factory on the same site as the kitchen, two industrial factories, the regional penitentiary and a day care centre for people with physical and mental disabilities. Ingredients and partially prepared food were delivered to the kitchen from a central facility. Documentation on transportation and food storage abided by the required standards. The French salad was prepared on site with potatoes, peas, cucumbers, carrots and mayonnaise. Fast refrigeration of the salad was obtained with the use of blast chillers and stored at <5 °C before serving. Critical control points for preparation of fried chicken pane included core temperature measurement and hot hold temperature checks. At two of the venues, where transport time was longer than 1 hour, temperature of the food was checked upon arrival.

No irregularities in the critical control point logbooks and in the implementation of the HACCP plan were detected at the time of inspection. Reports from previous inspections (the last one on 4 May 2012) did not show any irregularities. Practices during preparation, hygiene, cooking, storage, handling and distribution of food followed the required standards. The implementation of the use of blast chillers in the cooling processes was recent. All environmental samples tested negative. The national surveillance system did not detect any other possibly related outbreak in the same time frame.

4 DISCUSSION

This paper presents the results of an investigation of a point source *C. perfringens* outbreak conducted among kitchen staff and guests of five venues served by the same kitchen. From the results of our cohort study, at least 104 persons fell ill. Microbiological analysis confirmed that *C. perfringens* was the causative organism in this outbreak. Molecular genotypisation showed an indistinguishable profile among the isolate form the cook and among cases from four different venues. This suspected index case had symptom onset time on the day before the incriminated food was served and did not consume food prepared by the kitchen on the day the outbreak occurred. These results were indicative of a common source and of a probable origin of food contamination. Despite the absence of food samples, the results from the univariate analysis suggested that the French salad and chicken pane were the most likely vehicles of infection. After multivariable analysis, only the French salad retained statistical significance. Despite the fact that no practice violations were detected during the environmental risk assessment, preparation processes violation or tempera-

ture abuse of the prepared food items would have been necessary to allow the occurrence of the outbreak. It is important to note that *C .perfirngens* has one of the fastest doubling times known (19). Small missteps in temperature control of foods could have led to sufficient growth to cause illness. Based on these results, we implemented the following control measures: all ill persons were advised to stay at home until recovery; persons with a higher risk for spreading the disease were advised to be excluded from work for 48 hours after symptoms resolution; additional education of all kitchen staff on the importance of correct hygiene practices, exclusion from work when sick and temperature control of foods was performed. We gave particular importance to the critical control points of both implicated items. As refrigeration processes with the use of blast chillers were of recent implementation and our analytical study incriminated the French salad, we focused our advice on processes involving refrigeration and cold-holding. We planned an additional inspection after the conclusion of the outbreak investigation. This later revealed that education of staff on hygiene practices continued. Additionally, the kitchen acquired new equipment (more blast chillers) to improve refrigeration and temperature control processes.

According to our national surveillance data, this was the largest *C. perfringens* outbreak in Slovenia to date and the first in the last ten years. A possible explanation for the relatively low occurrence is a well regulated and controlled HACCP system established across the country. Food operators are required to implement and follow procedures based on those principles (20), regularly verified by internal checks and official controls. Other explanations could include the lack of routine testing for the pathogen, the fact that cpe is only detectable for two days after illness onset (21) and the usually mild and short clinical picture. To enhance detection of outbreaks caused by *C. perfringens*, emphasis should be placed on surveillance, fast response, acquisition of detailed clinical picture and aimed laboratory analysis.

Recommended laboratory criteria (1, 16–18) in conjunction with biological plausibility (clinical picture, incubation time, duration of illness) were used to identify *C. perfringens* as the causative agent. The detection of cpe in the stool samples provided additional confirmation on the causative agent. When cpe is detected in at least some of the cases, it is considered a reliable indicator for implicating *C. perfringens* as the etiologic agent in food-borne outbreak (21–23).

The lack of food samples proved a challenge for providing indisputable scientific evidence, besides analytical results, linking disease occurrence with the same mode of transmission at all venues. PCR and PFGE were performed on stool samples of cases from kitchen staff and four venues. In all tested samples, PCR analysis identified a type A *C. perfringens* with the enterotoxin gene, which is commonly produced by <5% of type A clostridia (5,24) such as antibiotic-associated and sporadic diarrhea, associated with plasmid-borne cpe-positive strains, may be food-related; (4. PFGE is useful in an outbreak situation for helping to identify which isolates have identical molecular genotypes and, therefore, might have a common source (3, 25). Our PFGE analysis confirmed the same genotype in all the analysed samples, giving us robust scientific proof on the connection with the implicated kitchen.

The main objective of our cohort study was to identify the vehicle of infection. From analysis of the epidemic curve and considering the median incubation time of 12 hours, the most probable time of infection was the cafeteria meal served on Wednesday, 16 May 2012. After univariate and multivariable analysis of the food items served, only the French salad remained significantly associated with the disease (RR: 6.35; 95% CI: 1.62-24.90; p = 0.008). According to the European Food Safety authority guidelines, such confirmation with analytical tools represents for the European Union Food-borne Outbreak Reporting System a reportable outbreak with strong epidemiological evidence (statistically significant association in well-designed analytical epidemiological study) (26). Despite statistical exclusion, we feel that due to the high biological plausibility of poultry as a vehicle, this item could not be ruled out completely; as such, control measures aimed at avoiding reoccurrences were directed to hazard control points critical for both items.

Regarding the source of food contamination, a cook who did not consume food on the implicated date reported a symptom onset time on Tuesday, 15 March 2012, indicating a possible index case. This statement was changed afterwards to a later onset time. Even though we ensured privacy during the interview, this change of statement could be explained by information bias due to prevarication caused by the fear of repercussions at the workplace. Three asymptomatic kitchen staff members had spore counts higher than 10^6/g. Such high spore counts can be found in healthy adults (27) but are to be evaluated with care in case of an outbreak scenario as humans, in case of hygiene practice violations, should be considered a risk factor for spread of *C. perfringens* (28). We hypothesise the possible index case as the most likely source of contamination, as clinical symptoms and higher shed of bacteria would have vastly increased the chances of contamination. Molecular genotypisation showing an indistinguishable profile in the stool sample from this case compared to the other 4 samples collected at different venues of the outbreak is supportive evidence of our hypothesis. This guided our decision to improve the knowledge of the staff on *C. Perfingens* transmission and hygiene practices.

In addition to the questionnaire, to avoid losing cases that could have been too sick to be at work and hence receive the questionnaire, we alerted the regional doctors of family practice and emergency departments to report such cases to us directly. Although no cases were reported this way, such an effort in conjunction with monitoring at a national level was of importance in the early stages of the investigation when there was no solid evidence that the outbreak was limited to one kitchen. We observed a low response proportion probably due to the lack of interest of non-affected persons, the absence of a constant presence of a formal authority and some social unrest present

at some venues. This could have led to an overestimation of our general AR but should not have affected the food specific RR nor the direction of the association. To limit non-response bias during data collection, we collaborated with the management personnel at each venue to promote the highest possible response proportion from all the attendees while extending the data collection period up to 5 days. Despite this 5 day window, we collected the majority of the questionnaires on the second day of investigation, avoiding as much as possible spreading rumours about particular food items that would bias the response of exposed and non-exposed persons altering the resulting measures of association obtained. In the future, the use of new technologies such as online questionnaires and smartphones could be used to increase response and improve the speed of data analysis at the same time.

In 42% of cases, the symptoms of disease persisted for more than 24 hours (median 22.5 hours (range: 4-59 hours). The commonly accepted range of duration of disease in the general population is under 24 hours (1,2). This finding is consistent with recent articles (29,30) suggesting that the range of duration of *C. perfringens* illness in the general population could be extended up to two or three days. Our data were collected with a self-administered questionnaire, therefore the influence of individual judgment and information bias cannot be excluded. We tried to minimise those influences on our estimations with clear symptom description in the questionnaire and fast execution of the investigation. Our findings could be of importance in other outbreak investigations while formulating hypotheses regarding the causative agent based on clinical signs. Additional research should try to confirm the results, attempting to prioritise the objective measurement of symptoms and their duration.

A possible limitation of our laboratory analysis was that, due to technological constraints, the location of the expressed *cpe*-gene could not be identified. Numerous papers discuss the distinction between a chromosome encoded cpe, usually implicated in food-borne outbreaks, and a plasmid encoded cpe, usually found in other cases of gastroenteritis (sporadic out-patient or nosocomial antibiotic associated) (3, 27, 31). This demarcation is not so well defined anymore, since plasmid encoded clostridia had been recently found implicated in food-borne outbreaks and knowledge on their epidemiological differences is still evolving, providing new ground in the analysis of *C. perfringens* related outbreaks (31–36)

5 CONCLUSIONS

We investigated the largest food-borne outbreak due to *C. perfringens* to date in Slovenia. As in many real life outbreak investigations, we were faced with a lack of food samples available for testing. Nevertheless, a proper epidemiological study in combination with detailed microbiological investigation with genotypisation enabled us to confirm the outbreak and identify the causative agent, the source of contamination and the vehicle of infection. We hypothesise the source of food contamination being a cook with an earlier onset of symptoms. The cook did not consume the incriminated food and tested positive for the presence in the stools of a strain of *C. perfringens* Type A positive for *cpe*-gene that was indistinguishable by PFGE analysis from the other strains detected during the outbreak at the different venues. Analytical data analysis allowed us to prove our hypothesis that a food item prepared at the kitchen was implicated in the outbreak. Despite the fact that no hygiene and practice violations were detected during the environmental risk assessment, some food preparation and storage violations would still be required to allow the organism to cause illness. With this consideration, we based our recommendations on the obtained results, suggesting control measures aimed at improving hygiene practices (source of food contamination) and temperature control of foods (vehicle of infection). This has led to the kitchen acquiring new equipment and improving staff knowledge of risks and processes, thus reducing the likelihood of future reoccurrences.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

This research received no grant from any funding agency in the public, commercial or not-for-profit sectors.

ETHICAL APPROVAL

Slovenian regional public health institutes have general consent to conduct comprehensive outbreak investigations as regulated by the Slovenian Communicable Diseases Act, 2006. Activities include, but are not limited to, contact tracing, interviews, sampling of human and environmental specimens, notification of reportable diseases, communication of the results to the scientific community and general public. Collected data were used only for the purposes of this outbreak investigation. Specific approval was not needed but the scope of use for the data collected was explained at each venue; the participation was voluntary.

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