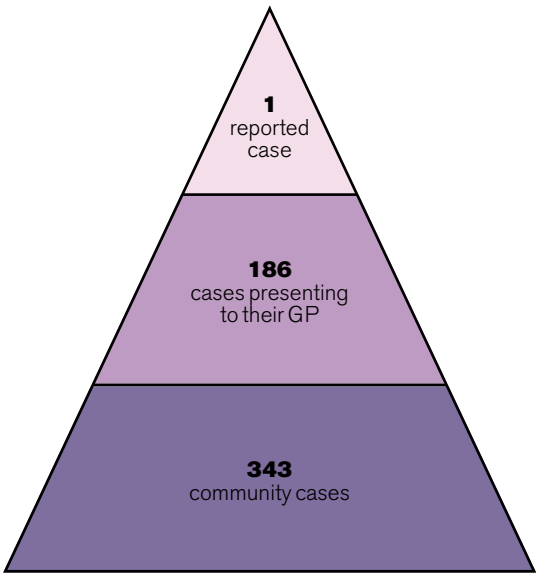
Clostridia and food-borne disease

Michael W. Peck

Food poisoning due to *Clostridium perfringens*

Spores of *Clostridium perfringens* are distributed widely in the environment and are present frequently in the intestines of humans and many domestic and feral animals. Food poisoning is most commonly associated with *C. perfringens* type A and is generally due to temperature abuse of cooked meat or poultry dishes. It is often linked to institutional catering establishments (e.g. school cafeterias, hospitals, nursing homes, prisons) where large quantities of food are prepared several hours before serving. The spores survive normal cooking and are able subsequently to germinate, leading to rapid multiplication during slow or inadequate cooling of the product. Ingestion of large numbers of vegetative cells leads to sporulation and associated enterotoxin production in the small intestine. Symptoms include diarrhoea and acute abdominal pain (but rarely vomiting). The incubation period is 8–22 h (usually 12–18 h), and the illness is usually over within 24 h, but less severe symptoms may persist for 1 or 2 weeks.

C. perfringens featured as a significant pathogen in the recent study of infectious intestinal disease in England, and from this study it can be estimated that there is a total of 144,000 cases per year in the UK. Considerable under-reporting is also indicated; it is estimated that for every 343 community cases, only one is reported to the Communicable Disease Surveillance Centre of the PHLS (Fig. 1). A related study has estimated a total of 248,520 cases per year in the USA, with 41 requiring hospitalization and 7 of the cases fatal. The UK Food Standards Agency has a target of reducing the incidence of food-borne illness by 20% by April 2006. This is to be measured as a 20% reduction in laboratory reports of disease due to five of the major food-borne pathogens, one of which is *C. perfringens*.



Food-borne botulism

Botulism affects humans, animals and birds. The most common forms of botulism in humans are food-borne, infant and wound (Fig. 2). Food-borne botulism results from consumption of pre-formed botulinum neurotoxin (as little as 30 ng may be sufficient). Infant and wound botulism are infections. The microflora in the intestinal tract of infants under 1 year old is unable to repel neurotoxigenic clostridia that colonize and produce toxin *in vivo*. Six cases of infant botulism have been confirmed in the UK; the most recent was in June 2001 and involved a 5-month-old baby. The cause was confirmed as *Clostridium botulinum* neurotoxin type B. Subsequent tests showed that an opened and an unopened can of an infant formula milk powder both contained organisms that produced *C. botulinum* neurotoxin type B, raising the possibility that the case was linked to consumption of infant formula milk powder. A conclusive link, however, remains to be established. Overseas, some cases of infant botulism have been linked to consumption of honey containing spores of neurotoxigenic clostridia.

Six physiologically and phylogenetically distinct clostridia produce the botulinum neurotoxin. Only three of these, proteolytic *C. botulinum* (Group I *C. botulinum*), non-proteolytic *C. botulinum* (Group II *C. botulinum*) and very occasionally neurotoxigenic *C. butyricum*, have been associated with food-borne botulism (Table 1). Whilst rarer than some other forms of food-borne illness, the severity of botulism makes it a serious concern. The consumption of as little as 0·1 g of food in which these organisms have grown and produced neurotoxin can result in illness. Initial symptoms of food-borne botulism may include impaired vision, dry mouth, nausea, vomiting and slight diarrhoea followed by constipation and intestinal pain. The symptoms can then progress to muscle weakness and flaccid paralysis which affects the respiratory muscles and can result in death if not treated. Proteolytic *C. botulinum*, non-proteolytic *C. botulinum* and neurotoxigenic *C. butyricum* survive and grow under different conditions, and thus cause problems in different types of foods (Table 1). To understand the conditions required to prevent growth of

Clostridium perfringens or organisms that form the botulinum neurotoxin and lead to food-borne botulism are the most frequent causes of clostridial food-borne illness. While food poisoning associated with *C. perfringens* is relatively common and relatively mild, food-borne botulism is rare, but very severe.

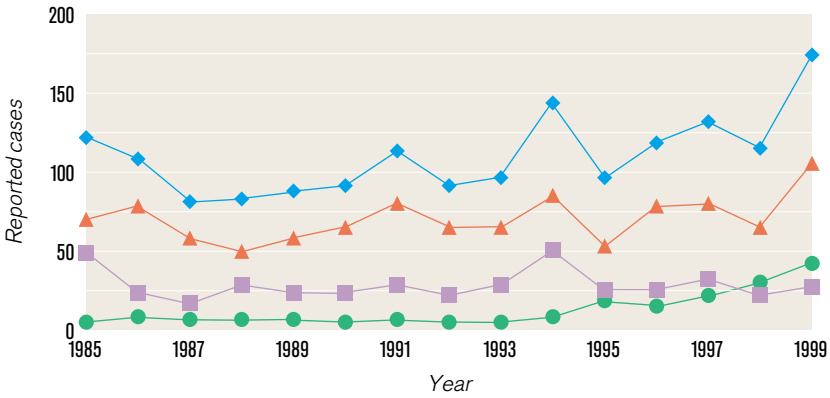


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Fig. 1. Reporting pyramid for *Clostridium perfringens*.
CALCULATED FROM *A REPORT OF THE STUDY OF INFECTIOUS INTESTINAL DISEASE IN ENGLAND* (2000) (LONDON: THE STATIONERY OFFICE)

ABOVE
Fig. 2. Summary of reported cases of human botulism in the USA (1985–1999). ◆, Total number of cases of botulism; ▲, cases of infant botulism; ■, cases of food-borne botulism; ●, cases of wound botulism.
SOURCE OF DATA: *MORBIDITY AND MORTALITY WEEKLY REPORTS* **47**, 1–116 (1999)

Table 1. Characteristics of the three physiologically distinct clostridia associated with food-borne botulism

Characteristic	Proteolytic *C. botulinum*	Non-proteolytic *C. botulinum*	Neurotoxigenic *C. butyricum**
Neurotoxins formed	A, B, F	B, E, F	E
Minimum growth temperature (°C)	10–12	3.0	10-15
Minimum growth pH	4.6	5.0	4.0–5.2
Spore heat resistance ($D_{100°C}$) (min)	>15	<0·1	<1–5
Foods involved in botulism outbreaks	Home-canned foods, faulty commercial processing	Fermented marine products, dried fish, vacuum packed fish	Vegetable-based foods in Asia
Potential food problems	Canned foods	Refrigerated processed foods with a long shelf life	??

Not extensively tested.

Table 2. Examples of recent outbreaks of food-borne botulism

Outbreak	Food	No. cases/deaths	Factors contributing to outbreak	Organism: toxin type
1989, UK	Commercially produced hazelnut yoghurt	27/1	Underprocessing of canned hazelnut conserve	Proteolytic *C. botulinum*: type B
1991, Egypt	Commercially produced uneviscerated salted fish ('faseikh')	>91/18	Putrefaction of fish before salting	Non-proteolytic *C. botulinum*: type E
1992, Spain	Commercially produced green beans/artichokes	4/1	Underprocessing (?)	Proteolytic *C. botulinum*: type B
1993, Italy	Commercially prepared aubergine in oil	7/0	Underprocessing, anaerobiosis	Proteolytic *C. botulinum*: type B
1994, USA	Potato dip 'skordalia' and aubergine dip 'meligianoslata'	30/0	Foil-wrapped, baked potatoes left at room temperature before use in dip	Proteolytic *C. botulinum*: type A
1994, China	Home-made salted and fermented paste of soybeans and wax gourds	6/3	Unsafe process/storage	*C. butyricum*: type E
1996, Italy	Commercially prepared mascarpone cheese	8/1	Unsafe process/storage	Proteolytic *C. botulinum*: type A
1996, India	Sevu (crisp made of gram flour)	34/3	Unsafe process/storage	*C. butyricum*: type E
1997, Iran	Traditionally made cheese preserved in oil	27/1	Unsafe process	Proteolytic *C. botulinum*: type A
1997, Argentina	Home-cured ham	6/0	Unsafe process/storage	Non-proteolytic *C. botulinum*: type E
1998, UK	Bottled mushrooms	2/1	Unsafe process/temperature abuse	Proteolytic *C. botulinum*: type B
1999, Morocco	Meat and chicken dish	80/15	Temperature abuse	*C. botulinum*: type B*
2001, USA	Fermented beaver tail and paw	3/0	Unsafe process/storage	Non-proteolytic *C. botulinum*: type E

Only toxin identified – unclear whether proteolytic C. botulinum type B or non-proteolytic C. botulinum type B.

these neurotoxigenic clostridia requires knowledge of their differing physiology. For example, proteolytic *C. botulinum* produces spores of high heat resistance, and the canning process for low-acid foods is designed to inactivate spores of this organism. Non-proteolytic *C. botulinum* can multiply and form toxin at temperatures as low as 3·0 °C. Botulism outbreaks have occurred, most frequently with processed fish, when the cold chain has not been maintained. A current concern is refrigerated processed foods with a long shelf-life. Electron micrographs of non-proteolytic *C. botulinum* are shown in Fig. 3.

Although food-borne botulism was recognized as a clinical entity several centuries previously, it was Emile van Ermengem who isolated the causative organism (initially called *Bacillus botulinus*) in 1897 from raw, salted ham and from the spleen of a victim. It is likely that the isolated strain was non-proteolytic *C. botulinum*. Over the next three decades a great number of outbreaks were identified across the world. This included the first UK outbreak at Loch Maree in August 1922, which involved consumption of wild duck-paste sandwiches containing type A neurotoxin. There were eight cases of botulism, all fatal. In a 7-year period, from 1918 to 1924, there were 107 outbreaks in the USA, involving 367 cases of which 230 were fatal. Many of these were associated with the home canning of vegetables. One particularly unfortunate outbreak occurred in Albany, Oregon, in 1924. All 12 members of the Gerber family died after consuming home-canned string beans containing type A neurotoxin (Fig. 4). In the early 1920s, the importance of botulism as a disease of animals on a scale even greater than that for human botulism had become apparent. So great were these worries about botulism that verses entitled *It's botulism* appeared in a veterinary journal in 1922 (Fig. 5).

Through the understanding and implementation of effective control measures, the incidence of botulism today is generally much lower than previously. However, there was a high incidence in Poland in the 1960s, 1970s and 1980s. In Russia in 1998 there were 374 outbreaks

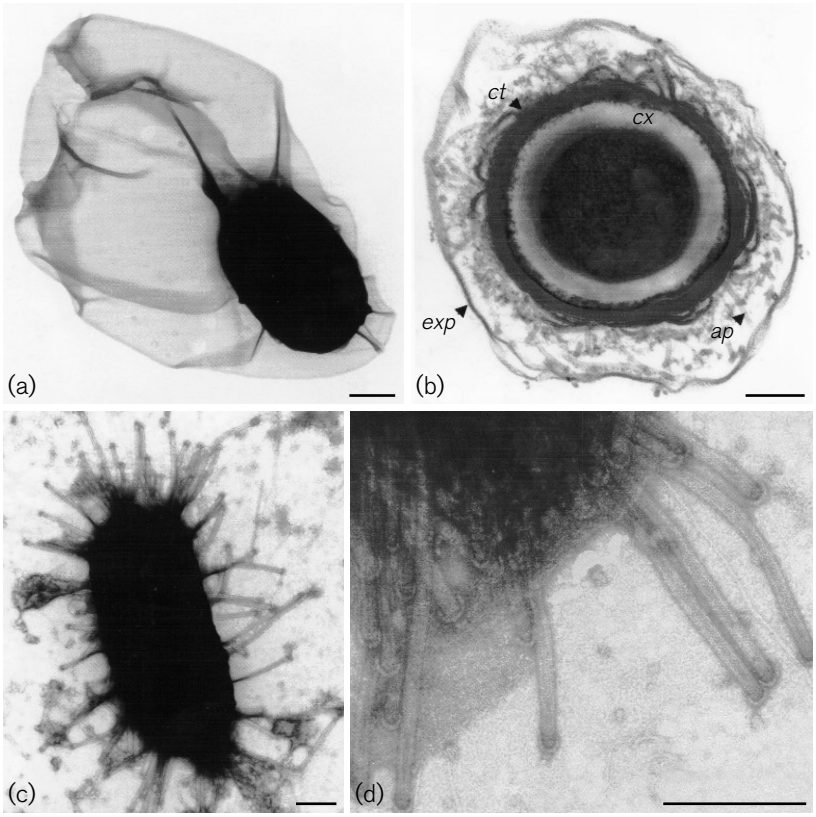

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Fig. 3. Electron micrographs of spores of non-proteolytic *C. botulinum*. (a) Spore of strain Eklund 17B, type B, negatively stained with saturated, aqueous uranyl acetate, showing membranous exosporium. (b) Section through a spore of strain Foster B96, type E, with 0.075% ruthenium red included in the fixatives and stained sequentially with uranyl acetate and lead citrate, showing appendages (ap) between the spore coat (ct) and exosporium (exp). cx is the cortex. (c) Spore of strain Sebald P34, type E, after removal of exosporium, negatively stained as for (a), showing appendages. (d) Spore of strain Hazen 36208, type E, negatively stained as for (a), showing detail of appendages. Bars, 200 nm.
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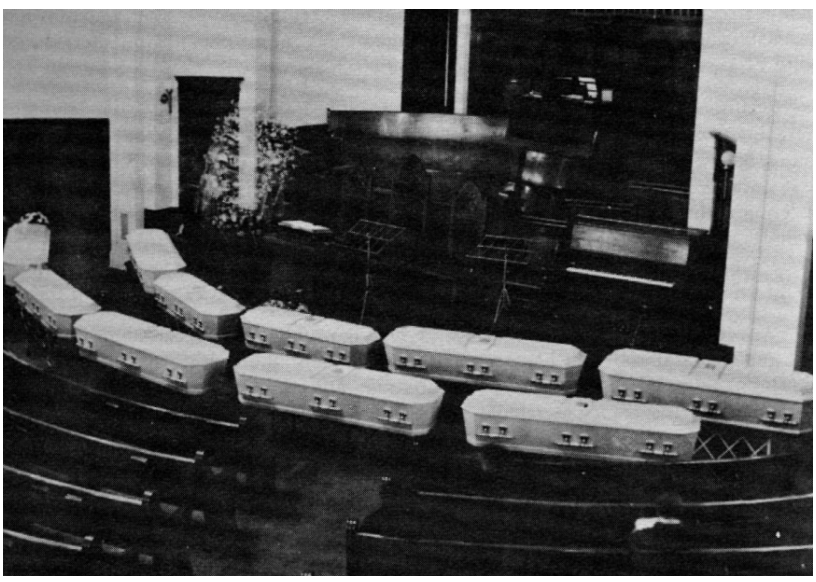
giving rise to 501 cases of which 41 were fatal. These high incidences were associated with adverse economic conditions. Cases of food-borne botulism occur either when there is failure to apply known control measures, or when foods are introduced without effective control measures. Including the initial outbreak at Loch Maree, there have been 11 outbreaks of food-borne botulism in the UK, with 58 cases of which 19 were fatal. The two most recent outbreaks are included in Table 2. Over the past 20 years, the reported incidence of food-borne botulism is approximately 35 cases per year in Italy (e.g. home-prepared vegetables in oil), 35 cases per year in Germany (e.g. salted hams), 30 cases per year in USA (e.g. home-canned vegetables, fermented marine products), 25 cases per year in France (e.g. salted hams), and 10 cases per year in Spain (e.g. home-canned vegetables). Again the number of reported cases is likely to underestimate the total number of cases. Most cases are associated with home-prepared foods, when known control measures have not been implemented. Food-borne botulism involving commercial processing is uncommon, but the consequences of outbreaks are likely to be severe. The fatality rate associated with food-borne botulism has fallen considerably in recent years because of rapid treatment with antitoxin and supportive therapy. It is now approximately 10% of cases, a proportion that is still high for a food-borne illness. The medical and economic consequences of botulism in commercial foods can be enormous. For example, it has been estimated that in the USA the cost per human case of botulism associated with commercial products is approximately $30 million, compared with $10,000–12,000 for each case of illness associated with *Listeria monocytogenes* and *Salmonella*. Examples of recent outbreaks of food-borne botulism are given in Table 2.

A recent discovery has been that of neurotoxigenic strains of *C. butyricum*. In view of the ability of these strains of *C. butyricum* to produce type E neurotoxin, the severity of any resulting illness (and the potency of the neurotoxin) must be considered equivalent to that caused by *C. botulinum*. Neurotoxigenic strains of *C. butyricum* were first described in the mid-1980s as being associated with infant botulism. Subsequently, neurotoxigenic *C. butyricum* has been associated with food-borne botulism. The first outbreak of food-borne botulism was reported in China in 1994, when six persons became ill (three of whom died) having consumed a home-made salted and fermented paste of soybeans and wax gourds. In the aftermath of this outbreak, it was established that two earlier outbreaks of type E botulism in China in 1973 and 1983, involving soybean dishes, were also associated with neurotoxigenic *C. butyricum*. A fourth suspected outbreak was reported in India in 1996 and involved 34 young students (three of whom died) who had eaten sevu (a crisp made with gram flour) at a school cafeteria. It has also been proposed that neurotoxigenic *C. butyricum* may have been responsible for outbreaks of type E food-borne botulism, where it had been previously assumed that non-proteolytic *C. botulinum* type E was the agent.

Concerns for the future

Much current research is focused on ensuring the continued safe development of refrigerated processed foods with an extended shelf-life. These foods meet

BELOW:
Fig. 4. Funeral of the Gerber family wiped out by botulism caused by consumption of home-canned string beans at Albany, Oregon, USA in 1924
FROM C.E. DOLMAN (1964) BOTULISM AS A WORLD HEALTH PROBLEM: IN *BOTULISM: PROCEEDINGS OF A SYMPOSIUM*, EDITED BY K.H. LEWIS & K. CASSEL (AMERICAN PUBLIC HEALTH SERVICE PUBLICATION NO. 999-FP-1)



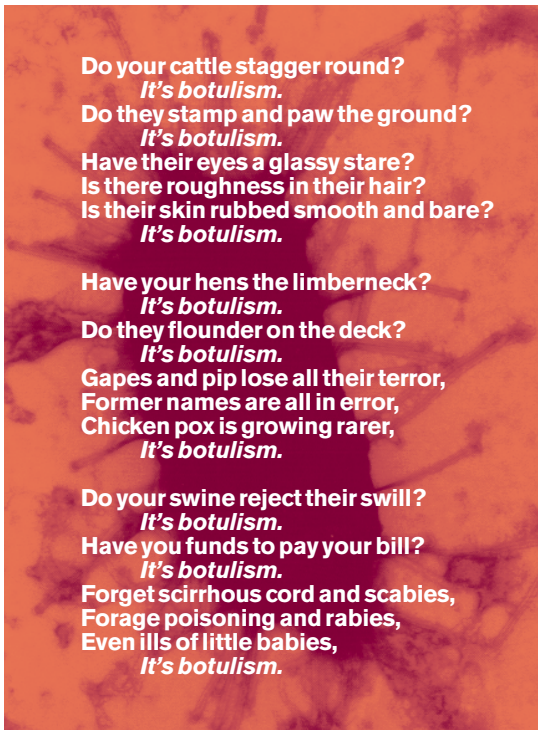


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Fig. 5. *It's botulism.*
FROM *J AM VET MED ASSOC* (1922) **14**, 117–118

consumer demand in that they require minimal preparation time compared with conventional meals, are of high quality, contain few preservatives and are minimally heat-processed. The heat process (typical maximum of 75–95 °C) is much less than for canned foods and is intended to minimize loss of sensory and organoleptic quality. After heating, the food is cooled rapidly and stored at refrigeration temperatures (1–8 °C). These foods are not sterile and product shelf-life is dependent on the heat process, storage temperature and possibly also the intrinsic properties of the food. Additionally, many of the foods are packed under vacuum or in an anaerobic atmosphere, restricting growth of aerobic, but not anaerobic, bacteria. These conditions favour colonization by micro-organisms that produce heat-resistant spores and grow in the absence of oxygen at refrigeration temperatures. In particular, concern exists about the potential for growth and toxin production by non-proteolytic *C. botulinum* in the absence of competition from other micro-organisms, and the associated food-borne botulism hazard. Current research is targeted at identifying combinations of environmental conditions that provide the relevant protection factor to ensure the continued safe development of these foods. A further issue is that of neurotoxigenic *C. butyricum*. In view of its association with recent outbreaks of food-borne botulism in Asia, it is now timely to determine environmental conditions that control growth and toxin production to ensure that the risk of food-borne botulism presented by neurotoxigenic *C. butyricum* is appropriately controlled in all foods.

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Further reading

Lund, B.M. & Peck, M.W. (2000). *Clostridium botulinum*. In *The Microbiological Safety and Quality of Food*, pp. 1057–1109. Edited by B.M. Lund, A.C. Baird-Parker & G.W. Gould. Gaithersburg: Aspen.

Labbé, R.G. (2000). *Clostridium perfringens*. In *The Microbiological Safety and Quality of Food*, pp. 1110–1135. Edited by B.M. Lund, A.C. Baird-Parker & G.W. Gould. Gaithersburg: Aspen.

Micro Shorts

HIV and AIDS in the UK

An epidemiological review: 2000

The UK Communicable Disease Surveillance Centre (CDSC) has just published the results of this survey into the incidence of HIV and AIDS. It shows that while homosexual men have the greatest chance of acquiring infection in the UK, there are currently more diagnoses of heterosexually acquired HIV, but the infection has usually taken place in Africa. The UK focus of HIV infection is London, with over two-thirds of cases. The incidence of total cases is rising and it is estimated that over 11,000 people in the UK are infected, but undiagnosed. Advances in treatment are reducing the number of deaths from AIDS, but the infection still remains incurable. A free copy of the report is available from CDSC, 61 Colindale Avenue, London NW9 5EQ. Any queries should be emailed to **jmortimer@phls.org.uk**

Researchers' website

The Office of Science and Technology has launched a new interactive website for researchers. It aims to give them a neutral forum where they can air their views, 'chat' with other workers, make contacts and promote their research. The site is open to all researchers, whether they work in academia or in industry. To register, log on to **www.researchersforum.gov.uk**

Foot-and-mouth disease

With the last UK county – Northumberland – declared free of the disease at midnight on 14 January, the The Institute for Animal Health (IAH) Pirbright Laboratory reported that it had completed its one-millionth test for the virus. The total number of samples tested throughout the outbreak was more than 2·75 million, mainly checked using ELISA-based tests. With the epidemic now apparently over, IAH, which trained staff from other labs during the crisis, will cease its sero-surveillance activities, but will continue to supply other institutions with the necessary reagents as well as carrying out virus neutralization testing on samples found positive by ELISAs. IAH will maintain its role as the only laboratory to perform diagnostic serology and viral detection assays. Continued vigilance over foot-and-mouth will be necessary, according to Chief Veterinary Officer Jim Scudamore, who warned at the SGM/ESCV/ESVV conference on 11 January that unless affected sites were thoroughly cleansed and disinfected, the disease could flare up again when farms were restocked. During the outbreak, there were 2,026 confirmed cases of disease and 4 million animals were slaughtered on almost 10,000 farms. Scientists and government advisers are now evaluating their handling of the epidemic with a view to the lessons to be learnt. Several official reports are expected.