Chapter 12
Botulism as a Disease of Humans

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Abstract Human botulism presents in several different forms which provide unique challenges to public health. Foodborne botulism, while still an issue with home-processed foods, is sometimes associated with restaurants and commercially produced (particularly chilled) foods. The diversity of food products available to consumers, which are widely distributed, requires an integrated, multiagency response approach to contain, evaluate and develop appropriate prevention measures. Wound and adult intestinal toxemia cases are difficult to confirm, and so the total illness burden is likely unknown. Infant botulism highlights the diversity of the neurotoxin-producing *Clostridium* sp. and associated toxins which can cause botulism worldwide. Finally, an astounding number of adverse events associated with toxin injections remind us of the hazards of botulinum toxin even as its therapeutic benefits expand.

Keywords Botulinum neurotoxin · Botulism · Foodborne botulism · Wound botulism · Infant botulism · Iatrogenic · Toxinfection

12.1 Introduction

Botulism, since its discovery in the eighteenth century, has resulted in dramatic public health response efforts to understand, identify, and control this rare but potentially fatal disease. Between 1735 and 1802, the number of outbreaks associated with blood sausage in Württemberg, Germany caused the government to provide instructions on safe preparation methods; by 1820, botulism was a mandatory reportable disease [1]. Originally thought to occur only with meat products, botulism caused by vegetables caught the world by surprise in 1904 when beans caused an outbreak in Darmstadt, Germany. While largely ignored initially, botulism due to consumption of preserved vegetables commonly occurs worldwide. Although botulism cases occurred with regularity in California, a multistate outbreak in 1919 caused by contaminated, commercially processed and packed olives brought national attention to the risk of botulinum toxin-contaminated foods. These events

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K. A. Foster (ed.), *Molecular Aspects of Botulinum Neurotoxin,* Current Topics in Neurotoxicity 4, DOI 10.1007/978-1-4614-9454-6_12,

represented a turning point in regulated food protection in the USA and resulted in coordination between federal, state and university researchers to develop commercial canning standards to protect consumers from this deadly illness. As a result, large outbreaks of botulism due to commercial food products declined, but small outbreaks began to increase during World War I and World War II as home-canning practices increased [1]. Even as public health officials began to develop educational material for those who prepare home-processed food, *Clostridium botulinum*, the causative organism that produces the botulinum toxin, still had a few tricks to show us. In 1951, botulism was recognized as being caused by contamination of a wound with in situ toxin production [1]. Originally the result of injuries and occasionally surgery, wound botulism has increased around the world due to certain illegal drug-use practices. In 1976, infant botulism, currently the leading botulism form in the USA, was recognized as a distinct syndrome as a result of intestinal colonization [2, 3]. The recognition of infant botulism introduced us to an astounding diversity of neurotoxin-producing *Clostridium* sp. New botulinum toxin-producing species, *C. baratii* type F and *C. butyricum* type E, were identified. Additionally, some *C. botulinum* isolates were found with the capacity to produce more than one toxin serotype; usually, one of the two toxin genes produce more toxin than the other (i.e., Af, Ab, Ba, Bf, Ae, where the lower case letter reflects a lower level of toxin produced). In 1988, the first cases of intestinal colonization of adults were reported in the USA; diagnosis of this form of botulism continues to perplex physicians and public health officials. Early work on purification, stabilization and characterization of botulinum toxin by researchers at the US Department of Defense and later at the University of Wisconsin laid the groundwork for the discovery of unique therapeutic properties of this deadly toxin [4]. While new therapeutic uses are continuing to be discovered, botulinum toxin reminds us of its danger through reports of localized and systemic paralytic effects following injections (iatrogenic botulism). This chapter highlights the surprises and diversity of human botulism.

12.2 Surveillance and Control

Countries with a relatively high number of botulism cases, such as the USA, Canada, Argentina, UK, Germany, Italy, France, Poland, China, Thailand and Japan, conduct surveillance and have a response network to investigate and control botulism. However, there is no worldwide reporting mechanism to accurately capture the global incidence of botulism. Limited understanding of global occurrence of botulism is probably due to a number of factors, including availability of inadequate resources to identify botulism cases, differences in internal reporting requirements, language barriers and limited external accessibility to data collected by public health agencies of individual countries. In 1999, a summary of responses from 14 European countries concerning botulism surveillance was published [5]. This publication revealed that 13 of the 14 countries surveyed included botulism as a notifiable disease; most had required reporting of botulism for decades. However, most did not report in-

Table 12.1 2008 European Commission Case Classification for botulism. [7]

	Clinical criteria	Laboratory criteria	Probable	Confirmed
Foodborne	At least one of the following: Bilateral cranial nerve impairment (e.g., diplopia, blurred vision, dysphagia, bulbar weakness) Peripheral symmetric paralysis	Detection of botulinum toxin in a clinical specimen	Any person meeting the clinical criteria and with an epidemiological link	Any person meeting the clinical and the laboratory criteria
Wound	At least one of the following: Bilateral cranial nerve impairment (e.g., diplopia, blurred vision, dysphagia, bulbar weakness) Peripheral symmetric paralysis	Detection of botulinum toxin in a clinical specimen Isolation of *Clostridium botulinum* from wound	Any person meeting the clinical criteria and with an epidemiological link	Any person meeting the clinical and the laboratory criteria
Infant	Any infant (< 12 months of age) with at least one of the following: Constipation Lethargy Poor feeding Ptosis Dysphagia General muscle weakness	Detection of botulinum toxin in a clinical specimen Isolation of *Clostridium botulinum* from stool	Any person meeting the clinical criteria and with an epidemiological link	Any person meeting the clinical and the laboratory criteria

dividual botulism cases, only total number, and many of these were reported as "food poisoning" rather than specifically for botulism. Additionally, there was little uniformity in the actual case definition used; some only used clinical criteria and others required both clinical and laboratory data. Currently, 30 countries within the European Union (EU) or European Economic Area (EEA) participate in voluntary reporting of botulism cases using a set of standard definitions as described in Table 12.1 [6, 7]. Since 2007, the European Centre for Disease Prevention and Control (ECDC) has collected and published an annual report summarizing the incidence of botulism. Participating countries mainly report to the ECDC through passive surveillance. Each country collects data through a compulsory reporting requirement primarily from laboratories, physicians and hospitals. Worldwide, a single suspect case of foodborne botulism is considered to be a public health emergency, because the first case may signal the occurrence of an outbreak. In EU countries, public health authorities quickly withdraw incriminated commercially distributed food products. Product alerts are delivered through the Rapid Alert System for Food and Feed (RASFF) and Early Warning and Response System (EWRS). This system provides a mechanism for rapid notification of potentially risky food exported to both EU and non-EU countries.

In the USA, the Centers for Disease Control and Prevention (CDC) maintains 24/7 epidemiology and laboratory consultation services through the CDC Emergency Operations Center (770 488–7100) to support state/local response to suspect cases [8]. CDC staff work with both state/local epidemiologists and hospital personnel to try and quickly identify clinical specimens and potential food sources which may need to be recovered for laboratory tests. If commercially produced products are suspected, then CDC ensures that the proper food regulatory agency (Food and Drug Administration, FDA or US Department of Agriculture, USDA) is involved to facilitate product recalls and to evaluate production records. CDC provides antitoxins for suspect adult botulism cases either directly or through established distribution systems located in both California and Alaska. Previously, the available therapeutic antitoxin products (a licensed A/B product, and an Investigational New Drug type E product) were limited to the three toxin serotypes (A, B and E) which primarily cause human botulism; however, an equine-derived heptavalent botulism antitoxin (BAT, Cangene Corp.), approved in the USA by FDA in March 2013, is currently used to treat adult cases and covers all known serotypes (A, B, C, D, E, F and G) which could occur naturally or as a result of an intentional act [9]. A human-derived antitoxin product (BabyBIG®) is available from The California Infant Botulism Treatment and Prevention Program (CA IBTPP) and is approved for the treatment of type A and B infant botulism cases [10]. Additionally, CA IBTPP provides 24/7 consultation (510–231–7600) for infant botulism cases throughout the USA and provides laboratory support for infants residing in California. CDC collates morbidity and mortality surveillance data from a variety of sources, including epidemiology and laboratory investigations reports from state/local health departments, FDA or USDA food investigations reports, CDC clinical consultations, antitoxin distribution reports and CA IBTPP antitoxin release summaries [8]. Each year, CDC prepares a summary of laboratory-confirmed botulism cases to help identify trends and potential high-risk areas for education and control. Since 1992, the definition of a botulism outbreak includes events involving two or more persons, whereas previously, a single case met the definition of an outbreak as long as a food source was also identified. Consensus definitions for different botulism types are developed through the Council of State and Territory Epidemiologists (CSTE) to ensure uniform reporting across the USA; the current definitions are provided in Table 12.2 [11]. CDC's annual summary report identifies the form of botulism as defined by CSTE for each case. Laboratory confirmation of botulism is achieved through detection of toxin or identification of a neurotoxin-producing *Clostridium* sp. in clinical specimens or by detection of toxin in foods as described for the different forms of botulism in Table 12.2 [12]. In addition, at CDC and other federal facilities such as FDA and USDA, laboratory testing occurs in state health departments and select county or city public health laboratories. A number of investigators have conducted studies to try and find an alternative to the mouse bioassay [13, 14]. However, laboratory confirmation in the USA continues to depend on the mouse bioassay because there is no alternative method approved or cleared by FDA for clinical specimen testing. Methods for detection and control of botulinum neurotoxins are discussed in Chap. 11.

Table 12.2 2011 US CSTE Position Statement for Botulism. [11]

	Clinical Description	Laboratory Criteria	Probable	Confirmed
Foodborne	Ingestion of botulinum toxin results in an illness of variable severity. Common symptoms are diplopia, blurred vision and bulbar weakness. Symmetric paralysis may progress rapidly	Detection of botulinum toxin in serum, stool or patient's food or Isolation of *Clostridium botulinum* from stool	A clinically compatible case with an epidemiologic link (e.g., ingestion of a home-canned food within the previous 48 h)	A clinically compatible case that is laboratory confirmed or that occurs among persons who ate the same food as persons who have laboratory-confirmed botulism
Infant	An illness of infants, characterized by constipation, poor feeding and "failure to thrive" that may be followed by progressive weakness, impaired respiration and death	Detection of botulinum toxin in stool or serum, or Isolation of *Clostridium botulinum* from stool	None	A clinically compatible case that is laboratory confirmed, occurring in a child aged less than 1 year
Wound	An illness resulting from toxin produced by *Clostridium botulinum* that has infected a wound. Common symptoms are diplopia, blurred vision and bulbar weakness. Symmetric paralysis may progress rapidly	Detection of botulinum toxin in serum or Isolation of *Clostridium botulinum* from wound	A clinically compatible case in a patient who has no suspected exposure to contaminated food and who has either a history of a fresh, contaminated wound during the 2 weeks before onset of symptoms or a history of injection drug use within the 2 weeks before onset of symptoms	A clinically compatible case that is laboratory confirmed in a patient who has no suspected exposure to contaminated food and who has a history of a fresh, contaminated wound during the 2 weeks before onset of symptoms or a history of injection drug use within the 2 weeks before onset of symptoms
Other	See Botulism, Foodborne	Detection of botulinum toxin in clinical specimen or Isolation of *Clostridium botulinum* from clinical specimen	None	A clinically compatible case that is laboratory confirmed in a patient aged greater than or equal to 1 year who has no history of ingestion of suspect food and has no wounds

12.3 Foodborne Botulism

In the USA, 1,184 foodborne botulism events (single cases and outbreaks) involving 2,727 persons were reported between 1899 and 2009 [8, 15, 16, 17]. The mean annual incidence of foodborne botulism in the USA is ~0.1 cases per 1,000,000 persons [8]. However, the annual incidence rate in the Alaska Native communities is 800 times greater than that in the contiguous states [18]. The number of foodborne botulism events in the USA has remained relatively stable (104 ± 33 events per decade) for the past five decades (Table 12.3). One obvious trend is the reduction in the number of foodborne botulism events in which toxin type was not determined. During the 1950–1959 reporting period, toxin type was determined in only 23% of events but by the 1980–1989 reporting period, almost 97% of events were categorized by toxin type. This trend is likely reflective of the improvements in awareness of this disease, accompanied by earlier clinical recognition of botulism and earlier collection of appropriate test samples. It also may be the result of the incorporation of new laboratory testing recommendations to include both detection of toxin and isolation of *C. botulinum* from stool [19]. In the USA, the majority of events (~60%) are caused by botulinum toxin type A; type E was identified in 30–45% of foodborne botulism events, primarily in Alaska, since 1980 (Table 12.3). Only two foodborne botulism events, both in California, have been caused by type F toxin; one caused by *C. botulinum* type F involving three persons and the other a single case caused by *C. baratii* type F toxin [20, 21].

There have been no major trends in the USA over the past 20–30 years in the major food types which cause botulism [8]. Vegetables and fish/aquatic animals continue to be responsible for the majority of foodborne botulism cases (~80%). Common home-preserved foods associated with botulism in the USA, excluding Alaska, include asparagus, green beans, peppers and mushrooms [8, 22]. In Alaska, botulism is associated with the consumption of Alaska Native traditional foods of fish (48%), marine mammals (47%) and beaver (6%) [18]. A Hispanic traditional food (*sierra en escabeche*) caused the only reported botulism outbreak (type A) in Puerto Rico [23]. This unusual food vehicle consists of pan-fried fish marinated with oil, vinegar, onions, peppercorns and bay leaves; the marinated fish is stored in large glass jars at room temperature for several days. Three of ten persons who ate the food over an 18-day period became ill. One surprising feature of this outbreak was that the food was acidic (pH 4.4) which is inhibitory to the growth of *C. botulinum*. While rare, botulism associated with acidic foods does occur, an interesting case of type B botulism due to pickled eggs occurred in 1997 [24]. Freshly hard-boiled eggs were covered with commercially canned beets, hot peppers and vinegar in a large jar and stored at room temperature in occasional sunlight. Just prior to closing the jar, a toothpick from the kitchen counter was used to pierce each egg so that juices could enter the center of the egg. The laboratory investigation demonstrated, perhaps not surprisingly, that the toxin level in the yolk of the egg was 1,000 times higher than in the liquid surrounding the eggs. No individual ingredient used to prepare the product contained *C. botulinum* spores, so spores in

Table 12.3 US foodborne events by toxin type, 1950–2009[a]

	1950–1959[b]	1960–1969[b]	1970–1979[b]	1980–1989[b]	1990–1999[c]	2000–2009[d]
A	14 (13.5%)	12 (15.4%)	68 (53.5%)	48 (60.8%)	72 (47.7%)	59 (60.8%)
B	3 (2.9%)	10 (12.8%)	28 (22.0%)	13 (16.5%)	23 (15.2%)	9 (9.3%)
E	7 (6.7%)	9 (11.5%)	15 (11.8%)	16 (20.3%)	51 (33.8%)	25 (25.8%)
F	0	1 (1.3%)	0	0	0	1 (1.0%)
Unknown	80 (76.9%)	46 (59.0%)	16 (12.6%)	2 (2.5%)	5 (3.3%)	2 (2.1%)
Total	104	88	127	56	151	97

[a] A foodborne event involving one or more cases
[b] Data from ref [12]
[c] Data from ref [22]
[d] Data from ref [8, 15, 16, 17]

the kitchen were likely to have been introduced into the center of the egg through the insertion of the toothpick (CDC unpublished data). Cases of botulism associated with traditional foods from other countries also have occurred in the USA. In 2006, home-prepared fermented tofu, commonly prepared in Asia and a common cause of botulism in China, caused type A botulism in an Asian woman living in California [25]. In 2012, two unrelated botulism cases (1 confirmed type B) in New York were suspected to be associated with a vendor-prepared tofu purchased at different times from a local market. The tofu was independently fermented by each of them. Type B toxin was detected in the tofu fermented by one of the patients [26]. In 2005, five individuals in New Jersey became ill, two were confirmed with type E botulism, after consuming a traditionally prepared Egyptian salted fish; one of the patients previously had botulism from consuming a similar product in 1992 [27]. Other unusual home-processed foods, such as peyote tea (a traditional drink of some American Native populations prepared from cactus) and pruno (a contraband alcoholic beverage prepared by prisoners), have caused botulism [8]. Surprisingly, even foods considered fresh (e.g., consumed soon after preparation, such as baked potato, potato salad and sautéed onions) rather than preserved have caused botulism (8). A common feature of these "fresh food" events is that each of these products usually consumed immediately was held at room temperature for 1 or more days, under at least partial anaerobic conditions (e.g., foil-wrapped potato, under an oily layer), prior to consumption with minimal or no heating.

Most foodborne botulism events in the USA occur as single cases, involve family members, or in defined social gatherings so the food source is relatively easy to identify even if unavailable for testing. However, several large outbreaks have occurred in restaurants which have challenged both epidemiologic and laboratory investigations. The largest outbreak (59 persons) in US history occurred in 1977 over a 1-week period in Michigan; all patients ate a meal at a single restaurant [28]. The investigation of the cause of botulism was complicated because of the number of people who ate at the restaurant and the number of foods served during the probable exposure period. However, type B toxin was detected in a jar of home-canned jalapeno peppers used to prepare various foods. The peppers were canned using an open kettle method (without pressure) approximately 5 months before the

outbreak; interviews with employees indicated that only a few jars were available since many exploded several months earlier. A very complicated type A outbreak occurred in New Mexico in 1978, involving 34 persons who ate a buffet dinner at a country club [29]. A commercially canned three bean salad was epidemiologically linked to the cases but was not available for testing. Both potato salad and coleslaw retrieved from the buffet were positive for botulinum toxin type A; however, all patients did not report eating potato salad and no ill persons reported eating coleslaw. An extensive investigation was unable to establish a clear mechanism for cross-contamination between foods served on the buffet although all three items were stored in the same refrigerator. Seven cases of type A botulism also occurred in 1978 in Colorado [30]. Potato salad, prepared from unwashed aluminum foil-wrapped baked potatoes held at room temperature for several days, was epidemiologically implicated as the cause of the outbreak, but left over food samples were negative for botulinum toxin. In 1994, 30 persons became ill after eating potato- or eggplant-based dip [31]. Botulinum toxin type A was detected in both dips; the epidemiologic investigation suggested that the eggplant-based dip was cross-contaminated by the potato-based dip through a shared spoon. Both dips were prepared using aluminum foil-wrapped baked potatoes that were stored at room temperature for at least 18 h. Studies conducted at the University of Wisconsin demonstrated that botulinum toxin was produced in aluminum foil-wrapped baked potatoes stored at 37 °C for 3–4 days. However, botulinum toxin was not produced in foil-wrapped potatoes held as long as 10 days at either 21 °C or 50 °C nor in foil-wrapped potatoes held up to 21 days at 4 °C [31]. In 1993, botulinum toxin type A surprisingly was detected in a cheese sauce served in a restaurant which caused eight botulism cases in Georgia [32]. Investigators suspected that the cheese sauce, which had expired 1 year prior to the outbreak, was contaminated with type A spores from baked potatoes served in the restaurant; just prior to the outbreak, the restaurant reopened after an extended closure. Although denied by the owners, the cheese sauce served in the outbreak was likely held in the restaurant since the closure. Since more than 50 cases of botulism were reported to be caused or suspected to be caused by baked potatoes, the FDA classified baked or boiled potatoes as “potentially hazardous food” [31]. Another surprising outbreak of 28 cases of type A botulism occurred in Illinois in 1983, as a result of consumption of restaurant-prepared sandwiches served with sautéed onions which were held throughout the day in a warm pan. Leftover food was not available from the restaurant, but a wrapper that previously contained one of the sandwiches was retrieved from the home of one of the patients; type A toxin was identified from washings of the wrapper [33].

Outbreaks in the USA also have occurred through consumption of commercially processed foods. One such type A outbreak (15 cases) in 2001 involved a commercially processed frozen meat-based chili [34]. The risk associated with this frozen meat-based chili was determined to be caused by mishandling of the product by a retail establishment rather than a manufacturing error; the product was frequently removed from the freezer and placed on a discount sale table outside the store. Another outbreak involving a commercially produced meat-based chili occurred in 2007 [35]. The 2007 outbreak had the capacity to be one of the largest foodborne

botulism outbreaks in the USA because it involved a commercially canned product that was widely distributed. Sixteen of 17 cans tested by the FDA were found to be positive for botulinum toxin type A. Significant deficiencies with the manufacturer's retort process resulted in the recall of 111 million cans of food involving 91 different products and the closure of the manufacturing plant. This was the first botulism outbreak associated with commercial canning in the USA in over 30 years, and it was the largest outbreak associated with commercial canning since 1919. From 1950 until this outbreak, only four botulism events (canned tuna, liver paste, vichyssoise and beef stew) in the USA were attributed to deficiencies in a commercial canning process [8, 35]. Remarkably, considering the number of botulinum toxin positive cans of chili identified by the FDA, only eight botulism cases, from three states, occurred as a result of this product failure [35]. Most consumers may have appropriately heated this product which destroyed the toxin and limited the number of persons exposed. The underlying cause of this canned chili outbreak was different from an outbreak earlier involving canned beef stew. The chili outbreak occurred as a result of a catastrophic breakdown of the retort process such that millions of cans were not properly heated. The beef stew outbreak in 1974, also type A, likely occurred as a result of either a two-line process which allowed cans to bypass the retort process or a lack of venting which may have created air pockets causing inconsistent heating [36]. No beef stew cans, other than the one consumed by the two patients, were positive for botulinum toxin, suggesting that this was a single can incident. Additionally, the contents of the can were reported by the consumers to be only warmed before eating. Three cases of type A botulism occurred in the USA in 1989 from the consumption of commercially prepared garlic in oil [37]. This outbreak followed a type B outbreak involving 36 persons in Canada with a similar product [38]. In response to these outbreaks, the FDA required new safety standards for these types of commercially prepared products [37]. A minimally processed commercial carrot juice product was the cause of an international type A outbreak in 2006 involving six total cases in Florida, Georgia and Ontario, Canada [39]. Except for the leftover product retrieved from the homes of the patients, no other bottles were identified with botulinum toxin, including bottles from the same lot consumed by the patients; however, several were found to contain *C. botulinum* type A spores. It was likely that the commercial carrot juice was inadequately refrigerated during transport, storage by the retailer or by the patients; however, at least two of the patients reported meticulous refrigeration control of the product following purchase. In 2011, two unrelated cases of type A botulism in different states were reported to CDC; both cases consumed commercial chilled soup sold by different companies that was left unrefrigerated by the consumers for several days [40]. A recent review of minimally heated, chilled foods suggests that these types of food products may be an emerging issue for botulism outbreaks [41]. Recent botulism events involving chilled foods, such as carrot juice and soup, which are designed to be consumed without heating or which are minimally heated even if required, unfortunately demonstrate that these products bring new challenges for public health, including assuring adequate labeling by the manufacturer and education of the consumer.

Foodborne botulism occurs worldwide, and rates of reporting vary by continent. However, the reasons for continental or country variations in reporting are not known. Foodborne botulism could be rare in some countries because of cultural differences in food preparation and consumption (low risk for botulism) or a result of limited medical and public health capacity in some areas (unrecognized cases). From publically available information, the toxin type shows some geographical distribution with type B predominating in Europe and type A predominating in Asia [42]. Botulism is rare in Canada but it is the second highest country in North America to report cases [43, 44]. From 1985 to 2005, 91 outbreaks (outbreak defined as ≥1 case) involving 205 cases with 11 known deaths were reported in Canada [43]. Most (~85%) foodborne outbreaks in Canada are similar to those that occur in Alaska in that they occur in native communities, primarily Inuit or First Nations, and involve traditional preparations of foods, including fish and marine mammals [43, 44]. All outbreaks in Canada involving native communities were caused by type E toxin. Most (64%) outbreaks in nonnative communities were caused by home-preserved foods; 50% type A, 34% type B and 14% type E. Two outbreaks in restaurants (chopped garlic in oil previously mentioned and bottled chanterelle mushrooms) accounted for 72% of all foodborne botulism cases in nonnative communities during the 1985–2005 reporting period [43]. Eight outbreaks or single cases occurred in Brazil from 1981 to 2001; the majority was caused by type A botulinum toxin in home-preserved foods [45]. In 2011, Brazil reported an outbreak among seven persons who consumed commercially produced sausage [46]. Foodborne botulism is considered a public health threat in Argentina; the first case occurred in Mendoza in 1922 due to home-canned asparagus [47]. Forty two cases were reported between 1992 and 2004; foods involved included home-processed ham, chili peppers, eggplant, cucumbers, heart of palm, tomatoes, peaches, spinach, cheese with onions, pickled octopus, asparagus, canned fish, sweet corn and rodent vizcacha. The only worldwide known outbreak due to an unusual dual toxin-producing strain (*C. botulinum* Af; type A produced at a higher level than type F) occurred in Mendoza and was caused by home-prepared pickled trout [48]. In 1998, type A botulism occurred in nine bus drivers who ate *matambre*, a traditional meat roll prepared with vegetables, spices and egg; this event increased awareness of foodborne botulism in Argentina and helped support a national surveillance system to include an antitoxin stockpile [49].

Reports of foodborne botulism are rare in the continent of Africa. The highest numbers have occurred in South Africa; however, only five events (3 type B, 1 type A, 1 unknown) have been reported [50]. All, in which a food was identified, have been associated with a meat product. A type A outbreak involving two young persons resulted from a damaged commercially canned fish with tomato sauce which was given to their economically distressed family. In 1991, a large outbreak of type E botulism occurred in Egypt which was traced to consumption of a traditional fish dish, *faseikh* (8). Recently, CDC provided assistance to two separate foodborne outbreaks one type B, affecting 32 persons in Rwanda and one type A, affecting three persons in Uganda. Although the food sources were not confirmed, a rice and bean mixture was epidemiologically linked to the Rwanda cases, and a home-prepared

herb-in-oil condiment was the suspected source in the Uganda outbreak (CDC unpublished data).

Botulism occurs frequently in various parts of Europe. As stated in Sect.12.2, 30 countries participate in the ECDC annual collection of botulism surveillance data. In 2011, the ECDC reported that 185 cases of botulism occurred among the 30 countries; however, the report does not delineate the number of cases attributed to foodborne botulism [6]. Four countries (France, Italy, Poland and Romania) accounted for 70 % of all reported cases. Sixteen participating countries reported no botulism cases during at least 1 of the 4 years of the reporting period; eight countries had no reported cases for the entire 4 years. The first reported foodborne outbreak in the UK occurred in 1922, involved eight persons, and was caused by type A botulinum toxin in duck paste [51]. Until 1999, ten additional events occurred involving 50 persons; three were type A, 3 type B, 1 type E and 3 unknown. The largest outbreak (type B), involving 27 persons, was caused by a hazelnut purée which was prepared with a sugar substitute. The UK reported 10–13 confirmed cases of botulism to the ECDC between 2006 and 2009, except for 2008 when only one case was reported [6]. In 2011, three children in a single family were confirmed with type A botulism; the outbreak was caused by a commercially produced product (korma sauce) only available in the UK [52]. The implicated batch of korma sauce was quickly withdrawn from sale. Additionally, alerts were issued throughout the UK and other areas of Europe through EWRS and RASFF. No other cases occurred. A dessert (tiramisu) containing contaminated mascarpone cheese caused a type A outbreak among eight young persons in Italy in 1996 [53]. Italy reported 12–32 botulism cases per year to the ECDC between 2006 and 2009 [6]. Most outbreaks in France are associated with canned ham, but recently nine cases were associated with home-canned asparagus [54]. Although most foodborne botulism cases in France are caused by type B toxin, at least 16 cases of type E botulism have occurred [55]. These type E cases in France differ from type E cases in North America, because they are primarily due to vacuum-packed fish and seafood products rather than “fermented” foods. France reported 4–23 confirmed botulism cases to the ECDC during the period of 2006–2009 [6]. In 2011, two type A outbreaks involving a total of nine cases were determined to be caused by a commercially prepared green olive paste (containing green olives, garlic, capers, and olive oil), produced in a small batch (total of 60 jars) [56]. The jars were primarily distributed to a small geographic area, but were also sold through the Internet. An investigation determined that the product was not sterilized correctly. Coincidently, during the same time period, Finland reported two cases of type B botulism confirmed to be caused by commercially prepared olives stuffed with almonds [57]. These are the only cases of foodborne botulism that have occurred in Finland except for two persons who had botulism from eating vacuum-packed smoked fish. Poland reports some of the highest number of foodborne botulism cases in Europe [58]. From 1988 to 1998, almost 2,000 cases have occurred with increases observed during times of social disruption and food shortages. The most common foods that cause botulism in Poland are home-canned pork, sausage, cheese and bacon; type B toxin was identified in > 80 % of cases. Recently, a case of type B botulism caused by home-preserved marinated mushrooms

was reported indicating that botulism in Poland is not restricted to home preservation of meat and cheese products [59]. Poland reported 15–24 annual cases of botulism during the period of 2006–2009 to the ECDC [6]. Presumably, all cases were foodborne since there have been no reports of other forms of botulism in Poland. Botulism in Romania from 1990 to 2007 was similar to Poland in that 93 % of foods involved were ham, pork, bacon and sausage; only type B outbreaks have been reported [60]. Romania reported 14–31 annual cases of botulism to the ECDC during 2006–2009 [6]. As in Poland, all reported cases are presumed to be food related. Foodborne botulism is rarely reported in Austria; only 15 cases have been reported since 1990 [61]. Botulism is rare in Denmark but usually involves type E toxin from the consumption of fish products [42]. However, a type B outbreak occurred in three Danish tourists on a mini cruise in Turkey; cheese rolls and fish were epidemiologically linked to the cases but no food was confirmed [62]. Notably, type F toxin first was discovered following an outbreak of three persons on the Danish island of Langeland [63]. While not part of the ECDC surveillance report, botulism is also rare in Turkey but three cases of unknown toxin type were epidemiologically linked to home-canned roasted mushrooms [64]. The Republic of Georgia has one of the highest incidences of foodborne botulism in the world (up to 6.7 per 100,000), primarily attributable (80 %) to unsafe home-preserved vegetables (85 % type B), while smoked fish and other food products also cause some illnesses [65].

As with Republic of Georgia, foodborne botulism is relatively common in Asia. From 1958 to 1989, 745 outbreaks involving 2,861 cases have occurred in China [66]. Over 60 % of these events were caused by home-preserved bean products, including bean curd and bean paste; most cases occur between February and May when these products are mainly prepared and consumed. Although toxins A, B and E have been identified, most cases (~ 79 %) were caused by type A. In contrast to type E cases in Alaska and Canada which occur along coastal waters, type E cases in China occur inland [67]. Surprisingly, one type E outbreak was found to be caused by *C. butyricum* type E in home-preserved soybeans [68]. Another outbreak caused by this rare *Clostridium* sp. occurred in India in 1996; the food epidemiologically associated with this outbreak was *sevu*, a crisp made from flour [69]. Japan first reported botulism in 1951 [70]. Although less common now, 86 outbreaks involving 351 cases occurred between 1955 and 1998. Most (88 %) of the outbreaks were due to type E botulinum toxin; only six were due to type A and three due to type B. All of the type E outbreaks were caused by *Izushi* (fermented fish and cooked rice) or *Kirikomi* (fermented fish without rice). The non-type E outbreaks occurred from commercially produced foods some of which were prepared using materials exported from other countries. In 1969, 23 type B cases occurred from food manufactured using bottle caviar imported from Germany. Eighteen type B botulism cases occurred in 1998 from the manufacturer of a food product using green olives imported from Italy. In 1984, type A botulism occurred in 36 individuals after eating vacuum-packaged deep-fried mustard stuffed lotus root. Botulism is rare in Thailand; however, since 1997, three outbreaks (all type A) have occurred from the consumption of community-prepared bamboo shoots [71, 72, 73]. The first two outbreaks affected < 12 persons each; however, the 2006 outbreak affected

209 individuals because the bamboo shoots were consumed during a highly attended religious event. The locally prepared bamboo shoots in 2006 were prepared by placing bamboo shoots in large tin containers over an open fire. Some of the bamboo shoots added last were likely not heated adequately, and the containers were filled to capacity likely creating an anaerobic environment when the cans were sealed. Another botulism outbreak occurred in Thailand in July 2006; 21 persons were affected after consuming a traditional meal that included raw deer meat. CDC provided laboratory assistance and identified type F toxin in the stool from one patient and from the samples of raw deer meat; *C. baratii* type F was identified in both the stool and the deer meat (CDC unpublished data). Commercial products have been responsible for some foodborne botulism in Asia. Type A toxin caused an outbreak in Taiwan involving nine persons who consumed commercially canned peanuts from an unlicensed manufacturer [74]. Recently, a single type E botulism case was reported in Taiwan associated with commercially vacuum-packaged dried bean curd [75].

Other than consumption of home-canned or home-processed food, there are no known specific risk factors for foodborne botulism; men and women appear to be affected equally. In the USA, foodborne botulism occurs primarily in persons aged 30–60 years probably due to the higher consumption of home-processed food products in this age group [8]. However, foodborne botulism affects all age groups. Foodborne botulism occurred in a 6-month-old US infant after eating botulinum toxin-contaminated home-canned baby food [76]. In 2011, Italy reported a case of type A foodborne botulism in an 8-month-old infant; the infant was exposed to improperly preserved turkey [77]. These two cases demonstrate that persons of all ages, even infants, are susceptible to this form of botulism. There has been speculation that younger persons may be less susceptible to the effects of botulinum toxin because lower case–fatality ratios are reported in this population, but the number of cases of foodborne botulism in younger persons is too low to establish a significant difference [8]. The trend observed simply may be due to fewer complications during intensive care respiratory support in younger patients rather than to inherent resistance to the effects of botulinum toxin [28].

Home-processed foods (served in homes, social settings and restaurants) account for 95 % of foodborne botulism outbreaks in the USA [8, 22]. Temperatures obtainable only with a pressure cooker are usually necessary to kill *C. botulinum* spores, particularly spores of proteolytic strains. The toxin itself is heat labile; heating to 80°C for 10 min is sufficient to destroy the toxin. However, some foods are not heated before being eaten, and others may not be heated sufficiently to reduce botulinum toxin below hazardous levels. A recent review of food preparation practices associated with three different home-processed foodborne outbreaks revealed that there was no evidence that a pressure cooker was used to preserve the foods, and in at least one outbreak, food was not adequately heated prior to the meal [78]. In 2010, five of seven persons that consumed a meal consisting of home-preserved food were determined to have type A botulism suggesting that even in Europe, additional education is needed to prevent this potentially deadly illness [79]. Public health investigators identified increased numbers of botulism cases in the USA dur-

ing periods of economic crisis, such as during its participation in World War I and II and the depression [80, 81, 82]. Both Polish and Romanian public health officials observed similar trends in their countries during times of social and political strife including post-communism eras [58, 60]. While home-canning practices, at least in the USA, have reduced in the past few decades, there is a fairly new trend around the world that encourages individuals to accept individual responsibility for preparedness against all types of disasters, such as hurricanes, tornados, earthquakes, even The End of The World as We Know It (TEOTWAWKI) campaigns. These "preppers" have created networks in various countries to serve as resources for those who want to prepare for upcoming disasters. One such group in the USA is the American Prepper Network [83]. A review of this website shows a wide range of advice on disaster planning. Unfortunately, the website includes outdated (circa 1912–1915) resources on home-canning practices. One of the documents provided on the website does mention pressure cooking as a means for home canning, but it only provides this as a suggestion to reduce the time to prepare the food rather than being a requirement for safe food production. Use of these documents by Network member or website visitors may result in an increase in the number of foodborne botulism cases around the world over the coming few years. While accepting individual responsibility for preparedness should be encouraged, perhaps networks such as this should be targeted for educational messages that make sure home-based disaster planners prepare safe food.

12.4 Wound Botulism

Wound botulism results from the growth of *C. botulinum* spores in a contaminated wound with *in situ* toxin production. The first report of a wound botulism case (occurred in 1943) was published in 1951 on a 15-year-old female who sustained a compound fracture of the leg and ankle during a fall [84]. Neurological symptoms began 14 days after the injury, and the patient died. A review of 18 other wound botulism cases in the USA resulting from injury showed that botulism symptoms began within 4–17 days [85]. Only 27 wound botulism cases were reported between the first case in 1943 and 1982 when the first wound botulism case associated with illegal drug abuse was reported in New York [86]. Between 1943 and 2009, 461 cases of wound botulism were reported in the USA [8, 15, 16, 17]. In cases in which toxin type was identified, 408 (88%) were type A, 35 (8%) type B, one (<1%) a mixture of type A and type B organisms, and 17 (4%) had serum quantity insufficient to do toxin-specific neutralization. Forty-seven wound botulism cases were reported between 1951 and 1990 [87]. Only 15% of the cases were associated with injection drug use; the remaining cases were associated with trauma or post-surgery complications. Recently, the fifth case of post-surgery botulism case (type A) was reported in a 54-year-old who had a laparoscopic appendectomy; this patient's risk factor for wound botulism was suspected to be due to broad-spectrum antibiotics used to treat an abdominal abscess in the patient 6 days after surgery [88]. In con-

trast, 389 wound botulism cases were reported to CDC between 1990 and 2009; 97% were due to injection drug use [8, 15, 16, 17]. The change in wound botulism in the USA is largely due to changing trends in California. A review of California botulism cases showed that prior to 1988, wound botulism occurred < 1 per year; 10 years later, 28 cases occurred in 1998 alone [89]. Prior to 1988, the rare cases of wound botulism were primarily male with median age of 28. After 1988, the median age increased to 40, 45% were women, a higher proportion (57%) occurred in the Hispanic population, and almost all were associated with injection drug use, primarily "skin popping" black tar heroin. The risk of wound botulism in injection drug users does not seem to be a behavioral deterrent. Seventeen wound botulism patients previously treated in California returned to the hospital within 1–71 months with additional episodes of wound botulism, presumably from continued exposure to contaminated illegal drugs; one case had three wound botulism episodes [90].

Wound botulism has been reported in Europe, Asia, Australia and South America; rarely, these cases are associated with trauma [91, 92]. Wound botulism was not recognized in the UK and Republic of Ireland prior to 2000 [93, 94]. Between 2000 and 2004, 88 cases were reported (80% occurred in England) all in injecting drug users. Similar to cases in California, most laboratory confirmed cases (88%) were caused by *C. botulinum* type A. In 2004, a geographic clustering (36 of 40 total cases) of wound botulism cases occurred in England [94]. This unusual cluster suggested that the patients were linked; however, a causal association could not be identified. To date, no laboratory has provided a definitive link between wound botulism in injection drug users and any contaminated drug source. Sixteen cases of wound botulism were reported to German public health officials between October 13 and December 5, 2005, when only one wound botulism case was reported for all the previous 3 years [95]. The sudden surge in the numbers of wound botulism cases prompted an extensive epidemiological investigation, but no link could be identified. However, seven *C. botulinum* type B isolates from six patients (two isolates were obtained from two wound sites on a single patient) had indistinguishable pulsed-field gel electrophoresis (PFGE) patterns suggesting a possible link.

In spite of recent reporting increases, the number of wound botulism cases may be underestimated. Wound botulism is laboratory confirmed by detection of toxin in serum and/or isolation of *C. botulinum* from a wound. Investigators in California reviewed medical records and laboratory data for 73 patients with illness compatible with wound botulism. Their review showed that serum was positive by mouse bioassay in only 68% of the cases [96]. Similar difficulties in laboratory confirming wound botulism have been reported elsewhere. Only 58% of suspect cases were laboratory confirmed by either toxin detection in serum or isolation of *C. botulinum* from tissue or pus in the UK between 2000 and 2002 [93]. Similarly, 58% of patients associated with a wound botulism cluster in Germany were laboratory confirmed by toxin detection or positive culture results [97]. Only 33% of 36 suspect wound botulism cases in England in 2004 were laboratory confirmed [94]. In Ireland, only 17% of cases were laboratory confirmed in 2008 [98]. The California study also showed that of the 23 serum negative patients, 26% did not have "botulism" or "suspected botulism" in their discharge summaries, in spite of

having classic botulism symptoms, such as ptosis, dysphagia, dyspnea and bilateral, descending muscle weakness. The lack of documentation of a diagnosis of botulism in the medical records for clinically consistent patients with negative test results suggests that wound botulism may be significantly under reported worldwide. As a result of this study, a change was made in 2011 to the National Notifiable Diseases Surveillance System CSTE Position Statement for Botulism (Table 12.2) to include a definition for a probable case for wound botulism which previously only contained a classification for confirmed cases.

12.5 Intestinal Toxemia Botulism

As early as 1937, investigators in the former Soviet Union recognized a secondary type of botulism sometimes termed "toxinfection" which resulted from growth of organisms and toxin production in the intestinal tract; however, this form of botulism was universally discounted for decades [99]. The Russian investigators demonstrated this in experimental animals and described extended (>14 days) detection of botulinum toxin in several patients and reoccurrence of severe disease over 3 months after initial exposure to a contaminated food product in the mid- to late-1950s. Two forms of intestinal toxemia botulism are now recognized worldwide: infant botulism and intestinal colonization botulism (adult and child) even though there are no standard measures (clinical or laboratory) to definitively define the adult form. Infant botulism is the most frequently recognized form of botulism in the USA, although it is rare in other countries [8].

12.5.1 Infant Botulism

Infant botulism as a unique syndrome first was reported in the USA in 1976 [2, 3]. Within 2 years of the first report, infant botulism became the most common form of botulism reported in the USA; currently, 80–100 cases are reported each year representing a fourfold to fivefold increase over reports of foodborne botulism [8]. The total number of US infant botulism cases reported to CDC between 1976 and 2009 was 2,709 [15, 16, 17, 100]. In 2008, Rhode Island became the last state in the USA to report its first infant botulism case (type B) [16]. Since 1985, 39% of all infant botulism cases have been reported from California.

Recently, a review of worldwide infant botulism from 1976 to 2006 showed that this illness has been reported from almost every continent; no cases were reported in Africa [100]. Only 18% of all cases occurred outside the USA. Continent-specific clustering also occurred. Most cases (99%) on the continent of North America occurred in the USA; only 13% of countries reported any cases with Canada reporting the second highest number of cases. Almost all (99%) cases in the South American continent occurred in Argentina, with only three countries (25%) reporting

even a single case. Thirteen of 47 (28%) countries spread throughout the European continent reported infant botulism cases; however, 40% of these cases occurred in Italy. Interestingly, there were no reports of infant botulism cases in Poland or in the Republic of Georgia both of which represent high incidents of foodborne botulism on this continent [6, 58, 65]. A third infant botulism case in Denmark was recently reported; however, foodborne botulism was also suspected since the commercially produced infant food, not available for testing, provided to the infant was reported by the mother to smell bad [101]. No organisms were recovered from the infant stool, and toxin was only detected in the serum. A product recall was issued although no toxin was detected and no *C. botulinum* contamination was identified in an unopened identical product. Only 6 of 44 (14%) countries on the continent of Asia reported infant botulism cases; 76% of the cases occurred in Japan [100]. Recently, investigators in Iran reported their first case of laboratory-confirmed (type A) infant botulism in a 6-month-old girl with a history of consuming honey 3 weeks prior to illness [102]. While a full food history was not provided, other than exposure to honey, type A toxin was detected in the stool and no other family members were reported to be ill. This report in Iran adds to the growing number of countries reporting cases of infant botulism. Australia, the largest of 14 countries consisting of mostly small islands on Oceania and the only one with reports of infant botulism, had 32 cases; 56% of these occurred in South Australia and Victoria [100].

All known botulinum toxin types, except types D and G, have caused infant botulism, and dual toxin-producing *C. botulinum* strains have been identified [100]. Toxin types A and B are the most commonly reported (98.6% of cases with known toxin type) cause of infant botulism worldwide (Table 12.4). An additional 45 cases were reported to be caused by a single strain which produced both type A and B toxin. The percentage of type A versus type B cases was nearly equivalent in most continents except for Asia, where the number of type A cases was approximately two times the number of type B cases and South America, where 99% of cases were type A. Type E toxin was the next highest toxin type reported in Europe; four cases of infant botulism due to *C. butyricum type E* were reported from Italy [100]. *C. butyricum* type E was also identified in one case in Japan and in one case in the USA. A second type E case was identified in 2007 in the USA and is the first case of infant botulism caused by *C. botulinum* type E [103]. The first type F infant botulism case was described in 1975 and determined to be caused by *C. baratii* type F [104]. To date, nine type F infant botulism cases in the USA were either suspected or proven to be caused by *C. baratii* type F, the most recent was identified in 2008 in Colorado [16, 100]. Another case of infant botulism due to *C. baratii* type F occurred in Hungary and represents the only reported infant botulism type F case outside the USA [100]. A single case (5½-month-old female) of infant botulism due to *C. botulinum* type C was reported in Japan in 1990 [105].

Infant botulism is reported in the USA most commonly in the second month of life and occurs somewhat earlier in cases of type B disease (median 9 weeks) than in cases of type A disease (median 11 weeks); type F cases have been reported in infants as young as 1–2 days old [10]. There is no sex predilection and no apparent pattern of seasonal variation. Little is known about how infants are exposed

Table 12.4 Global distribution of reported infant botulism toxin type

Area	A	B	C	E	F	Ba[b]	Bf	Ae	Unknown	Total
North America	1,213 (44%)[a]	1,489 (54%)		2 (0.0 7%)	9 (0.33%)	16 (0.58%)	5 (0.18%)		2 (0.07%)	2,736
USA[c]	1,191 (44%)	1,484 (55%)		2 (0.07%)	9 (0.33%)	16 (0.59%)	5 (0.18%)		2 (0.07%)	2,709
Canada	22 (81%)	5 (19%)								27
South America	368 (99%)	1 (0.27%)							1(0.27%)	370
Europe	20 (30%)	27 (41%)		4 (6%)	1 (1.5%)	1 (1.5%)	2 (3%)	1 (1.5%)	10 (15%)	66
Oceania	12 (37%)	15 (47%)				1 (3%)			4 (12%)	32
Asia	14 (48%)	8 (28%)	1 (3%)	1 (3%)					5 (17%)	29
Africa	0	0	0	0	0	0	0	0		0
Total	1,627 (50%)	1,539 (48%)	1 (0.03%)	7 (0.2%)	10 (0.3%)	18 (0.6%)	7 (0.2%)	1 (0.03%)	22 (0.7%)	3,233

[a] Percentage calculated from total number of cases for each continent or country

[b] Most identified as predominately B with minor level of type A toxin; in one case, the relative toxin levels were not available [100]

[c] Data for the USA from refs [15, 16, 17, 100]; all data from other countries/continents were from ref [100]

to neurotoxin-producing *Clostridia* sp. Although spores have been found in some foods, only honey has been identified as a specific risk to infants [8]. Several studies from the late 1970s and early 1980s showed that more than 20 % of affected infants in the USA had ingested honey before the onset of botulism. Additionally, *C. botulinum* spores of the same type as those isolated from feces were cultured from honey known to be fed to the infant in a few cases suggesting a causal relationship. However, since most infants with infant botulism, at least in the USA, have had no exposure to honey, the risk factors and vehicles of transmission of *C. botulinum* for the majority of cases remain unclear [8, 100]. A survey of foods revealed *C. botulinum* in samples of corn syrup as well as honey, but in no other tested category of foods. Some infant botulism cases, particularly those < 8 weeks old received no other food then breast milk. Some environmental studies demonstrated that the same serotype of *C. botulinum* that caused disease could be isolated from the soil in an infant's yard and from vacuum cleaner dust; investigators have also frequently noted environmental conditions that might expose infants directly to environmental sources of *C. botulinum* spores, such as a shared crib, dusty or windy locales, nearby building construction or outdoor activities [8]. *C. botulinum* type A was isolated from a left over rice pudding and *C. botulinum* type B was isolated from both opened and unopened powdered infant formula (PIF) fed to a type B infant botulism case in the UK [106]. Ten *C. botulinum* type B isolates obtained from the opened PIF were clustered into four amplified fragment length polymorphism (AFLP) patterns, and nine *C. botulinum* type B stool isolates were clustered into two patterns; two of the AFLP patterns from the opened container of PIF were indistinguishable from the two AFLP patterns from stool isolates. Cultures of 25 unopened cans of PIF (same brand and batch as fed to infant) resulted in only one can with *C. botulinum* type B; the AFLP pattern of the isolates from this unopened container was distinct from both the AFLP patterns from the stool and the four AFLP patterns obtained from the opened food. PFGE studies confirmed that some isolates from the opened PIF were indistinguishable from stool isolates, but the isolates from the unopened PIF were unique [107]. Both these related studies demonstrate that simple identification of the same toxin type (type B in this case) in cultures of stool and implicated food cannot be used to assign a causal relationship between infant botulism and foods known to be consumed by these cases. Isolation of *C. botulinum* type A from the left over rice pudding demonstrates that simply isolating a neurotoxin-producing *Clostridium* sp. from a leftover food also cannot be used as proof of source of exposure in infant botulism cases, since this infant was confirmed to have type B disease. Additionally, infants may be colonized with several different strains of *C. botulinum* and products, such as PIF fed to infants, may contain several unique strains. The studies also showed that *C. botulinum* type B spores with indistinguishable AFLP and PFGE patterns from the stool isolates were present in the opened container of PIF suggesting that spores in this opened food may have caused infant botulism in this case. However, the studies did not prove that contamination of the PIF by the strain which caused illness occurred during manufacturing, since an unopened container of PIF of the same brand and batch number contained spores which were clearly distinct from those which caused this infant botulism case. The source of the

spores found in the opened container is not known, but could have occurred after the product was opened by the consumer. While these studies demonstrated that it may be possible to use AFLP or PFGE to analyze products containing *C. botulinum* spores and to suggest that a particular product may be the source of infant botulism, additional studies are needed to establish the diversity of *C. botulinum* before they can be used to establish a causal relationship between isolates recovered from clinical samples and suspect food products.

12.5.2 Intestinal Colonization Botulism (Child or Adult)

Intestinal colonization botulism in an adult or child is an elusive disease to classify. These are isolated cases of botulism in patients >1 year of age in which extensive investigations failed to implicate a specific food as the cause of the disease and for which an infected wound could not be identified and illegal drug use was not suspected. In the USA, the initial investigation of these is usually recorded by CDC as cases of "undetermined origin" or "other." There is currently no standardized CSTE position statement for these cases and so these are nationally reported as "Other" if laboratory confirmed (Table 12.2). Since 1976, 73 botulism cases could not be definitively classified as either foodborne or wound botulism (CDC unpublished data). Of the 64 for which toxin type was known, 63% were type A, 13% were type B and 25% were type F. This somewhat contrasts with the relative distribution of toxin type associated with foodborne botulism in the USA from 1975 to 1992: type A (57%), type B (18%), type E (21%) and type F (0.3%) [42]. Although there has been speculation on the matter since the 1920s, careful investigation has now demonstrated that some of these cases of "undetermined origin" are caused by colonization of the gastrointestinal tract by neurotoxin-producing *Clostridia* sp. with *in vivo* production of toxin, analogous to the pathogenesis of infant botulism [8, 108, 109, 110].

Remarkably, 25% of the USA cases of "undetermined origin" were associated with type F toxin. The first such case in 1987 was determined to be caused by *C. baratii* type F [111]. A review of botulism cases from 1981 to 2002 identified 13 type F adult cases [112]. *C. baratii* was isolated from stool for 9 of these 13; 8 stool cultures produced *C. baratii* type F. One type F case from California was due to foodborne botulism since type F toxin was detected in an implicated food consumed by the patient; *C. baratii* type F was also isolated from the food [21]. Toxin type F was detected in stool culture of two additional cases but the causative organism was not identified [112]. Five of the nine patients in which botulism was caused by *C. baratii* type F, including the California foodborne botulism case, had a medical history of major gastrointestinal surgery, recent gastrointestinal procedures, and/or recent antimicrobial treatment consistent with other reported intestinal colonization botulism cases. Few cases of intestinal colonization botulism are reported outside the USA. Two unrelated cases, both caused by *C. butyricum* type E, were reported in Italy [113]. Both cases presented to the hospital with acute abdominal pain and

underwent surgery for suspected appendicitis; during surgery, each was found to have inflamed Meckel's diverticulum which was resected. *C. butyricum* type E was isolated from stool 11–16 days from symptom onset. Five cases of suspected adult intestinal colonization were identified between November 2006 and May 2008 in or around Toronto, Ontario, Canada; three of which were described in detail in 2012 [114]. Two of the three patients were confirmed as type A botulism; both had medical histories significant for gastrointestinal complications. The third patient (type B botulism) had no significant medical history to suggest a higher risk for intestinal colonization. In 2003, investigators in Japan reported an intestinal colonization botulism case in a 12-year-old girl following foodborne botulism involving consumption of vacuum-sealed hashed beef [115]. The description of this case in Japan is similar to early reports by Russian investigators in which prolonged botulism followed a shorter typical foodborne botulism episode [1]. *C. botulinum* type Ab was isolated from the patient's stool up to 122 days from hospital admission; serum collected at day 250 was able to neutralize the effects of type A toxin, suggesting that the patient produced protective antibody to the toxin produced in the gut [115]. Support for the diagnosis of botulism from intestinal colonization can be obtained by the demonstration of the prolonged excretion of toxin- and neurotoxin-producing *Clostridium* sp. in the stool [109]. Unfortunately, few laboratories collect and test additional clinical specimens on these cases. Definitive classification of this form of botulism is difficult and in most cases relies on the absence of association with a botulinum toxin-contaminated food or a reliable history of consuming home-canned foods. Intestinal colonization botulism patients may produce detectable antibody due to the long-term exposure to low levels of toxin [109, 115]. However, identification of circulating antibody is rarely, if ever, done. The limited data available on intestinal colonization botulism cases have not identified any trends in specific food risks and except for alterations in the gastrointestinal tract, no other specific risk factors have been identified [8].

12.6 Iatrogenic Botulism

Remarkably, one of the most potent toxins known has brought incredible relief to individuals with a wide range of conditions. As with all pharmaceuticals, there are potential risks in therapeutic administration of the botulinum toxin. Several products are currently approved for use in both the USA and Europe for defined conditions, such as blepharospasm, strabismus, cervical dystonia, severe primary axillary hyperhidrosis, spasticity and for the temporary improvement in the appearance of moderate to severe facial frown lines. However, off-label use of botulinum toxin for the treatment of other conditions occurs. Adverse events associated with botulinum toxin injections that lead to unanticipated local or systemic effects consistent with botulism symptoms are classified as iatrogenic botulism, although there is no formal definition in the CSTE guidelines (Table 12.2).

Almost all approved products have caused some adverse effects. A 3-year-old female with cerebral palsy was off-label treated in multiple limbs with a total of 400U onabotulinumtoxinA (BoTox BT/A) (marketed by Allergan) [116]. Within 3–4 weeks of injection, the child experienced worsening dysphagia, excessive drooling and aspiration of liquids. Respiration would sometimes cease when the child was sleeping and required resuscitation by her mother. Severe generalized weakness lasted for 6 weeks and she was unable to lift her head for 3 months. A 10-year-old boy was treated in multiple limbs with a total of 19,000U rimabotulinumtoxinB (Myobloc BT/B; marketed by Solstice Neurosciences) for spastic quadriplegia [117]. Within 1 week, the patient began experiencing problems breathing when sleeping and progressed to cranial nerve symptoms by week 5; full recovery did not occur until 6 months from the injections. An adult patient treated with 18,500U of type B toxin (product not identified) for cerebral palsy-related leg spasticity developed severe dysphagia that required hospitalization for 12 days; full resolution of symptoms did not occur for 75 days [118]. In 2010, adverse events in three adult patients with spasticity treated with 1,000–1,500U abobotulinumtoxinA (Dysport BT/A; marketed by Ipsen Biopharm Ltd.) were reported [119]. Two of the patients presented within 1–3 weeks from injection with symptoms of botulism (dysphagia and moderate to severe weakness of the upper body; one required intubation); the adverse symptoms resolved within 2 months. The third patient did not show systemic symptoms (dysarthria, dysphagia, shortness of breath, mild weakness of head, neck and upper extremity muscles) until 1 week after the fourth injection (identical injections were spaced 3–4 months apart); adverse symptoms did not resolve for 6 months. An interesting case of iatrogenic botulism was reported in a weight lifter [120]. The patient was initially treated with 1,000U Dysport BT/A in his lower limbs for a gait disorder. On follow-up, the patient reported a tremendous improvement in gait coordination but also some feeling of fatigue and generalized weakness that started about 2 weeks after injection. Three months later, the patient was injected with a lower dose (600U) and asked to keep a diary of his weight lifting capacity. His meticulous documentation showed almost a 50% reduction in both upper and lower limb strength 6 weeks after injection, but strength capacity returned by week 12. While adverse symptoms were fairly mild and the benefit of treatment clearly outweighed the risks, this report demonstrates that even low doses of botulinum toxin migrate beyond the injection site and can affect distant muscles. Some adverse events may be more localized. A review of 211 patients injected into extraocular muscles showed that ptosis occurred in 8.7% of patients; most events were mild and most recovered within 6 weeks [121].

Only a small number of peer-reviewed publications report on adverse events associated with either therapeutic or cosmetic injections. Perhaps there is reluctance on the part of a physician to report in the scientific literature adverse events for off-label use or changes in recommended dosing of pharmaceuticals. Additionally, suspected iatrogenic botulism cases are rarely reported (<eight reports documented) in the USA to CDC's Botulism Surveillance System or other state health departments, and none have been laboratory confirmed. However, in the USA, many of these events are reported through MedWatch, a voluntary system for reporting adverse

events, product quality problems or product use errors. A review of adverse events reported to MedWatch during and following administration of licensed botulinum toxin was conducted in 2005 [122]. Serious adverse events reported (1,437) in patients from 1989 to 2003 treated with licensed toxin for therapeutic reasons (for both labeled and unlabeled indications) covered a wide variety of reactions, including death, ptosis, diplopia, dysphagia, dysarthria, dystonia, fatigue, and generalized muscle weakness, muscle spasm, allergic reactions, flu-like syndromes, gastrointestinal symptoms, cardiovascular system-related symptoms, respiratory system-related symptoms and seizure.

In January 2008, the Public Citizen's Health Research Group, a US consumer advocacy group, petitioned the US FDA to follow and expand on the EU 2007 safety recommendations and require manufacturers of therapeutic botulinum toxin products to add warning labels on product vials and to provide health-care providers and consumers information on potential risks to toxin injections [123]. This group's petition included an analysis of adverse events reported between 1997 and 2006. Their analyses identified 658 adverse events with 27% ($N=180$) reported as aspiration, dysphagia or pneumonia. Almost 50% of these more serious events required hospitalization and 16 deaths were reported; four deaths were in children < 18 years of age. As a result of the petition, FDA reviewed available data from clinical trial studies, published reports and MedWatch adverse events reports. In April 2009, the FDA determined that these adverse events warranted the requirement of a "Black-Box" warning label to be applied to all botulinum toxin products approved for use in the USA [124, 125]. In addition, the FDA required manufacturers to develop a risk mitigation strategy (RMS) to include information documents for both practitioners and patients. FDA determined that the RMS documents must have plain language which informs patients of possible adverse events and also language that makes it clear to practitioners the importance of following manufacturers' suggestions for dosing. FDA's review did not identify any deaths in patients treated with toxin type A for cosmetic purposes, but commonly reported reactions included headaches, focal facial paralysis, muscle weakness, dysphagia, flu-like syndromes and allergic reactions [122, 124, 125]. However, FDA's requirement included "Black-Box" warning labels and an RMS for products intended for cosmetic use only. This action by FDA may reduce the number of adverse events reported in the USA, since patients will be provided clear information on the risks of treatment, and practitioners will be provided with guidance on approved uses and information on the potential inequitable results when using identical dosing using different products.

It is not clear whether all adverse events are directly related to botulinum toxin. The summary report by FDA investigators in 2005 is clear in that not all adverse events reported to the FDA can be clearly identified as caused by the product [122]. A recent study [126] summarizes a prospective evaluation of adverse events, following botulinum toxin injection of 334 pediatric cerebral palsy patients. This study suggests that when used properly, even in severely affected cerebral palsy patients, the improvements in quality of life, following botulinum toxin treatment, outweigh the risk of primarily temporary adverse events. However, it is the physician's responsibility to know and understand the risks, including any maximum dose recom-

mendations [127]. Additionally, they need to understand that there are no dosing recommendations for pediatric patients since botulinum toxin is not approved in the USA for this population. Instituting strong requirements for "Black-Box" warning labels and development of a product RMS in all countries should provide physicians with the information they need to clearly explain the risks and benefits so that the patient's caregivers can make informed decisions on treatment options involving botulinum toxin.

An additional risk factor for iatrogenic botulism is in counterfeit or other non-approved materials marketed for human use and the availability of bulk research toxins not intended for human use. A list of several counterfeits marketed as alternatives to legitimate therapeutic toxin products was recently reported [128]. Independent laboratory analyses of these products indicated that most did not meet toxin potency as indicated on the vial; some were much lower, but even more problematic was that some were found to be more potent than the label indicated. Use of these bogus products could result in overtreatment of patients with subsequent severe consequences. A recent report by security analysts suggests that the botulinum toxin counterfeit market is growing around the world [129]. The groups marketing these products primarily through the Internet, in many cases, use deceptive practices to make their material resemble licensed products. Unfortunately, commercial industry and the health-care field contain unethical individuals who potentially profit from the production and administration of these bogus materials.

Perhaps the most incredible outbreak of iatrogenic botulism reported to date occurred in 2004, when a physician, on a suspended license, injected himself and three others with a research grade botulinum toxin type A product to treat moderate to severe facial frown lines; each patient was exposed to as much as 20 μg of type A complex through 4–6 injections in the facial area [130]. Onset of symptoms of dysphagia, diplopia and generalized weakness began within 1–2 days; all required mechanical ventilation within 3 days following injections. All patients, except for the physician who administered the injections (permission to collect clinical specimens was not provided by this patient), were laboratory confirmed by detection of botulinum toxin type A in serum; stools when available did not contain toxin, and *C. botulinum* was not isolated. All four patients survived; one patient, who was followed up to 10 months after hospitalization, reported persistent mylagias, muscle weakness, shortness of breath, and persistent pain that required treating with an analgesic medication [131]. The toxin used in these cases was labeled as a laboratory research product, not licensed or intended for human use, and was ordered as a single 100-μg vial and shipped from a legitimate manufacturer. The physician was sentenced to 3 years in prison for misbranding a drug [130]. Other vials of non-approved botulinum toxin were recovered from the physician's office but records were not available on whether this product was administered to any individuals visiting this doctor's office. The owners of a business, Toxin Research International, Inc (Tucson, AZ, USA) pled guilty in 2006 to charges of mail/wire fraud, misbranding a drug and defrauding the US government related to the packaging and distribution of vials of bogus Botox [129]. The greed of some individuals, unscrupulous doctors or other health-care providers, the availability of "bogus" improperly tested/labeled therapeutic products and the continued practice of "off-label" use of even approved

products by inexperienced practitioners may only increase the number of iatrogenic botulism cases reported to authorities.

12.7 Summary

Botulism is a rare and often surprising illness. While once thought to only occur as a foodborne intoxication, human botulism now occurs worldwide as one of the several unique forms (foodborne, wound, infant, adult colonization, iatrogenic and, rarely, inhalational) [8]. All provide unique challenges for diagnosis and control. Originally named because the illness was thought to be solely a problem of sausage, surveillance worldwide over the past 200 years has demonstrated the wide variety of foods, including sausage, fish, marine mammals, vegetables of all types, which may cause botulism. The identification of recent outbreaks due to commercial products suggests that this illness may not be on the decline around the world. Minimally processed, chilled foods may present a new era in foodborne botulism and provide opportunities for enhanced control measures. Additionally, wound botulism, infant botulism and adult colonization cases are beginning to be recognized in many countries, suggesting that reports of these botulism forms may become more frequent. Finally, a disturbing number of adverse events are being reported related to therapeutic toxin injections. These events are particularly challenging for public health because: (1) some of these events may be unrelated to toxin injections, (2) there is no current mechanism to capture this botulism form through public health surveillance, (3) no known laboratory tests can differentiate between true iatrogenic botulism and non-related causes of symptoms and (4) counterfeit products may be used by unscrupulous health-care providers which may be difficult to trace to their source.

Botulism has provided surprises to investigators through the identification of unusual *Clostridium* sp. that produce botulinum toxin. Other neurotoxin-producing species may be lurking in the environment waiting to be discovered. Seven serotypes of botulinum toxin so far have been identified but others may exist. Recent studies have demonstrated a diversity within the known toxin serotypes which is discussed in Chap. 10 but, subtype-specific risks to humans are currently unknown. Future surprises in botulism outbreaks, discovery and further study of toxin serotypes/subtypes, and characterization of the neurotoxin-producing Clostridia involved will help us understand and perhaps eventually control human botulism.

Acknowledgments The author thanks Dr. Peter Gerner-Smidt, Centers for Disease Control and Prevention (CDC) for translation of the Danish article. The findings and conclusions in this report are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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