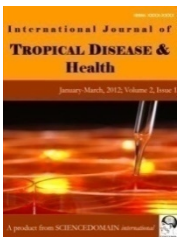

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Food Borne Outbreak at a Salad Eatery, Ghana - 2009

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Authors' contributions

This work was carried out in collaboration between all authors. Author JD designed the study, took part in data collection and analysis, laboratory analysis of samples and also writing the manuscript. Authors BA and FD took part in data collection, laboratory analysis and drafting of the manuscript. Authors OA, FW, EA and CO reviewed the manuscript for intellectual content. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: We investigated a foodborne outbreak to determine its magnitude, source of infection and causative agent using laboratory confirmation.
Study Design: Descriptive cross-sectional study
Place and Duration of Study: Koforidua Township of the New Juaben Municipality, Eastern Region, 5th - 8th November 2009
Methodology: A case was defined as any person presenting with abdominal cramps, diarrhea and or nausea to the Eastern Regional Hospital between 5th and 8th November, 2009 and had eaten salad from the salad eatery. All the cases that reported to the hospital were interviewed and medical records reviewed. Four stool samples, portions of the different vegetables (cabbage, carrots, green pepper and onion) used in preparing the salad and a mixed salad portion were collected for laboratory diagnosis. Environmental assessment at the salad eatery was conducted. We assessed the site were the vegetables were prepared and the transportation process of vegetables to the salad eatery.

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Results: A total of 40 cases were identified with an attack rate of 0.26/1000 population with no fatalities. This was a point source outbreak with an incubation period of 7-20hours. The most affected were cases aged 21-30years (35%) and females (55%). Laboratory diagnosis confirmed *Clostridium perfringens (C. perfringens)* as the probable causative agent in two stool samples. *C. perfringens* was also confirmed in the mixed salad portion and the cabbage. The spore count for the mixed salad was 10^7 CFU/gram of salad and the cabbage was 10^9 CFU/gram of cabbage. The vegetables were washed with ordinary water only and transported to the salad eatery with poor temperature control of warm cabbage.

Conclusion: We confirmed an outbreak probably caused by *C. perfringens* food poisoning. The inadequate washing of vegetables and poor temperature control of warm cabbage was the probable source of the outbreak. Education of food vendors on strict food hygiene was conducted in the Koforidua Township and the inspection of food eateries re-enforced.

Keywords: Food poisoning; clostridium perfringens; outbreak; laboratory confirmation; Ghana.

1. INTRODUCTION

Globally, it is difficult to estimate the incidence of foodborne diseases because most cases are markedly underreported [1]. In Africa where most countries are of low income status, foodborne illnesses may cause a billion illnesses and 4-6 million deaths each year [2].

Food borne illnesses are caused mainly by bacteria, viruses, parasites or chemicals [3]. A suspected outbreak of probably *Clostridium perfringens (C. perfringens)* food poisoning was reported in the Eastern region of Ghana. *C. perfringens* is a bacterium that causes foodborne illnesses. Itsfood poisoning is quite common [4] and it is an important cause of outbreaks worldwide [5]. When high vegetative spore counts greater than 10^5 CFU/gram of *C. perfringens* are consumed, the bacterial cells can sporulate and produce enterotoxin in the human small intestine which results in illness. Signs and symptoms include sudden onset of abdominal cramps, diarrhea, and nausea [6] which are usually mild but can be deadly depending on the immune status of a person. It has a short incubation period of 8-22 hours with median of 10-12 hours [7]. The economic impact of *C. perfringens* food poisoning is substantial [2] because it leads to loss of work hours due to morbidity.

Most *C. perfringens* food borne outbreaks are caused by improper food handling practices and inadequate food preparation procedures [6]. Heating foods to temperatures above 45ºC caninactivatevegetative cells of *C. perfringens* but under circumstances of poor temperature control especially slow cooling and insufficient reheating, spores can germinate in contaminated food [8].

In the Eastern region of Ghana, most foodborne outbreaks are rarely detected and are not confirmed with laboratory methods. An outbreak of suspected *C. perfringens* food poisoning occurred in Koforidua, Eastern region among persons who ate salad from a salad eatery on 5th November 2009. Initially, four persons reported to the Eastern Regional Hospital with diarrhea and abdominal cramps with claims of eating salad from the eatery. The Public Health unit of the hospital was informed and a team was formed to investigate the outbreak. Food eateries are fast springing up in Koforidua with little or no supervision of their activities by environmental health officers.

We investigated this outbreak of food poisoning in the Koforidua Township to determine its magnitude, identify the source or vehicle of infection and causative agent using laboratory confirmation.

2. MATERIALS AND METHODS

2.1 Case Definitions

A probable case was defined as any person presenting with abdominal cramps, diarrhea and or nausea to the Eastern Regional Hospital between 5th and 8th November, 2009 and had eaten salad from the salad eatery. A confirmed case was a probable case with *C. perfringens* isolated from their stool sample by bacteriological culture.

2.2 Study Design

This was a descriptive cross-sectional study conducted to characterize the outbreak. All suspected cases that reported to the Eastern Regional Hospital were interviewed with a structured questionnaire to obtain demographic data, information on whether they ate at the salad eatery and the time they ate the salad. Medical records of cases were reviewed to extract presenting symptoms, date of onset of illness and treatment given. The owner of the eatery was also interviewed to obtain information on how the salad was prepared, source of vegetables, knowledge on food hygiene and medical certificate for food vendors.

2.3 Study Area and Population

This outbreak occurred at a salad eatery in Koforidua, an urban town in the New Juaben Municipality of the Eastern Region which is about 85km from Accra, the capital city of Ghana. The salad eatery is located in the central business part of the town and serves a population of 154, 998 persons living in the Koforidua Township. About 44.5% of the population work in the formal government sector, 28.1% work in the agriculture sector and about 27% work in industry.

2.4 Data Analysis

Data generated was entered into Microsoft Excel spreadsheet and analyzed in SPSS version 16. Univariate analysis was conducted to generate frequencies and percentages with appropriate measures of central tendency.

2.5 Laboratory Investigations

Four stool samples of cases who had not taken antibiotics were collected for laboratory diagnosis. One portion each of the different vegetables (i.e. cabbage, carrots, green pepper and onion) used in preparing the salad and a portion of the mixed salad were also collected for laboratory diagnosis. All samples were collected into sterile containers and transported on ice packs to the laboratory within 24 hours of collection. The samples were cultured at the bacteriology unit of the Regional Hospital. Stool samples were inoculated in cooked meat medium and incubated at 37ºC overnight. The broth was then sub cultured on Blood Agar (Oxoid Limited, Basingstoke UK) and Chocolate Agar (Oxoid Limited, Basingstoke UK) and incubated anaerobically using anaerobic jars with gas packs at 37ºC for 24 hours. Isolated colonies were confirmed by Gram stain, catalase test, oxidase test and reverse CAMP test

according to standard bacteriological procedures [9]. Colony count of isolated *C. perfringens* was performed by serial dilution of the bacteria in peptone water and grown on nutrient agar. Colony forming units (CFU) on nutrient agar were counted and multiplied by the dilution factor to obtain the viable count of the bacteria [10].

Each vegetable sample was blended into a homogeneous mixture and cultured on Blood agar (Oxoid Limited, Basingstoke UK) and Chocolate agar (Oxoid Limited, Basingstoke UK). The inoculated agar plates were incubated anaerobically using anaerobic jars with gas packs at 37ºC overnight. Isolated colonies were confirmed by Gram stain, catalase test, oxidase test and reverse CAMP test. Colony count of isolated *C. perfringens* was performed by serial dilution of the bacteria in peptone water and grown on nutrient agar. Colony forming units (CFU) on nutrient agar were counted and multiplied by the dilution factor to obtain the viable count of the bacteria [10].

2.6 Environmental Assessment

This was done at the salad eatery and at the vegetables preparation site which was at a different location from the eatery. Cleanliness of the eatery, the vegetable preparation site and storage containers of the salad were determined by inspection with a checklist. Hand washing practices of the vendor, the salad preparation process and temperature control for stored salad during transportation to the salad eatery were observed.

3. RESULTS

3.1 Descriptive Study

A total of 40 cases were recorded with an overall attack rate of 0.26/1000 population with no fatalities. The age range for cases was 13-67years with a median age of 31years.

Females were mostly affected 55.0% (22/40) compared to males 45.0% (18/40). Generally, cases mostly affected were aged 21-30 years 35.0% (14/40) and the least affected were aged 61-70 years 2.5% (1/40) (Table 1).

Table 1. Age and sex characteristics of food poisoning cases, Koforidua, Nov 2009

	Number of cases (% of total cases) n = 40		
Age (yr.)	**Female**	**Male**	**Total**
11-20	4 (10.0)	2 (5.0)	6 (15.0)
21-30	5 (12.5)	9 (22.5)	14 (35.0)
31-40	7 (17.5)	4 (10.0)	11 (27.5)
41-50	3 (7.5)	1 (2.5)	4 (10.0)
51-60	3 (7.5)	1 (2.5)	4 (10.0)
61-70	0 (0.0)	1 (2.5)	1 (2.5)
Total	**22 (55.0)**	**18 (45.0)**	**40 (100)**

It was a point source outbreak with an incubation period for cases that ranged between 7-20 hours. The mean incubation period was 12 hours. Only one case (2.5%) developed symptoms 7 hours after eating the salad with most of the cases, 11each (28%), developing symptoms 10 hours and 12 hours after eating the salad. None of the cases developed symptoms between 17-19 hours and only one (2.5%) developed symptoms 20hours after eating the salad (Fig. 1).

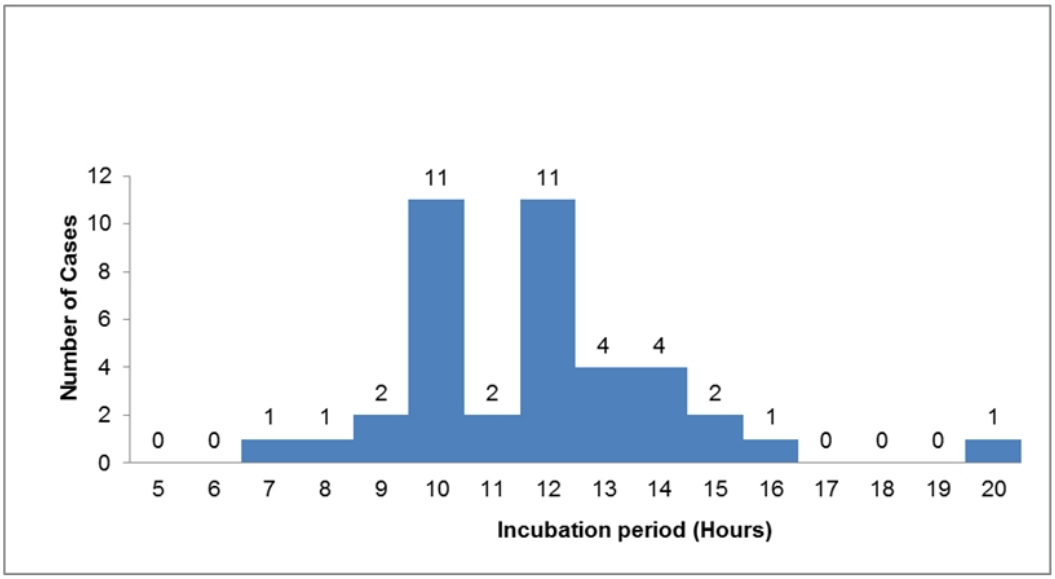

Fig. 1. Incubation period of food poisoning cases in Koforidua, 5th – 8thNovember 2009

The commonly reported symptom was abdominal cramps (37/40, 93%) followed by diarrhea (28/40, 70%) and 50% of cases reported nausea (Table 2).

Table 2. Signs and symptoms of food poisoning cases, Koforidua, Nov 2009

Signs/Symptoms	Number of cases (n=40)	Percentage (%)
Abdominal cramps	37	93
Diarrhea	28	70
Nausea	20	50
Chills	9	23

The cases resided in several suburbs scattered around the salad eatery (Fig. 2). Most cases (8/40, 20%) were from Betom, a suburb that was very close to the eatery. Also, a relatively large number of cases (5/40, 12.5%) were from a suburb called Adweso that is on the main road that passes by the salad eatery.

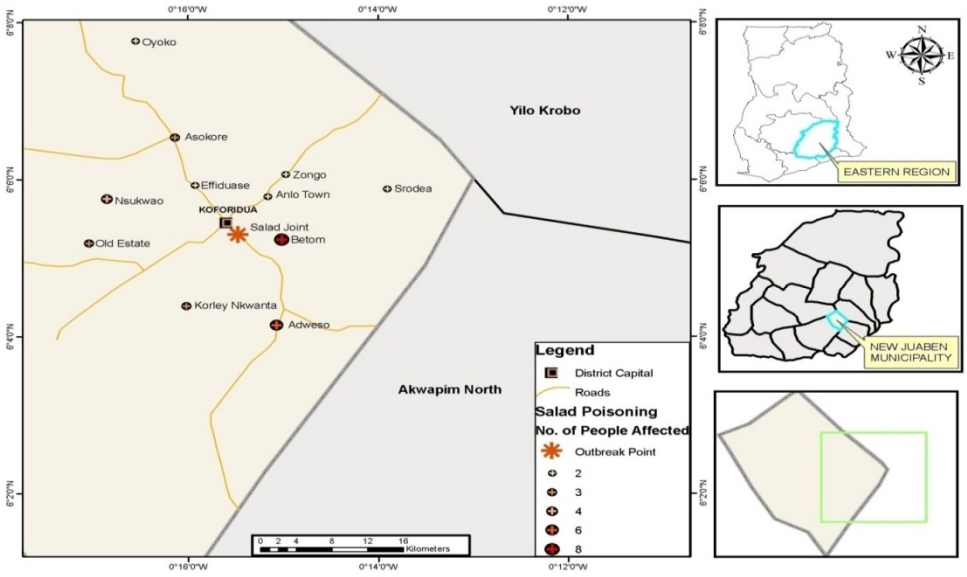

Fig. 2. Distribution of food poisoning cases by surburbsin Koforidua, November 2009

3.2 Index Case

The index case was a 34year old gentleman who bought salad from the eatery in the morning of 5th November 2009 at 7:00 am. Twelve hours after, he started experiencing abdominal cramps with diarrhea and reported to the Regional Hospital that night. He was hospitalized and treated. Two hours after he was hospitalized, three other persons reported to the hospital with similar symptoms of abdominal cramps and diarrhea. All claimed they had eaten salad from the salad eatery earlier in the day.

3.3 Laboratory Investigation

Of four stool samples cultured in the laboratory, *C. perfringens* was isolated from two while the other two samples yielded *Staphylococcus spp* and *Salmonella spp*. *C. perfringens* was also isolated from the cabbage used in preparing the salad and the mixed salad portion. The spore count for *C. perfringens* isolated from the mixed salad was 10^7 CFU/gram of salad and for the cabbage was 10^9 CFU/gram of cabbage. *Bacillus spp* were isolated from the carrot sample with viable count of 10^2 CFU/gram of carrots.

3.4 Environmental Assessment

The salad eatery was operated by the owner and one employee. The owner of the eatery was a 32year old woman who had sold salad for 2years. She had a medical certificate issued by the Municipal Assembly to prove she was medically fit to operate the eatery.

The salad eatery was located in the central business part of town near a major road where there was heavy vehicle and pedestrian movement. The vegetables for the salad which include cabbage, carrots, green pepper and onion were bought from two farms different from

the farms she usually buys from. These vegetables were prepared at the house of the eatery owner.

The surrounding at the salad preparation site was clean but the surrounding at the salad eatery was inadequately clean. The storage containers for the salad and accessories served with the salad were not covered exposing them to flies and dust from the road side. There was no hand washing facility at the eatery for the vendor or customers to wash their hands.

3.5 Food Assessment

The vegetables were washed with pipe-borne water but were not washed thoroughly and vinegar was not added to the wash water to ensure microorganisms were killed. All the vegetables were diced and stored separately in plastic containers. Only the cabbage was par-boiled; there was insufficient cooling of the warm cabbage to temperatures below 43ºC to 45ºC (109ºF to 113ºF) before stored in poly-bags. All the vegetables including the poor temperature controlled stored cabbage were transported over a distance of 3.5km to the salad eatery for sale. At the salad eatery (sales point), portions of each of the prepared vegetables were mixed with salad crème, baked beans and corned beef. The mixed salad was sold with bread.

4. DISCUSSION

This outbreak was a point source outbreak probably caused by *C. perfringens* that occurred among 40 recorded persons who ate salad from a salad eatery in the central business part of the Koforidua Township. Cabbage used in preparing the salad was the likely vehicle of infection. The inadequate washing of vegetables and storage of insufficiently cooled cabbage in poly-bags transported over a distance of 3.5km with no temperature control resulted in contamination of the salad. There was no evidence that this outbreak resulted from human-to-human transmission.

The epidemiological analysis as well as biological plausibility for this outbreak suggests that the likeliest causative agent was *C. perfringens. C. perfringens* is a bacterium that is widely distributed in the environment and frequently occurs in the intestines of humans and many domestic animals [11]. Its food poisoning is characterized by intense abdominal cramps and diarrhea which begin 8-22 hours with a median of 10-12 hours after consumption of foods containing large numbers of the bacteria capable of producing the food poisoning toxin [7,11] and illness is usually brief [12]. In this outbreak the incubation period was 7-20 hours with most cases (60%) developing symptoms 10-12 hours after eating salad. In addition abdominal cramps and diarrhea were the most reported symptoms (Table 2) and cases had stopped reporting within 48 hours of the index case. These are all consistent with *C. perfringens* food poisoning. Although *Staphylococcus spp* and *Salmonella spp* are also bacteria that can cause food poisoning were isolated from stool samples of two cases, the clinical findings, incubation period and duration of illness of cases were consistent with *C. perfringens* food poisoning. Moreover, the isolation of *C. perfringens* spores from two stool samples, cabbage and mixed salad portions supports the conclusion that *C. perfringens* was the probable causative agent.

Our findings were comparable to findings in a clostridium outbreak that occurred among Inmates at a County Jail, Wisconsin where *C. perfringens* was isolated in stool and food samples [13]. Another outbreak of *C. perfringens* food poisoning occurred among

membersof the Cedar Heights Church in Iowa, USA where clinical findings and incubation periods of cases were similar to our findings [14].

It is known that sufficient heat inactivates *C. perfringens* vegetative cells, however, its spores can survive and germinate in contaminated food under circumstances of poor temperature control, particularly slow cooling and insufficient reheating [8]. The likely vehicle of infection in this outbreak was the cabbage because *C. perfringens* was isolated from the cabbage sample and not from the carrots, green pepper or onion samples. Although *C. perfringens* was also isolated from the mixed salad, the spore count (10^7 CFU/gram) was less than spore count (10^9 CFU/gram) for the cabbage. This confirms that *C. perfringens* contamination was in the cabbage and it was the presence of the cabbage in the mixed salad that caused contamination of the mixed salad.

Spores of the bacteria from the soil or water (if contaminated by feces) used in watering the cabbage could have settled on the cabbage. The inadequate cleaning of the cabbage with ordinary water with no vinegar added did not ensure that spores of *C. perfringens*were washed off completely. The insufficient cooling of par-boiled cabbage to temperatures below 43ºC to 45ºC (109ºF to 113ºF) before storage in poly-bags and poor temperature control during transportation to the salad eatery created an ambient temperature for rapid multiplication of the bacteria to quantities greater than10^6 CFU/gram of salad which is the infective dose required to produce toxins in the human intestine for clinical disease [15]. The optimal temperature for growth of *C. perfringens* vegetative cells is 43ºC to 45ºC (109ºF to 113ºF) [8,16]; therefore, cooling food to below these temperatures is important to prevent contamination [17]. A similar outbreak of *C. perfringens* occurred in Japan where vegetables (spinach) were implicated [18].

In this outbreak, *Bacillus spp* were isolated from the carrot sample. *Bacillus spp are* bacteria that are widely found in the environment and can be isolated from the soil [19]. One of the species, *Bacillus cereus,* causes food poisoning that is similar to that caused by *C. perfringens* but in this outbreak though *Bacillus spp* were isolated from the carrots, the viable count of 10^2CFU/gram of carrot was not significant to have caused an infection as the infective dose is 10^6 CFU/gram.

A lot of fast food eateries are present in Koforidua however activities of these eateries are not regularly monitored by environmental health officers. This leads to improper handling, preparation and storage of food by vendors causing contamination of foods which can eventually lead to outbreaks. Moreover, most outbreaks of food poisoning origin are undetected because cases are underreported [20] and the few that are detected are not investigated with laboratory confirmation to detect problems in the food handling chain for implementation of appropriate control measures. However, in this outbreak *C. perfringens* was detected as the probable causative agent and problems in the vegetables preparation, storage and transportation process were identified.

Most of the cases were from Betom and Adweso. This could be because these suburbs were very close to the salad eatery. Also the main route for these suburbs to the central business part of town was by the salad eatery therefore, the eatery was easily accessible to persons from these suburbs moving to and from the central business part of town compared to other suburbs in the town.

One of the main challenges of this investigation was the inability to detect if the *C. perfringens* strain identified carried the cpe-gene which encodes the enterotoxin that causes

food poisoning. This is because the regional hospital laboratory lacked the capacity to do it, however, with the incubation period for cases and signs and symptoms reported in this outbreak, *C. perfringens* could be the probable cause of the outbreak.We had a difficulty in obtaining stool samples from cases because most had taken antibiotics before reporting to the hospital and since the diagnostic technique was bacteriological culture, it was not possible to use samples from these cases for isolation of bacteria. Moreover, the attack rate obtained in this outbreak could be lower than the actual attack rate because the denominator used in computing the attack rate was the population of the suburbs surrounding the salad eatery and not population of those who ate in the eatery. This is because we could not obtain any record of persons who ate from the eatery during the outbreak period. For security reasons, active case search could not be conducted in the Koforidua township thus only cases that reported to the regional hospital were investigated.

Following this outbreak when inadequate washing of vegetables, insufficient cooling of the cabbage before storage and poor temperature control during transportation was detected, the eatery was closed down. The owner and her employee were educated on strict food hygiene and temperature control before they were allowed to operate the eatery. Environmental health officers then supervised and monitored their activities to ensure proper salad preparation, storage and transportation.

5. CONCLUSION

A point source outbreak probably caused by *C. perfringens* food poisoning occurred with no fatality among persons who ate spore-contaminated salad at a salad eatery. The probable vehicle of infection was the cabbage. The inadequate cleaning of cabbage, insufficient cooling of par-boiled cabbage before storage and poor temperature control during transportation to the salad eatery were risk factors identified in the food handling process.

Food vendors in the municipality have been educated on strict food hygiene to ensure food safety and periodic inspection of food eateries by environmental health officers to ensure vendors comply with standard protocols has been intensified.

CONSENT

All authors declare that informed consent was obtained from all cases interviewed during the outbreak investigation.

ETHICAL ISSUES

We sought permission to review medical records of cases from the regional hospital management team. This was a public health response to an outbreak therefore ethical committee review did not apply.

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COMPETING INTEREST

The authors declare that they have no competing interest

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