

# Two unlinked cases of foodborne botulism in Italy at the beginning of 2010

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## SUMMARY

The study provides data on two cases of foodborne botulism caused by consumption of commercial vegetable products: artichoke preserve and cream of vegetable soup. By mouse bioassay, *Clostridium botulinum* toxin in both the suspected food samples was detected and identified as type B toxin. The detection of *C. botulinum* toxin in the artichoke preserve indicates an inadequate food production technology while the presence of *C. botulinum* toxin in the vegetable soup appeared to be related to wrong behavior on the part of the consumer and to faulty food preservation. The study confirms that an early identification and reporting of suspected botulism cases is vital in the prevention of accidental widespread outbreaks.

**KEY WORDS:** *C. botulinum*, Foodborne, Commission Regulation No. 2073/2005

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## INTRODUCTION

Food-borne botulism is a severe and deadly intoxication caused by the consumption of food containing as little as 30 to 100 ng of preformed botulinum neurotoxin synthesized by a spore-forming anaerobic bacterium named *C. botulinum*. In addition, some strains of two other clostridia, *Clostridium baratii* and *Clostridium butyricum*, also form a botulinum neurotoxin (Peck, 2006). *C. botulinum* produces seven different toxins (A-G) of which types A, B, E and rarely F cause human botulism. These types have been reported as an important food safety hazard (Lindstrom, 2003; Hobbs and Roberts, 2005). Types A and B are found in soil and animal fertilizers, therefore they might be found in canned food products of vegetable origins, e.g., tomato, spinach and beans (Davis, 2003). Type E is found in aquatic envi-

ronments, sea food, and marine sediments (Boyer *et al.*, 2001).

*C. botulinum* spores are widely distributed in the environment. *C. botulinum* needs an anaerobic environment that is free of growth inhibitors, that has adequate nutrients, a suitable temperature, enough water, and adequate acidity to support the growth and production of toxins. The growth of *C. botulinum* in food products may be prevented by controlling the acidity (pH <4.6), water activity (Aw <0.93) and temperature (refrigeration) and by using chemical preservatives. Most moist foods, such as fresh meat, fruits and vegetables, have high Aw values and therefore require refrigeration (<4°C) to inhibit production of toxins by *C. botulinum* (Peck, 2002).

The neurotoxins block neuromuscular stimulation by interfering with cholinergic autonomic neuromuscular plaques at presynapsis (Dobbs and Austin, 1997).

Typical clinical symptoms of botulism include cranial muscle paralysis, such as double vision and dilated pupils, slurred speech, dry mouth, difficulty in swallowing and speaking, and facial paralysis. As the disease progresses, paralysis of the limbs and respiratory dysfunction become ap-

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parent. Respiratory muscle paralysis can lead to death. Recovery occurs upon the sprouting of transitory nerve endings that reside when the synaptic activity of the original nerve regenerates (Meunier *et al.*, 2002). Recovery may require several weeks to months and is dependent on the amount of toxin ingested and, to a lesser extent, on the toxin type in question. Type A toxin tends to be more potent than types B and E and causes prolonged disease (Foran *et al.*, 2003).

The classical form of botulism is food poisoning, an intoxication that follows the consumption of food containing preformed neurotoxin and presents an incubation period from 12 to 72 h. The food-borne form, in addition to the general signs of botulism, may be manifested by gastrointestinal symptoms such as nausea, vomiting, and constipation. The treatment consists mainly of intensive symptomatic care, including respiratory support (Bossi *et al.*, 2004).

The Italian health authorities have reported every year approximately 20 cases of food-borne botulism in humans, mainly caused by consumption of home-made vegetable and fishery preserves (Epicentro, 2008; Fencia and Anniballi, 2008). The present study describes the detection and identification of botulinum toxin and viable *C. botulinum* from commercially vegetable products (artichoke preserve and cream of vegetable soup), suspected to be the cause of two different *food-borne botulism* episodes in Lombardy region (Italy).

## MATERIALS AND METHODS

### Samples

Two samples of commercial products, an artichoke preserve and a cream of vegetable soup were sent from the Hospital Agency of the Lombardy region to the IZSLER (Istituto Zooprofilattico Sperimentale di Lombardia and Emilia Romagna) in March 2010. The samples were suspected to be associated with two epidemiologically distinct cases of food-borne botulism in human patients.

The artichoke preserve was an artisanal spread cream composed of artichoke, extra virgin olive oil, dried fruit, onion, garlic, spices and its flow diagram of production included a thermal treatment of pasteurization. The manufacturing com-

pany indicated in the labeling a shelf-life of two years and conservation at refrigeration temperatures was not recommended.

The cream of vegetable soup was pasteurized and the manufacturer indicated a shelf-life of 45 days. Labeling suggested conservation of the product at refrigeration temperature. The hospitalized patient declared that the cream was stored at room temperature for some hours before usage and that the container appeared swollen.

### Determination of pH and Aw in food samples

Two aliquots from each sample were removed for pH and Aw measurement to avoid contamination of the bulk of the food. Aw values were measured directly at the surface of food samples using surface probes (Testo 650 Humidity Meter), while mixed samples were used to evaluate the pH values (Hanna Instruments HI 223).

### Determination of toxicity in food

A 25 g aliquot of food sample supplemented with 15 ml of gel phosphate buffer, pH 6.5, was homogenized in a filter stomacher bag for 3 minutes and incubated overnight at 4°C. Filtered part was sampled and centrifuged for 60 minutes at ca. 4000 g (refrigerated centrifuge) and supernatant fluid was divided into 2 aliquots (ca. 5 ml of each aliquot).

0.5 ml of the first aliquot of supernatant fluid was intraperitoneally (i.p.) injected into two mice (16–24 g). In order to inactivate botulinum toxin, the second aliquot was heated for 10 minutes at 100°C, cooled and then 0.5 ml was injected i.p. into a second pair of mice. All mice were periodically observed for five days, recording symptoms of botulism and death (IZSLER method PM n. 23035.002, modified from Solomon and Lilly, 2001; AOAC, 1979).

### Toxin typization

Polyvalent antitoxin type ABE and monovalent antitoxin types A, B and E were used. 0.5 ml of each antitoxin and 0.5 ml of the first aliquot of supernatant fluid were contacted for 30 minutes. The extract was then injected i.p. into 4 pairs of mice, each with a different antitoxin. All mice were periodically observed for five days, recording symptoms of botulism and death (IZSLER method PM n. 23035.002, modified from Solomon and Lilly, 2001).

### Detection of viable *C. botulinum*

A 25 g aliquot of food sample was supplemented with 25 ml of buffered peptone water (BPW) (Oxoid) and homogenized in a filter stomacher bag for 1-2 minutes at low speed. The filtered part was sampled and centrifuged for 60 minutes at ca. 4000 g (refrigerated centrifuge). Then supernatant fluid was removed and the pellet was re-suspended in 6 ml of BPW and inoculated into 3 tubes of cooked meat medium (CMM) (Oxoid). Inoculated tubes were heated for 30 minutes at 80°C and then incubated anaerobically at 30°C. These cultures were centrifuged for 60 minutes at ca 4000 g (refrigerated centrifuge) after 5 days of incubation. The pellet was Gram-stained and microscopically examined; supernatant fluid was filtered and tested for botulinum neurotoxin by mouse bioassay (IZSLER method PM n° 23035.002, modified from Solomon and Lilly, 2001).

## RESULTS

The pH and  $A_w$  values monitored in the artichoke preserve and vegetable soup were 5.7-5.72 and 0.941-0.947, respectively.

By a mouse bioassay, *C. botulinum* toxin was detected in the artichoke preserve and cream of vegetable soup, as the mice developed typical clinical signs and the i.p. injected mouse died from respiratory failure. Type-B toxin was identified in both the suspected food samples.

Culturing in CMM and microscopic examination after Gram-staining disclosed Gram + bacilli with oval and subterminal endospores. The mouse bioassay carried out using the CMM cultures confirmed the production of the B toxin in the isolates.

## DISCUSSION

Wong *et al.* (1988) reported that all the strains of *C. botulinum*, irrespective of the type, are able to grow and produce toxins at pH 5.2. The pH values found in the artichoke preserve (5.7) and cream of vegetable soup (5.72) were therefore permissive for clostridial growth and for toxin production by *C. botulinum*. In addition, the  $A_w$  values of the artichoke preserve (0.941) and veg-

etable cream (0.947) were found to be permissive for the development of endospores and for synthesis of toxins (Baird-Parker and Freame., 1967; Peck, 2002). In order to decrease the risks of foodborne botulism, it is important to take into account that the development and growth of *C. botulinum* occurs only under particular conditions. Prevention may be achieved by inhibiting spore germination, by acidification and reduction of water content and by correct sterilisation of canned food product (Sobel *et al.*, 2004).

The detection of *C. botulinum* toxin in the artichoke preserve indicates that the pasteurization method was inadequate technology especially for a non-acidified preserve. At present food industry marketing perceives that consumers want less-processed natural food with no preservatives (Zink D.L., 1997), but the manufacturing companies cannot reduce the levels of food safety using unsuitable technology. Inappropriate (mild) thermal treatment combined with long storage at room temperature could account for *C. botulinum* growth and toxin production.

Food labelling on the artichoke preserve reported a 2-year shelf-life and it did not suggest conservation at refrigeration temperatures, although the Commission Regulation (EC) on microbiological criteria for foodstuffs No. 2073/2005 requires that the instructions on labelling for the use and conservation of food are compulsory when the lack of these instructions does not allow appropriate use of the food.

Production of *C. botulinum* toxin in the vegetable soup appeared to be related to wrong behavior of the consumer and to faulty preservation of food.

In agreement with Lagendijk *et al.* (2008) it is essential to recommend that perishable food not be left more than two hours at room temperature and consumer education is needed to reduce the risk of foodborne outbreaks.

However, the Commission Regulation (EC) No. 2073/2005 recommends that food safety criteria be applied throughout the shelf-life of the products during their distribution, storage and usage. Therefore, the survey suggests that a 45-day shelf-life for the vegetable cream was not appropriate. The Rapid Alert System for Food and Feed (RASFF) was activated in both cases of food borne botulism and the entire batches of artichoke preserve and vegetable soup were with-

drawn from the Italian market and the additional samples collected during the RASFF resulted negative for *C. botulinum* toxin analyses.

Early identification and reporting of suspected botulism cases is vital in the prevention of accidental widespread outbreaks and in order to guarantee food safety it is necessary to control all the steps of the food chain and to maintain food traceability during all the stages of production.

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