

## Large outbreaks of *Clostridium perfringens* food poisoning associated with the consumption of boiled salmon

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### SUMMARY

Five large outbreaks of food poisoning are described in which clinical, epidemiological or laboratory data indicated *Clostridium perfringens* as the causative organism. The foodstuff common to all incidents was boiled salmon served cold as an hors d'oeuvre. In all cases the fish had been subject to a long period of cooling or storage between boiling and consumption. It is thought that multiplication of the organism occurred during this time.

Recommendations are made for the avoidance of further similar incidents.

### INTRODUCTION

Food poisoning due to the organism *Clostridium perfringens* is usually associated with the consumption of meat or poultry. Bacterial spores introduced on the flesh following contamination from the gut contents can survive cooking processes, particularly if the spores are heat-resistant. Under suitable conditions the spores germinate, and bacterial growth occurs during subsequent holding of the food prior to consumption. Food must contain large numbers of vegetative cells of *C. perfringens* for there to be a risk of food poisoning (Gilbert, 1983). The risk is recognized to be greatest where large joints of meat, poultry carcasses or meat stocks are left to cool slowly after cooking (Roberts, 1982). Most fish dishes are

consumed hot soon after they have been cooked. Although *C. perfringens* may commonly occur in the gut contents or on the flesh of gutted or filleted fish (Taniguti & Zenitani, 1969), opportunity for growth of the organism between cooking and consumption is rare.

In this paper five outbreaks of illness are described in which boiled salmon served cold as an hors d'oeuvre was either shown to be or was thought to have been the most likely vehicle for *C. perfringens* food poisoning. Apart from a reference to the first two incidents mentioned herein (Turnbull & Gilbert, 1982) we are unaware of reports of previous incidents of this type occurring in this country. Two of the incidents are among the largest outbreaks of *C. perfringens* food poisoning recorded in the literature.

#### *Incident A*

Fifty guests who attended a festival supper in the County of Avon in June 1974 were taken ill with severe diarrhoea 16–20 h afterwards.

#### *Laboratory investigation*

*C. perfringens* was isolated from salmon mayonnaise remaining from the meal at counts of  $10^8$  per g. Six single-colony isolates of this organism from the fish were all shown to be of serotype 11 (7), as were 5 of 6 isolates from a faecal sample from a 9-year-old boy who had been taken ill with diarrhoea 18 h after eating a portion of the salmon taken home by one of the caterers. The count of *C. perfringens* in this faecal sample was  $2 \times 10^4$  per g. An atypical strain of *Bacillus cereus* (with a weak egg-yolk reaction) was also present in the salmon sample. There were large numbers of this organism on the surface of the fish ( $10^9$  per g), and smaller numbers ( $8 \times 10^5$  per g) in the interior flesh. This organism was not detected in the faecal sample. No other food poisoning organism was detected.

#### *Food preparation*

A large quantity of the fish had been boiled and left to cool overnight in its own juices. Although it is understood that a refrigerator was used it is likely that cooling of the fish was inefficient. After portioning, mayonnaise prepared on the day of the function was added to the fish just before serving.

#### *Interpretation*

It was concluded from the clinical history, the incubation period, and the counts and serotypes of *C. perfringens* in the fish and the faecal sample that this was probably a *C. perfringens* food poisoning, the organism being derived from the raw fish. The finding of *B. cereus* (in the food only) was thought to have been incidental, but the possibility of aerobic spore-bearing organisms (*Bacillus* sp.) contributing to illness following consumption of boiled fish or fish products should not be overlooked.

#### *Incident B*

Two hundred and nineteen guests attended a masonic dinner in Lancashire in February 1975. The main items on the menu were: an hors d'oeuvre, cold salmon with salad; roast beef in gravy; and lemon meringue pie and cream. Within 9–12 h

at least 56 of those attending had become ill with diarrhoea. The illness lasted 1–3 days.

#### *Laboratory investigation*

Food remaining from the function was available for laboratory examination. *C. perfringens* was isolated from two samples of cooked salmon at counts of  $6 \times 10^4$  and  $4 \times 10^5$  per g. No other food-poisoning bacteria were detected in the fish, and none was detected in samples of roast beef or gravy. Detailed examination of eight faecal samples from persons ill revealed heat-resistant *C. perfringens* to be present at counts between  $10^5$  and  $2 \times 10^8$  per g. No other pathogen was isolated. All but 1 of 16 isolates of *C. perfringens* from these 8 faecal samples were of serotype 1, as were all 18 isolates taken from samples of salmon.

#### *Food preparation*

Six whole frozen salmon each approximately eight pounds in weight had been used. They had been defrosted overnight, seasoned, wrapped in tin foil, and placed in three baking tins lined with foil. Sufficient cold water was added to cover the wrapped fish which were then brought to the boil over gas rings and kept boiling for an hour and a half. The fish, still wrapped, were left to cool in the pans of water at ambient temperature in the kitchen overnight. At noon on the following day they were unwrapped, skinned and portioned together with salad on plates ready for serving. The plates stood for a minimum of 2–4 h at room temperature prior to the start of the function. The cook was adamant that she had prepared salmon in this way for 25 years and had never previously had complaints of illness.

#### *Incident C, D and E*

These all occurred at a modern hotel in central London which specializes in large luncheon and dinner dance functions. Incident C occurred in February 1984. Incidents D and E affected two different groups of persons attending functions on the Saturday and Sunday of one weekend in February 1985.

#### *Incident C*

This occurred following a mid-day meal in which salmon mayonnaise featured on the menu. At least 60 of the 850 women attending the function were taken ill with diarrhoea and stomach cramps 12–16 h after the meal. Those attending lived over a wide area of the eastern counties of England, and most had returned home before becoming ill.

#### *Laboratory investigation*

Non-serotypable *C. perfringens* was isolated from faecal specimens from three patients, and was present in small numbers only ( $10^2$ – $10^3$  per g) in a sample of cooked salmon remaining. No other food-poisoning bacteria were detected in the fish or in a sample of mayonnaise.

#### *Food preparation*

Salmon had been defrosted on day one, boiled and cooked on day two, and consumed after portioning on day three. The other foodstuffs served on this occasion were beef bourguignon with rice and vegetables and a peach dessert.

### *Interpretation*

The paucity of laboratory data arising from this incident meant that the cause of illness was not clearly established at the time. In retrospect it closely parallels the incidents D and E occurring at the same hotel in February 1985, and gives credence to the belief that boiled salmon was the vehicle for a *C. perfringens* food poisoning.

### *Incident D*

Seven hundred and two persons attended a dinner/dance at the hotel on a Saturday evening in February 1985.

Within 24 h of the event a large number of those who had been present reported ill with gastro-enteritis, chiefly a watery diarrhoea, stomach cramps and nausea. Vomiting was not a marked feature. A questionnaire issued later revealed that the incubation period was 6–30 h, with the peak onset time 10–15 h after the meal. The illness lasted 1–3 days. The guests dispersed to their homes immediately after the function, making investigation more difficult.

### *Epidemiological and laboratory investigation*

The P.H.L.S. Communicable Disease Surveillance Centre (C.D.S.C.) was alerted by the organizers of the function on the Monday morning as soon as reports of illness started to come in, and a medically qualified epidemiologist from C.D.S.C. was invited to assist the Local Authority and Department of Community Medicine with the investigation. On preliminary enquiries it was estimated that about 80% of those attending had been ill, implying that as many as 500 persons may have been affected.

Faecal specimens were collected within 2–3 days of the onset of symptoms from 19 guests who had been ill and submitted to the local Public Health Laboratory.

Tests for *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp, were all negative, but 'heat-resistant' *C. perfringens* (the spores of which can survive boiling for at least an hour) were recovered from 10 of the 19 specimens. In addition 'heat-sensitive' strains of *C. perfringens* were isolated from a further 5 of these 19 specimens. Representative cultures of the organisms (three separate colonies from each of the faecal specimens that were positive) were submitted to the Food Hygiene Laboratory at Colindale for serotyping, together with the faecal specimens from which they were isolated for tests for the presence of *C. perfringens* enterotoxin (Bartholomew *et al.* 1985).

All cultures from 9 of the persons from whom 'heat-resistant' strains were isolated and those from all 5 persons from whom 'heat-sensitive' strains were isolated were shown to be of the same serotype, 1. In addition *C. perfringens* enterotoxin was detected in 9 of the 12 faecal specimens tested. These results indicate that the illness was due to *C. perfringens* food poisoning and suggest a common foodstuff as the vehicle for the organism.

### *Incident E*

Occurring at the same hotel on the day after incident D, it was investigated as a separate incident since a quite different group of guests, numbering 455, were involved. They had come to London from many different parts of the country.

Reports of illness in this group were first received on the Tuesday morning and indicated that the illness was of a similar nature to that in incident D. Of 371 persons replying to a questionnaire issued later by the organizer of this function, 284 (77 %) reported being affected.

#### *Laboratory investigation*

Attempts were made to ensure that faecal specimens from some of those ill were obtained for laboratory examination. The wide dispersal of the guests hampered rapid follow-up and, in the event, we learned of the examination of only a few faecal specimens in Public Health Laboratories in different parts of the country. *C. perfringens* was detected in 7 of the 11 specimens in which it was sought. No other intestinal pathogen was detected. Cultures of *C. perfringens* from 6 patients were submitted for serotyping, and those from 4 were found to be of serotype 1. No test was done for the presence of enterotoxin in faeces from this incident.

The clinical history, together with the isolation of *C. perfringens* serotype 1 from a relatively high proportion of the faecal specimens in which it was sought, strongly suggests, that this was a further outbreak caused by this organism.

#### *Illness in hotel staff at the time of incidents D and E*

From the Saturday evening through until the Monday morning, a total of 16 members of the hotel staff reported ill with diarrhoea. Faecal specimens were examined from 13 of these, primarily to exclude presence of infective pathogens (*Salmonella* spp., *Shigella* spp.). All specimens were negative for these pathogens. Six specimens collected early after the onset of illness were tested for *C. perfringens*. All six were positive for this organism, and five specimens were shown to contain *C. perfringens* serotype 1.

#### *Foodstuffs implicated in incidents D and E*

At both functions the great majority of the guests had partaken of food from a standard menu. Both menus contained salmon mayonnaise as an hors d'oeuvre and both contained a breast of chicken dish, though it was served in different ways and under different names on the two days.

Questionnaires were issued to guests at both functions to collect information on the foodstuffs each had consumed, and whether or not they had experienced illness. Interpretation of food-specific attack rates may be difficult under these circumstances. Table 1 shows the food-specific attack rates for incident D (Saturday). The only foodstuff for which there was a statistically significant difference in the attack rates, between those who consumed it and those who did not, was the salmon mayonnaise. This case is based on the recall of just three persons who were not ill, and stated that they had not eaten salmon cocktail.

Similar results for incident E (Sunday) are shown in Table 2. Statistically significant differences in the attack rates were found here between those who did or did not eat one or other of two foodstuffs, the salmon mayonnaise or the chicken. The numbers of individuals reporting that they did not consume one or other of these two items were small, but were considered sufficiently large to apply Cochran's Test for analysis of the occurrence of illness after eating one food independent of the other. Results of this test are shown in Table 3. They indicate

Table 1. *Incident D. Food-specific attack rates*

Food	Ate				Did not eat				P value
	Ill	Not ill	Total	Attack rate (%)	Ill	Not ill	Total	Attack rate (%)	
Salmon mayonnaise	208	36	244	85	0	3	3	0	0.0074
Cheese	202	35	237	85	5	4	9	55	N.S.
Chicken	202	36	238	85	5	3	8	63	N.S.
Sauce with chicken	200	35	235	85	3	2	5	60	N.S.
Potatoes	208	38	246	86	1	1	2	50	N.S.
Beans	201	37	238	84	5	0	5	100	N.S.
Dessert	201	38	239	84	6	1	7	85	N.S.

N.S., not significant.

Based on replies to 249 questionnaires. Only main food items consumed are listed.

Table 2. *Incident E. Food-specific attack rates*

Food	Ate				Did not eat				P value
	Ill	Not ill	Total	Attack rate (%)	Ill	Not ill	Total	Attack rate (%)	
Salmon mayonnaise	280	79	359	80	4	8	12	33	0.003
Soup	261	82	343	76	23	5	28	82	N.S.
Chicken	276	79	355	78	8	8	16	50	0.03
Vegetables	272	82	354	77	12	5	17	71	N.S.
Potatoes	271	81	352	77	13	6	19	68	N.S.
Peach	276	82	358	77	8	5	13	62	N.S.
Ice cream	256	72	328	78	28	15	43	65	N.S.

N.S., not significant.

Based on replies to 371 questionnaires.

Table 3. *Incident E. Cochran's test for significance*

Food eaten	Salmon			No salmon		
	Ill	Not ill	Total	Ill	Not ill	Total
Chicken	273	71	344	0	7	7
No chicken	7	7	14	2	4	6
Total	280	78	358	2	11	13

Cochran's test for chicken, independent of salmon,  $\chi = 1.74$ ,  $P > 0.05$  (not significant).

Cochran's test for salmon, independent of chicken,  $\chi = 4.12$ ,  $P < 0.05$  (significant).

that illness was significantly associated with consumption of salmon independent of the consumption of chicken, and not vice versa.

#### *Food preparation, incidents D and E*

The methods of food preparation had been ascertained at an early stage in the investigation from the hotel staff. The mode of preparation of those foodstuffs for

which the statistical analyses had shown a significant association with illness, or which were thought to be potentially risky, is now described.

*Salmon mayonnaise.* The salmon used was frozen Pacific salmon from Alaska. Gutting and dressing had been carried out on board ship in the Pacific. It was purchased, boxed and frozen, from a specialist wholesaler in South London, and delivered to the hotel 48 h before each function. Two hundred lbs was delivered on the Thursday prior to the Saturday function, and a further 90 lbs on the Friday. The fish was filleted at the hotel into slices approximately half an inch thick and placed in a cold water bouillon. The bouillon contained vinegar, onion, carrot and seasoning. The immersed fish was brought to the boil in large shallow pans and kept boiling for 15–30 min. It was then left to cool in the bouillon for 2–3 h at ambient temperature before being drained and placed, covered with sheets of paper, on trays. These trays were then placed in 'walk-in' cold rooms overnight. The following afternoon the cooked fillets were taken from the refrigerators, broken up by hand, and portioned with the addition of lettuce, capers and home-made mayonnaise (pH 4.0). The completed dishes were understood to have been refrigerated until the following night and served cold straight from refrigeration. No faults in the extensive refrigeration plant were revealed at the time of the kitchen inspection. Salmon for the Sunday function (E) was understood to have been prepared in a similar way 24 h later. The reported method of preparation did not reveal any immediately obvious cause for concern, but the quantities of food being processed could well have led to difficulty at the cooling stage.

*Chicken* (Function D). Fresh unfrozen portions of breast of chicken were delivered to the hotel on the day before the dinner. After overnight refrigeration they were prepared on the following morning. In the afternoon, portions were dipped in a wash of beaten egg and breadcrumbs and shallow fried. Prior to serving they were kept warm for 1–2 h in heated cabinets. No fault in the operation of these cabinets, which are intended to keep cooked food at a temperature above 65 °C, was known. The method of preparation was understood to have been similar for the Sunday function (E). A sample of chicken breast fried in breadcrumbs for the Sunday function was submitted to a Public Health Laboratory outside London for bacteriological examination. No *C. perfringens* or other food-poisoning bacteria were detected in this sample.

*Chicken sauce.* For the Saturday night function (D) only, the chicken portions were served on a layer of sauce made from thickened stock the day before and reported to have been kept boiling overnight in a stockpot. This sauce did not feature as a statistically significant food item (Table 1) and cannot be implicated as a food vehicle in function E.

At the time of the kitchen inspection none of those foodstuffs remained for submission for laboratory tests.

#### *Incidents D and E conclusions*

Apart from being gathered together in the same building the only common factor uniting all those who were ill was consumption of food prepared for the two functions. An 'incubation period' between assembly and onset of illness of, on average, 10–16 h excludes the possibility of food-borne or air-borne viral gastrointestinal infection. The clinical, laboratory and epidemiological investigations all

Table 4. *Summary of the five incidents*

Incident	Location	Year	Number of persons at risk	Number of persons ill	Suspected food vehicle
A	Bath	1974	Not known	50	Salmon mayonnaise
B	Preston	1975	219	56	Salmon salad
C	London hotel	1984	850	60+	Salmon mayonnaise
D		1985	702	209+	
E		1985	455	284+	

Table 5. *Summary of isolations of Clostridium perfringens from remaining cooked salmon and from stool specimens of patients*

Incident	Count in salmon (per g)	Serotype in salmon	Main serotype detected in stools	Number of stools with this serotype
A	10 <sup>8</sup>	11(7)	11(7)	1
B	10 <sup>4</sup> -10 <sup>6</sup>	1	1	8
C	10 <sup>2</sup> -10 <sup>3</sup>	N.T.	N.T.	3
D	N.A.	—	1	14*
E	N.A.	—	1	4

N.T., non-typable, N.A., food not available.

\* Tests for enterotoxin positive in 9 of 12 stools.

support the interpretation that the illness affecting guests at both functions, as well as some of the hotel staff, was a *C. perfringens* food poisoning. In these incidents it was not possible to verify this by recovery of the organism from remaining food.

From the food preparation histories supplied and the epidemiological data abstracted from returned questionnaires, the greatest suspicion falls on the salmon cocktail as the vehicle for the organism. It should be stated that the epidemiological data do not entirely eliminate the possibility that poultry or stock (sauce) was the vehicle in one or both of these incidents, but the statistical data render this possibility most unlikely.

#### DISCUSSION

The main findings are summarized in Tables 4 and 5. In each of the five incidents differing evidence was forthcoming in support of the conclusion that *C. perfringens* had been the causative organism and that salmon was the foodstuff in which it had multiplied.

In only two incidents (A and B) were large numbers of this organism cultured from the salmon. In three incidents (A, B and C) *C. perfringens* was isolated both from one or more faecal specimens from those ill and also from the salmon, and

serotyping of the organisms showed the cultures to be related or possibly so. In the two largest incidents (D and E) there was an almost complete lack of availability of food remnants for investigation. Dispersal of the guests also made rapid collection of faecal specimens more difficult. A positive result for the then recently introduced test for *C. perfringens* enterotoxin in faecal samples was the most conclusive indication of the cause of incident D. The similarity in clinical presentation and the isolation of *C. perfringens* of the same serotype from those ill suggests a common cause for incidents D and E. The implication that salmon was the foodstuff responsible for these last two incidents arose largely from the reported method of food preparation together with the results of the epidemiological investigations. It should be remembered that for this information one is reliant on the informants for accuracy and that no independent check is normally possible.

The uniting factor for all five incidents was the mode of preparation of the salmon. Whole fish or large quantities of thick-cut fillets were cooked by boiling, followed by a long slow cooling process, either at room temperature or for a part of the time in a refrigerated cold store.

Both the cooking and the rapid cooling of food in bulk can present difficulties. The larger the scale of the catering operation the greater the difficulties will be. In very large-scale catering increased facilities for efficient rapid cooling of food will be as important as increased facilities for efficient cooking. Food poisoning due to *C. perfringens* is most likely to occur where catering is on a large scale, where meat or, as shown here, fish cuts are large and difficulties are encountered at the cooling stage.

The following measures are recommended for the avoidance of similar incidents.

(1) Consideration should be given to the division of large whole salmon (greater than 3 kg, 6½ lbs weight) either prior to cooking or else soon after removal from boiling. This will facilitate speed of cooking and, of particular importance, the speed of cooling afterwards. For preparation of portioned salmon dishes it will be more appropriate to divide the fish into steaks not exceeding 5 cm (2 in) in thickness prior to boiling.

(2) After boiling, whether as whole fish, as sides, or steaks, the fish should be removed from the liquor and left to cool, covered but not wrapped, in a cool area of the kitchen, for not more than one and a half hours.

(3) If there is to be further holding prior to consumption rapid chilling is essential. The fish should be placed unwrapped and in layers not more than 5 cm (2 in) thick in stainless steel or aluminium trays for refrigeration in a cold store at 2–5 °C. Trays must not be close-stacked in a refrigerator or efficient cooling will be impossible.

(4) Where large quantities of fish (e.g. 45 kg, 100 lbs, or more) are regularly being prepared in this way specially designed rapid chilling equipment as used in cook/chill catering will be necessary. An electronic thermometer with a probe for accurately measuring the centre temperature of the chilled food would be desirable.

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## REFERENCES

- BARTHOLOMEW, B. A., STRINGER, M. F., WATSON, G. N. & GILBERT, R. J. (1985). Development and application of an enzyme linked immunosorbent assay for *Clostridium perfringens* type A enterotoxin. *Journal of Clinical Pathology* **38**, 222–228.
- GILBERT, R. J. (1983). Food-borne infections and intoxications; recent trends and prospects for the future. In *Food Microbiology: Advances and Prospects* (ed. T. A. Roberts and F. A. Skinner), pp. 47–66. Society for Applied Bacteriology Symposium Series No. 11. London and New York: Academic Press.
- ROBERTS, D. (1982). Factors contributing to outbreaks of food-poisoning in England and Wales 1970–1979. *Journal of Hygiene, Cambridge* **89**, 491–498.
- TANIGUTI, T. & ZENITANI, B. (1969). Incidence of *Clostridium perfringens* in fishes. II. On the detection rate of *C. perfringens* type A and heat resistance of isolated strains. *Journal of the Food Hygienic Society of Japan* **10**, 266–271.
- TURNBULL, P. C. B. & GILBERT, R. J. (1982). Fish and shellfish poisoning in Britain. In *Adverse Effects of Foods* (ed. E. F. P. Jelliffe and D. B. Jelliffe), pp. 297–306. New York: Plenum Press.