
Appendix B: Investigation and microbiological examination of samples from suspected food poisoning incidents

Introduction

Whenever a food poisoning incident is suspected every effort should be made to obtain accurate histories of food consumption from the individuals who have developed symptoms. Remnants of uneaten food associated with the incident should be taken from both the place of preparation and that of consumption. At the earliest opportunity as much detail as possible should be collected concerning the method of preparation, the cooking and the storage of all implicated food items, as memories are short. Even small delays in obtaining this information can hamper an epidemiological investigation.

Food-borne infections vary in their mode of action on the gastrointestinal tract. Those in which the infecting organism has multiplied to a large extent in the foodstuff before ingestion will have a shorter incubation period than those in which growth within the intestine has to occur before symptoms are experienced. Pre-formed toxin, in food, is likely to act on the stomach and cause rapid onset vomiting. The toxins of *Clostridium botulinum* are absorbed and produce more serious sequelae by affecting the central nervous system. The longest incubation times are associated with those organisms that subsequently invade the blood stream after entering the lower intestine. Intermediate delay periods of onset occur where the mode of action is by way of enterotoxins liberated only when the organisms begin to either lyse or sporulate.

Knowledge of the clinical details of the illness and the presentation of symptoms provide vital clues as to the likely food poisoning organism (Tables B.1 (infections) and B.2 (intoxications)).

It is rarely practicable or necessary to culture for all pathogens in all samples. Relevant tests will be selected in the light of available clinical and epidemiological information. For example a pathogen may already have been isolated from human specimens examined in parallel with the food. The residue of samples should be stored under refrigeration for possible further examination.

Figure B.1 illustrates a scheme to be considered when a suspect food arrives in the laboratory. Much of the work may be omitted or postponed if the clinical information gives a clear lead or if the pathogen has already been isolated from the patient. Detailed methods for the isolation and identification of the various

Table B.1 Microbiological food-borne infections: usual incubation periods and symptoms. Reproduced with permission, from [1].

Causative organism	Incubation period	Symptoms
<i>Salmonella</i> spp.	12–48 h	Diarrhoea, vomiting, fever, abdominal pain lasting for several days
<i>Salmonella typhi</i>	12–20 days	Fever, septicaemia and other systemic symptoms
<i>Campylobacter jejuni/coli</i>	2–5 days	Fever and malaise often precede abdominal pain and profuse diarrhoea (often bloody)
<i>Escherichia coli</i>		
EPEC, EIEC, ETEC, EaggEC	10–72 h (depending on group)	Diarrhoea, vomiting, fever, malaise
VTEC serotype O157	12–60 h	Haemorrhagic colitis, haemolytic uraemic syndrome
<i>Shigella</i> spp.	1–4 days	Abdominal cramps, diarrhoea and fever, dysentery
<i>Yersinia enterocolitica</i>	1–7 days (can be shorter)	Abdominal pain, fever, headache, diarrhoea, malaise and vomiting
<i>Vibrio parahaemolyticus</i>	12–24 h	Profuse diarrhoea, leading to dehydration, vomiting and fever
<i>Vibrio cholerae</i> -O1 and non-O1	48–72 h	Profuse watery diarrhoea
<i>Aeromonas</i> spp.	8–36 h	Diarrhoea, malaise
<i>Clostridium perfringens</i>	8–18 h	Abdominal pain, diarrhoea, nausea, rarely vomiting or fever
<i>Bacillus licheniformis</i> *	2–14 h	Predominantly diarrhoea, vomiting occasionally, abdominal pain
<i>Bacillus cereus</i> *	8–16 h	Predominantly diarrhoea with occasional vomiting
<i>Listeria monocytogenes</i>	1–7 days 1–10 weeks	Diarrhoeal symptoms (rare) Meningitis, fever, septicaemia, abortion
NLV	12–48 h (may be as long as 72 h)	Nausea, projectile vomiting, diarrhoea lasting 1–2 days
<i>Cryptosporidium parvum</i>	1–2 weeks	Diarrhoea, bloating
<i>Cyclospora cayentanensis</i>	1–2 weeks	Watery diarrhoea lasting 1–8 weeks, abdominal pain, bloating

*Members of the *Bacillus* group produce illness with a range of symptoms and incubation periods. The full mechanism of action has not been fully elucidated and so the *Bacillus* spp. included in this table have been allocated on the basis of their main symptom.

EaggEC, enteroaggregative *Escherichia coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; NLV, Norwalk-like viruses (also known as small round structured viruses, SSRV); VTEC, verocytotoxin producing *E. coli*.

Table B.2 Microbiological food-borne intoxications: usual incubation periods and symptoms. Reproduced with permission, from [1].

Causative organism	Incubation period	Symptoms
<i>Staphylococcus aureus</i>	2–6 h	Severe vomiting, abdominal pain, diarrhoea, occasionally severe dehydration leading to collapse
<i>Bacillus cereus</i> *	1–5 h	Acute vomiting; diarrhoea also common
<i>Bacillus subtilis</i> *	1–14 h (can be as short as 10 min)	Vomiting and diarrhoea
<i>Clostridium botulinum</i>	12–36 h	Fatigue, lassitude, dizziness, involvement of central nervous system causing blurred vision, difficulty with speech and breathing

*Members of the *Bacillus* group produce illness with a range of symptoms and incubation periods. The full mechanism of action has not been fully elucidated and so the *Bacillus* spp. included in this table have been allocated on the basis of their main symptom.

food poisoning organisms from food are to be found elsewhere in this manual.

Aerobic colony counts (total viable counts) and enumeration of indicator organisms (Enterobacteriaceae and *Escherichia coli*), expressed as colony forming units (cfu)/g, are complementary to the examination for specific food poisoning organisms. They may give an indication of whether effective hygiene and temperature control procedures have been applied during preparation, transportation and storage. Even microscopic examination of simple stained smears from homogenized food suspensions can reveal relative numbers of morphological types of organisms. It can also be useful as a rapid screening test when staphylococcal food poisoning is suspected.

The following notes on individual microorganisms are provided as an aid to laboratory workers in selecting relevant procedures and follow-up tests.

Organisms causing food-borne infections

Salmonella

With a few important exceptions, e.g. *Senterica subsp.* Typhi, *S. Dublin* and *S. Choleraesuis*, salmonellae show little host specificity and most can cause gastroenteritis when ingested by humans. In the investigation of outbreaks efforts should be directed towards demonstrating a common *Salmonella* type in patients and food and in establishing the reason for the presence of salmonellae in food. Incubation time is usually 12–48 h or longer since multiplication has to occur in the intestine.

Isolation of salmonellae from stool specimens in acute cases is usually possible by direct plating on suitable selective agars, although enrichment may

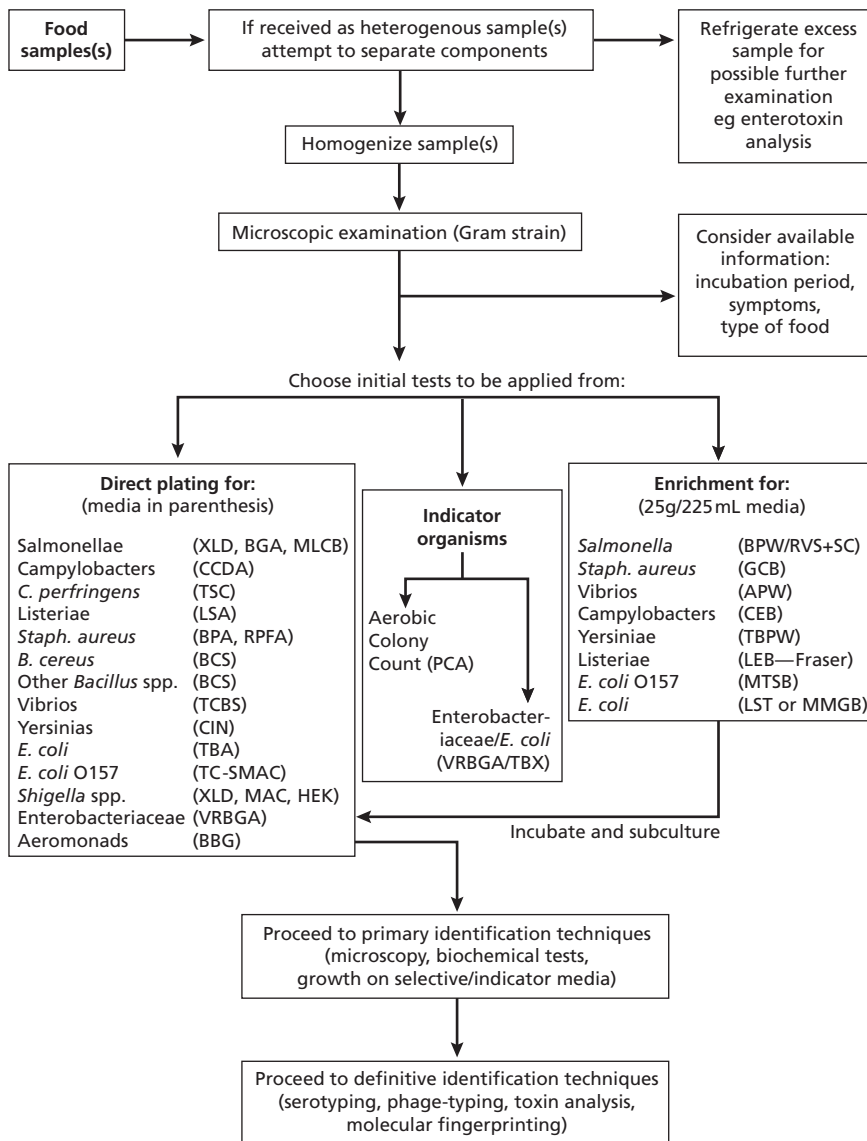


Fig. B.1 Suggested scheme for the examination of food specimens from food poisoning outbreaks [1].

Culture media:

APW, alkaline peptone water; BCS, bacillus cereus selective agar (e.g. PEMBA or MYP); BGA, brilliant green agar; BPA, Baird Parker agar; BPW, buffered peptone water; CEB, campylobacter enrichment broth; CCDA, cefoperazone charcoal desoxycholate agar; CIN, cefsulodin-irgasan novobiocin agar; HEK, Hektoen agar; GCB, Giolitti Cantoni broth; DCA, desoxycholate citrate agar; LEB, listeria enrichment broth (e.g. Fraser broth); LSA, listeria selective agar (e.g. Oxford); LST, lauryl sulphate tryptose broth; MAC, MacConkey agar; MLCB, mannitol lysine crystal violet bile agar; MMGB, minerals modified glutamate broth; MTSB, modified trypticase soya broth; PA, Preston agar; PCA, plate count agar; RPFA, rabbit plasma fibrinogen agar; RVS, Rappaport Vassiliadis soya peptone broth; SC, selenite-cystine broth; SEB, shigella enrichment broth; TC-SMAC, tellurite-cefixime sorbitol MacConkey agar; TBA, tryptone bile agar; TBPW, tris buffered peptone water; TBX, tryptone bile agar supplemented with BCIG; TCBS, thiosulphate citrate bile-salt sucrose agar; TSC, tryptose sulphite cycloserine agar; VRBGA, violet red bile glucose agar; XLD, xylose lysine desoxycholate agar.

be necessary for diagnosis of carriers and asymptomatic cases. Opinions differ on the number of *Salmonella* organisms that need to be ingested to form an infecting dose. In some outbreaks large numbers have been found in the implicated food while in others there is every indication that infection has resulted from ingestion of fewer than 100 salmonellae. In many food and milk-borne outbreaks the organisms cannot be recovered from the implicated source by direct plating techniques. For this reason many enrichment protocols have been described for the isolation of salmonellae from food, and many comparisons of these methods have been made. No single procedure has been found to be suitable for the recovery of all salmonellae from all types of food. Most of the methods described involve primary enrichment in non-selective broth, to allow recovery of sublethally injured organisms, followed by secondary enrichment in elective or selective broths. Secondary enrichment broths are subcultured after incubation on to selective agars. Suspect *Salmonella* colonies are checked for purity and their identity confirmed by biochemical and serological tests.

Salmonella Typhi and the *S. Paratyphi* A, B and C are worthy of special mention. These serotypes are host adapted to humans, but can be transmitted in food. The usual source of these organisms in food is by contamination from an infected food worker or by direct contamination from human sewage. The traditional techniques used for isolation of *Salmonella* from food may not be suitable for the detection of these host-adapted serotypes. Use of media containing brilliant green or malachite green dyes and methods using elevated temperatures (41.5°C) are particularly unsuitable. Procedures using other enrichment media (e.g. tetrathionate broth [formulations without brilliant green] or selenite) incubated at 37°C for 24–48 h and subcultured to xylose lysine desoxycholate agar (XLD) and bismuth sulphite agars are more likely to be successful. These organisms are categorized as Hazard Group 3 pathogens and therefore all work undertaken with high-risk samples and known positive cultures should be carried out in a Containment Level 3 laboratory.

Salmonellae are identified initially according to the serological reactions of their somatic O and flagellar H antigens. Strain diversity can also be demonstrated within each serotype. To assist in epidemiological investigations special methods of strain identification have been developed for some of the commoner *Salmonella* serotypes. For example, phage typing is available for *S. Typhimurium*, *S. Enteritidis*, *S. Hadar* and *S. Virchow*. Antimicrobial resistance patterns are also valuable, especially for characterization of strains of *S. Typhimurium* that have acquired multiple resistance. Application of molecular fingerprinting techniques has become commonplace. The gold standard is pulsed field gel electrophoresis (PFGE) [2] and single enzyme amplified fragment length polymorphism (SAFLP) is also proving to be a valuable technique [3].

***Campylobacter jejuni* (C. coli)**

Campylobacter jejuni is now well recognized as the major cause of bacterial

gastroenteritis in humans, but the epidemiology and mode of transmission of the organism has still not been fully determined. The route of infection is by ingestion and the incubation period is usually 2–5 days. Bloody diarrhoea with fever and abdominal pain are commonly the predominant features of the illness.

In several large outbreaks milk (either untreated or inadequately pasteurized) and water (from drinking water supplies or from recreational exposure) have been shown conclusively to be the vehicles of transmission. In the majority of sporadic cases the vehicle is not identified even when a particular food item is suspected. There are, however, strong associations between cases and either the handling of raw poultry or consumption of undercooked poultry.

It has been demonstrated that as few as 500 *Campylobacter* organisms can be an infective dose. Isolation of the organism from food and environmental sources is, therefore, attempted by enrichment culture. Enrichment broths are incubated at either 37°C or 41.5°C, or a combination of the two temperatures to allow recovery of sublethally damaged organisms, for 48–72 h and then subcultured on to selective agar media. These are further incubated under microaerobic conditions at 37°C or 41.5°C for 48–72 h. Suspect *Campylobacter* colonies on these media may be confirmed by giving a positive oxidase test and showing typical cell morphology by modified Gram stain and motility by either phase contrast or dark-field microscopy. Cultures may be identified and subdivided more fully into biotypes using several methods [4].

To aid epidemiological investigations and surveillance further characterization is necessary. In the UK this is currently achieved by a combination of serotyping and phage typing supplemented by molecular fingerprinting techniques [5–7]. PFGE and restriction fragment length polymorphism (RFLP) methods are most frequently used although gene sequence typing is now being introduced.

Escherichia coli

The historical association of acute gastroenteritis in infants below the age of 3 years with a number of serotypes of *Escherichia coli* is well founded. These serotypes, which are not specifically related to the production of verocytotoxin or identifiable enterotoxins, are usually designated enteropathogenic *E. coli* (EPEC). Outbreaks that were quite common in hospitals and nurseries a few decades ago are now very infrequent and this may be due to improvements in standards of hygiene.

A different group of *E. coli* serotypes produce an invasive type of diarrhoea similar to that caused by *Shigella dysenteriae* in which actual invasion of the colonic mucosa with ulceration occurs. Food-related outbreaks are infrequent, but when they do occur there appears to be no predilection for any particular age group. These are known as enteroinvasive *E. coli* (EIEC).

People who travel from countries with a high standard of hygiene to areas of the world with poor hygiene, particularly those with a tropical climate,

frequently fall victim to travellers' diarrhoea in which the most common cause is enterotoxigenic *E. coli* (ETEC). The source of the organism is contaminated food or water but the indigenous population are largely unaffected owing to immunity acquired by previous exposure. ETEC also invade and colonize the surface of the intestinal mucosa and then produce enterotoxins (similar to cholera toxins) that may be heat labile and/or heat stable.

Enteroaggregative *E. coli* (EaggEC) are so called because of their ability to adhere to HEp-2 cells in tissue culture in a characteristic manner. EaggEC have been associated with sporadic cases of diarrhoea and food-borne outbreaks in the UK. Another group of *E. coli* are the diffusely adherent *E. coli* (DEAC) but the pathogenicity of these organisms is poorly understood.

Direct plating on selective media such as MacConkey agar can be used for the isolation of these organisms from clinical cases but it is usually necessary to send faecal specimens to a reference laboratory for diagnosis and confirmation.

Isolation of these groups of *E. coli* from food can be done successfully by a combination of direct plating on selective media, such as MacConkey agar or violet red bile-salt lactose agar, and enrichment in minerals modified glutamate or lauryl sulphate tryptose broths. Isolates are confirmed by growth and indole production at 44°C. Demonstration of β -glucuronidase activity is frequently used for confirmation of *E. coli* isolated from food but this characteristic is not exhibited by all *E. coli* strains that cause gastrointestinal disease.

Many serogroups of *E. coli* produce toxins specific for vero cells in tissue culture. There are two main toxins VT1 and VT2 and, because they are closely related to shiga toxins found in *Shigella dysenteriae*, they are also known as ST1 and ST2. These verocytotoxins are associated with human cases presenting with diarrhoeal symptoms, haemorrhagic colitis and haemolytic uraemic syndrome. Hence, strains that produce these toxins are designated verocytotoxin producing *E. coli* (VTEC) or shiga toxin producing *E. coli* (STEC). Not all serogroups that produce verocytotoxins cause disease in humans although they are widely distributed in food-chain animals.

The major human pathogen in this group of verocytotoxin producing *E. coli* is serotype O157:H7. Isolation from clinical specimens is done by direct plating on cefixime-tellurite sorbitol MacConkey (TC-SMAC) agar incubated at 37°C for 24 h. Strains of this serotype are more readily detected by their lack of ability to ferment sorbitol in sorbitol MacConkey agar. Colonies are usually confirmed by latex agglutination with anti-O157 reagent and sent for confirmation of toxin production to a reference laboratory.

Gastrointestinal disease has been linked with many different foods but mainly with red meat and dairy products. Isolation of the VTEC O157 from foods is achieved by a combination of enrichment in modified trypticase soya broth (MTSB) incubated at 41.5°C for 24 h, immunomagnetic separation and plating onto CT-SMAC. Suspect colonies are confirmed as presumptive positive VTEC O157 by biochemical and latex agglutination tests. (Note: VTEC O157 isolates are usually β -glucuronidase negative.) Tests for toxin production and toxin typing usually require the facilities of a reference laboratory. For the

epidemiological investigation of outbreaks and sporadic cases additional characterization of isolates is needed. These tests, undertaken by the reference laboratory, include phage typing, toxin gene typing and PFGE.

VTEC are now categorized as Hazard Group 3 pathogens and therefore all work undertaken with high-risk samples and known positive cultures should be performed in a Containment Level 3 laboratory.

***Shigella* spp.**

This genus includes *Shigella dysenteriae*, *S. boydii*, *S. flexneri* and *S. sonnei*. These are host-adapted organisms and only infect humans and other primates. Infection with these organisms may produce a range of symptoms; the most severe cases develop dysentery, but milder forms may result in a self-limiting diarrhoeal disease. The infective dose is known to be low and the organisms can be spread via contaminated food and water, contact with contaminated environmental surfaces and by flies. Person-to-person spread is also important in institutional outbreaks. Food-borne transmission is usually the result of contamination of ready-to-eat foods by human sewage. Outbreaks of infection with *S. sonnei* and *S. flexneri* have been associated with contaminated salad vegetable crops and fruit.

Isolation from suspect food products is achieved by a combination of direct plating onto a suitable selective medium and by enrichment culture. The enrichment broths currently available are not specific for isolation of *Shigella* spp. but also grow other Gram negative enteric organisms.

Isolates should be referred to a reference laboratory for confirmation, specialist serotyping, phage typing (*S. sonnei*) and molecular typing.

Yersinia enterocolitica

Yersinia enterocolitica has been associated with a variety of human clinical disorders including diarrhoea, abscesses, septicaemia, arthritis, skin rash and symptoms similar to those of appendicitis. The organism has also been implicated in food poisoning where symptoms of abdominal pain, fever, vomiting and diarrhoea have been reported. The incubation period in food poisoning incidents is usually 24–36 h but can be longer. The organism can multiply in foods stored for prolonged periods at refrigeration temperatures (4°C) and also in acidic foods (pH 4.8). In such foods it may be found at high and therefore presumably hazardous levels.

The successful isolation of *Y. enterocolitica* from food and environmental samples is achieved by the use of enrichment techniques followed by plating onto selective media. A number of different enrichment procedures have been described and there is still debate on which enrichment and isolation procedure should be used to achieve the best recovery. One of the most productive has been enrichment in simple buffer solutions incubated at low temperatures, 4–9°C, for periods of up to 14 days with periodic subculture of the enrichment broth onto

cefsulodin-irgasan-novobiocin (CIN) agar for incubation at 30°C overnight. However, this prolonged incubation period is impractical and successful recovery using incubation at 21°C and 25°C has been described.

For epidemiological purposes a biotyping scheme that subdivides *Y. enterocolitica* into five biochemical groups has been described. Further subdivision of the species is possible using a serotyping scheme based on the somatic antigens and by phage typing. There are at least 58 serotypes of which serotypes O3, O8, O9 and O5,27 have been most commonly associated with human illness.

Vibrio parahaemolyticus

Vibrio parahaemolyticus, a halophilic Gram negative organism, was first recognized as a food poisoning organism in Japan. It has assumed prominence there as the commonest cause of food poisoning associated with the consumption of raw or processed seafoods in summer. In the United Kingdom the organisms should be considered and sought in all cases or outbreaks of gastroenteritis where seafoods are implicated and also in persons who have recently travelled abroad.

Enumeration of *V. parahaemolyticus* in foods should be attempted by inoculating and spreading dilutions of food suspensions on to the surface of plates of selective media followed by incubation overnight at 37°C. In addition a portion (25 g) of the food should be added to a suitable alkaline enrichment broth then incubated at 37°C followed by 41.5°C and subcultured after 6 h and 18–24 h on to selective agar plates. Following incubation suspect colonies are picked from the plates; those which produce oxidase and catalase and are sensitive to vibriostatic agent O129 (2,4-diamino-6,7-di-iso-propylpteridine) are classed as vibrios. Further identification of *V. parahaemolyticus* and its differentiation from other organisms is dependent on biochemical tests.

Serological typing of *V. parahaemolyticus* is important in epidemiological investigations: all strains have identical H antigens, but so far 11 'O' and 53 'K' antigens have been recognized, allowing identification of 54 different types.

***Vibrio cholerae* O1 and non-O1 cholera vibrios**

Traditionally, cholera and other choleraic infections caused by these organisms have not been considered in relation to food microbiology because the mode of transmission is primarily by water either directly or indirectly. The global movement of food products has created the potential for transmission of cholera to non-endemic areas. The Food Safety Act now embraces water used in the food industry so it is appropriate that mention is made of these organisms with regard to foods that come from an aqueous environment, or are washed in or irrigated with water that may be contaminated. Wet fish and non-acid fruits and vegetables should be examined when cholera infection is present in the place of origin of such foods. The same media and techniques are used as for the

detection of *V. parahaemolyticus*, but each organism has different cultural, biochemical and serological characteristics.

Aeromonas

The majority of clinicians and food microbiologists have yet to be fully convinced that this group of Gram negative bacilli are an established cause of food poisoning in humans. These organisms are present in all natural waters and moist environments and, not unexpectedly, in food submitted for laboratory examination, especially that stored at low ambient temperatures where *Aeromonas* spp. are still able to multiply rapidly.

Normal healthy humans rarely carry *Aeromonas* spp. in their faeces and when they do the strains appear to be similar to those in the environment. There is, however, growing evidence that individuals who have experienced incapacitating gastrointestinal symptoms, predominantly of diarrhoea, following the consumption of foods which can be heavily contaminated with *Aeromonas* spp. and in whom no other bacterial cause has been found, often yield heavy growths of these organisms if faeces are cultured on appropriate laboratory media. Enrichment in liquid media containing antibiotics is often employed since *Aeromonas* spp. tend to be inherently resistant to many antibiotics. They are also resistant to the chemical compounds used as disinfectants, including chlorine. It may well be that, rather than being the primary pathogens, *Aeromonas* spp. grow selectively and opportunistically in the gut of individuals in whom the balance of the natural flora is disturbed for some reason.

From knowledge of the pathogenicity of *Aeromonas* spp. it is not possible to give a precise incubation time for symptoms to develop although some guidance is given with the 8–36 h in Table B.1. It is important, however, to try to ascertain from the history of the food the likely source and to determine the particular item in which the number of *Aeromonas* organisms ingested would have been greatest. Outbreaks of enteritis due to aeromonads are unknown in the UK and the investigation of a sporadic case is often unrewarding.

Aeromonas hydrophila and *A. sobria* are the species most commonly isolated from meat products while *A. caviae* is more often associated with fish and fish products. Serotyping at a reference laboratory (available for strains from humans with or foods associated with gastroenteric infection) may lead to the elucidation of more of the epidemiology of infections due to these organisms.

Clostridium perfringens

Clostridium perfringens food poisoning outbreaks usually occur following the ingestion of large numbers (10^6 or more/g) of the organism in the suspected food. Investigation of the food preparation techniques will almost invariably reveal that the food has been held in conditions that allow spore germination

and subsequent proliferation, i.e. in the temperature range 20–55°C for at least several hours. Onset of symptoms is usually 8–18 h depending on the quantity of food ingested.

Clostridium perfringens is readily isolated from stool specimens by direct plating onto neomycin blood agar incubated anaerobically at 37°C overnight. When examining specimens from outbreaks it is useful to determine the spore count following alcohol shock treatment of a faecal suspension. Investigation of faecal specimens for *C. perfringens* enterotoxin can be done by tissue culture and immunoassay methods, but this is best done at a reference laboratory.

Isolation and enumeration of *C. perfringens* in suspected foods is important in the microbial assessment of the food. The current preferred method is a pour plate technique using tryptose sulphite cycloserine (TSC) agar incubated anaerobically at 37°C overnight. Black colonies, indicative of sulphite reducing clostridia, should be confirmed by inoculation into motility-nitrate and lactose-gelatine media. The numbers of vegetative *C. perfringens* decline rapidly in refrigerated foods; enrichment using cooked meat broth or reinforced clostridial medium may therefore be indicated if there has been a significant delay before examination in a food poisoning investigation.

To confirm the link between implicated food and cases it is important to characterize the isolates. It is useful to send multiple colony picks to a reference laboratory for serotyping and molecular fingerprinting using SAFLP.

Listeria monocytogenes

Listeria monocytogenes is widely distributed in nature and is the causative organism of several forms of human disease. While it is undoubtedly food-borne, *L. monocytogenes* is rarely associated with outbreaks of gastrointestinal disease. Infection with *L. monocytogenes* is manifested in many ways, from symptomless carriage to fatal septicaemia or meningitis. The initial isolate of *L. monocytogenes* is therefore usually made in pure culture from blood or cerebrospinal fluid and culture of intestinal content may be unproductive. Listeriosis in pregnancy is of importance because of the ability of the organism to cross the placenta to infect the foetus, resulting in neonatal infection or abortion. The predisposing factors which enable the organism to gain entry to the blood stream are not clearly understood, but immunosuppression associated with malignancies and reduced immunocompetence at the extremes of life are important, as is the number of *L. monocytogenes* bacteria ingested with food.

Although the infective dose of *L. monocytogenes* is unknown it is likely to vary greatly between individuals possibly owing to other predisposing factors such as immune status. However, on the basis of the frequency with which foods harbour low numbers of the organism and of the small numbers of reported cases of food-borne listeriosis it would be reasonable to suggest that the infective dose is high. In the rare instances where incriminated foods have been available from cases of listeriosis, the levels of *L. monocytogenes* recorded have been greater

than 10^3 /g. Foods containing greater than 100 cfu/g should be considered to be potentially hazardous to health.

As a result of epidemiological studies of outbreaks of listeriosis in the 1980s, dairy products have been established as one of the commonest sources of infection. Laboratory studies have failed to show that *L. monocytogenes* survives normal pasteurization processes. The consumption of dairy products containing unpasteurized or under-pasteurized milk such as soft cheeses, fresh cream and ice-cream before development of symptoms in infected individuals has been documented. Other implicated foods include meat pâtés, coleslaw, chicken, turkey, fin fish and shellfish. With knowledge of the history of the food it may be possible to target those items eaten from the categories listed above. Although the optimal temperature for growth is 34–35°C, *L. monocytogenes* continue to multiply at low temperatures when other flora are inhibited. Long shelf-life food products that have been held at refrigeration temperature for prolonged periods may therefore be more suspect than fresh foods.

Enumeration of *L. monocytogenes* in food suspensions is important and is achieved by plating directly onto plates of a suitable listeria selective agar (LSA) medium. Enrichment protocols to detect low numbers of *Listeria* include primary and secondary enrichment steps with subsequent subculture to LSA. For the isolation of *L. monocytogenes* culture media are incubated at 35–37°C; this temperature may inhibit the growth of other species of *Listeria* that have not been implicated in illness. Identification of *L. monocytogenes* and other species is by biochemical tests.

For the confirmation of outbreaks it is essential to send isolates to a reference laboratory for identification and subtyping using serotyping, phage typing and PFGE. Epidemiological studies suggest that certain recognizable serotypes, notably 1/2a, 1/2b and 1/4b, which occur more frequently in human disease, may be more pathogenic.

Organisms causing food-borne intoxications

Staphylococcus aureus

Staphylococcal food poisoning is a food-borne intoxication, and results from the ingestion of food in which *Staphylococcus aureus* has already grown to high numbers and produced exotoxin(s). There are currently 12 different staphylococcal enterotoxins (SEs) that can be detected either by immunoassay or by the polymerase chain reaction (PCR). The short incubation period and symptoms experienced closely resemble those seen with many chemical intoxicants. Bacteriological evidence is, therefore, essential for the confirmation of staphylococcal food poisoning outbreaks.

Diagnosis of human cases of staphylococcal food poisoning is straightforward and *S. aureus* can be isolated directly from stool samples by direct plating onto a selective agar. In outbreaks large numbers of *S. aureus* can be

isolated from suspected foods and it is important to enumerate the organisms using selective agar media incubated at 37°C for 24–48 h. If the food is known or suspected to have been handled or stored in a manner which may have affected the viability of the organisms, then enrichment broth culture followed by plating onto selective agar should be used for isolation. Traditionally, media containing high salt concentrations such as salt meat broth have been used, but these can be very inhibitory to stressed cells. Presumptive colonies from the selective agar cultures should be confirmed as *S. aureus* by a coagulase test or commercial equivalent and by desoxyribonuclease (DNase) production.

Isolates from food samples, clinical specimens and from food handlers should be further characterized by special tests carried out at the reference laboratory. These include phage typing, toxin typing and PFGE. Conclusive proof of a staphylococcal outbreak is provided by detection of indistinguishable strains from food and cases, by confirming the production of enterotoxin and, when possible, by detecting enterotoxin in the implicated food.

***Bacillus cereus* and other *Bacillus* spp.**

Bacillus cereus food poisoning usually results from the ingestion of food containing large numbers (10^6 – 10^8 /g) of the organism and pre-formed toxin (emetic type) and is therefore an intoxication. A less common diarrhoeal type of food poisoning is associated with infection followed by toxin production predominantly in the intestine. Vomit and faeces should both be examined if possible. Isolation from these samples is achieved by direct plating onto *B. cereus* selective agar (BCS), e.g. polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA) or phenol red egg yolk polymyxin agar (PREP or MYP) agar incubated at 30–37°C overnight. Both of these BCS agars contain mannitol and egg yolk emulsion. *Bacillus cereus* is distinguished from other *Bacillus* species by: (i) production of blue or turquoise to peacock blue colonies on PEMBA or pink colonies on MYP (PREP) due to the inability of *B. cereus* to ferment mannitol; and (ii) production of a zone of opacity around the bacterial growth due to lecithinase activity.

As large numbers of *B. cereus* are required to cause illness, isolation and enumeration in the examination of food samples associated with outbreaks can usually be achieved by direct plating onto the surface of BCS agar incubated aerobically at 30–37°C for 24 h. Selective enrichment is rarely necessary and the significance of *B. cereus* recovered by enrichment techniques is dubious.

Further confirmation of isolates requires biochemical tests. A serotyping scheme is available for *B. cereus* that is based on agglutination with sera raised against the H (flagellar) antigens. Serotyping, tests for toxin production and molecular finger printing methods are valuable in confirming outbreaks and are available from the reference laboratory.

Other members of the genus *Bacillus*, notably those of the *B. subtilis*–*licheniformis* group, have also been implicated in outbreaks of food poisoning.

Initial isolation and enumeration procedures are similar to those for *B. cereus*. Biochemical tests are necessary for confirmation; unlike *B. cereus* these organisms do ferment mannitol and do not produce lecithinase.

Clostridium botulinum

The name *Clostridium botulinum* is used to describe several phenotypically different clostridia that produce extremely potent exotoxins. These toxins are classified as A, B, C, D, E, F and G. The toxins cause a neuroparalytic illness, commonly referred to as botulism. *Clostridium botulinum* producing toxin types A, B, E and F are associated with botulism in humans.

The classical food-borne illness results from the ingestion of food in which *Clostridium botulinum* has multiplied and produced one of the neurotoxins. In view of the severity of the illness, rapid identification of the food source is essential in order to prevent further cases. Suspected foods, together with any containers, should be examined without delay. If a commercially manufactured product is suspected, as much information as possible about the product, such as brand name, batch code and package size, should be obtained. A health hazard warning may need to be issued pending the results of the laboratory examinations.

Serum and faecal specimens should be collected from suspect cases and sent immediately for toxin testing. Suspected foods together with rinses from containers should be examined for the presence of toxin and cultured by direct and enrichment methods using specialist media. Enumeration of organism is not usually considered necessary. In view of the potency of the neurotoxins, isolates of *C. botulinum* or suspected foods should be referred to a reference laboratory for these specialist tests.

Confirmation of a case of botulism is dependent on detecting neurotoxin in the clinical specimens. Food poisoning is confirmed by demonstration of toxin production by the isolates from the food together with the presence of toxin in the suspected food. Currently the only reliable method for the detection of biologically active toxin is the mouse bioassay. Although immunodiffusion tests and immunoassay techniques have been developed they lack sensitivity; they do not confirm the presence of functionally active toxin and hence cannot be used diagnostically.

Protozoa and viruses

It should be remembered that not all food-borne gastrointestinal disease is caused by bacterial pathogens. Both protozoa and viruses must be considered if the symptoms suggest the possibility and there is absence of one of the usual suspects.

Cryptosporidium parvum and *Cyclospora* spp. have been associated with food-borne transmission and outbreaks of disease. The foods implicated are usually salad vegetables or soft fruits that have been contaminated by sewage polluted

water. However, meat and milk have also been implicated in outbreaks of cryptosporidiosis [8].

Norwalk-like viruses (NLVs), previously known as small round structured viruses (SRSV), have been transmitted via food but this is usually due to contamination from a food handler excreting the virus. Shellfish are also recognized as a source of these viruses and become contaminated during filtration of sewage-infected water. Other viruses such as hepatitis A and rotavirus may also be associated with food-borne transmission [9]. Examination of clinical specimens and suspect foods require specialized techniques for detection which are outside the scope of this manual.

References

- 1 Department of Health. *Management of Outbreaks of Food-borne Illness. Guidance Prepared by a Department of Health Working Group*. London: Department of Health, 1994.
- 2 Threlfall EJ, Powell NG, Rowe B. Differentiation of salmonellae by molecular methods for epidemiological investigations. *PHLS Microbiol Digest* 1994; **11**: 199–202.
- 3 Peters TM, Threlfall EJ. Single-enzyme amplified fragment length polymorphism and its applicability for *Salmonella* epidemiology. *System Appl Microbiol* 2002; **24**: 400–4.
- 4 Bolton FJ, Wareing DRA, Skirrow MB, Hutchinson DN. Identification and biotyping of campylobacters. In: Board RG, Jones D, Skinner FA, eds. *Identification Methods in Applied and Environmental Microbiology. Society for Applied Bacteriology Technical Series No. 29*. 1992: 151–61. Oxford: Blackwell Scientific Publications.
- 5 Frost JA, Oza AN, Thwaites RT, Rowe B. Serotyping scheme for *Campylobacter jejuni* and *Campylobacter coli* based on direct agglutination of heat stable antigens. *J Clin Microbiol* 1998; **36**: 335–9.
- 6 Thwaites RT, Frost JA. Drug resistance in *Campylobacter jejuni*, *C.coli* and *C.lari* isolated from humans in England and Wales. *J Clin Pathol* 1999; **52**: 812–14.
- 7 Frost JA, Kramer JM, Gillanders SA. Phage typing of *Campylobacter jejuni* and *C.coli*. *Epidemiol Infect* 1999; **123**: 47–55.
- 8 Nichols GL. Food-borne protozoa. *Br Med Bull* 1999; **55**: 209–35.
- 9 Appleton H. Control of food-borne viruses. *Br Med Bull* 2000; **56**: 172–83.