Clostridium botulinum, and neurotoxigenic *Clostridia*

anses
French agency for food, environmental and occupational health & safety

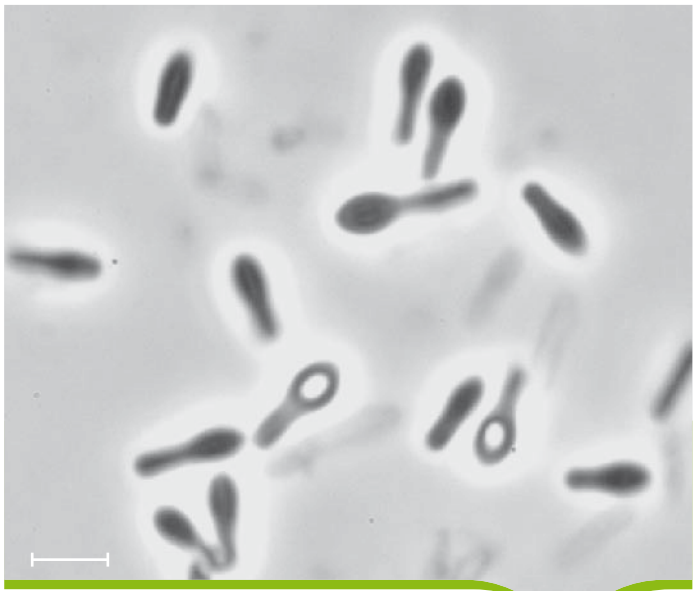
Clostridium botulinum and neurotoxigenic *Clostridia*
Family of *Clostridiaceae*

Bacterium

Characteristics and sources of *Clostridium botulinum* and neurotoxigenic *Clostridia*

Main microbial characteristics

Clostridium botulinum is a Gram-positive, strictly anaerobic, spore-forming bacillus. Strains of *C. botulinum* differ considerably by their cultural, biochemical and genetic characteristics and can be divided into four groups (Groups I to IV). Furthermore, certain atypical strains, only rarely isolated in Europe, belonging to other species of *Clostridium*, are neurotoxigenic: *C. butyricum* (type E botulinum neurotoxin) and *C. baratii* (type F botulinum neurotoxin). With only a few exceptions, each strain produces a single type of botulinum toxin. There are seven types of botulinum toxin (A to G) with different immunological properties, each of which is neutralised by a specific serum. In addition, depending on their amino acid sequences, sub-types are identified in each type of botulinum toxin (Table 1).



C. botulinum type A. © M. Popoff

Table 1. **Characteristics concerning survival, growth and toxin production of *C. botulinum***

	C. botulinum Group I (proteolytic)			*C. botulinum* Group II (non-proteolytic)			*C. botulinum* Group III (non-proteolytic)			*C. botulinum* Group IV (proteolytic)		
Toxins	A, B, F			B, E, F			C, D			G		
Sous-types de toxines	A1, A2, A3, A4, A5, B1, B2, B3, bivalent B (Ba, Bf, Ab), proteolytic F			E1, E2, E3, E6, non-proteolytic B, F			C, D, C/D, D/C			G		
Bactéries apparentées non toxinogènes	*C. sporogenes*			Pas de nom d'espèces			*C. novyi*			*C. subterminale*		
Croissance cellules végétatives	Min.	Opt.	Max.	Min.	Opt.	Max.	Min.	Opt.	Max.	Min.	Opt.	Max.
Temperature (°C)	10	35-40	48	3	18 - 25	45	15	37 - 40		/	37	/
pH	4.6	/	9.0	5.0	7.0	9.0	5.1	6.1 -6.3	9.0	4.6	7.0	/
a_w	0.94	/		0.97	/	/	0.97	/		0.94	/	/
NaCl concentration preventing growth	10			5								
Toxin production												
Min temperature (°C)	10			3			15			/		
Min a_w	0.94			0.97			0.97			0.94		
Stability and inactivation of toxins	The toxins are resistant to freezing (activity of toxins previously formed in the food not reduced by freezing) Destroyed after 10 min at 100°C or 30 min at 80°C											

Data sheet on foodborne microbial hazards
December 2010

Sources of the hazard

The reservoir for *C. botulinum*, as for the other *Clostridia* is the environment: soil, dust, marine or freshwater sediments, wastewater, slurry and, occasionally, the intestinal tracts of asymptomatic humans and animals.

Botulism is a human and animal disease but there are no reports of direct transmission from an animal infected with botulism to humans. Furthermore, no epidemiological link has been shown between outbreaks of human botulism and outbreaks of animal botulism.

Animal botulism mostly concerns birds and cattle and is most frequently due to types C or D. Birds and mink are especially sensitive to type C and cattle to type D. Type D botulism is also found amongst palmipeds. Fish in northern seas, especially the Baltic, are frequently asymptomatic carriers of *C. botulinum* E in their digestive tubes. Botulism in its clinical form is rare in pigs, but *C. botulinum* B can be found in the digestive tubes of asymptomatic pigs. Food is the principal source of contamination in animals and preventive measures consist in controlling feed contamination and the prompt disposal of carcasses.

Transmission routes

The disease is not transmitted between individuals, but is usually the result of the ingestion of contaminated food. Three forms of botulism are recognised, corresponding to the three principal routes for contamination.

Foodborne botulism is due to the ingestion of botulinum toxin preformed in a food. This is the form most frequently found in adults.

Intestinal toxaemia botulism caused by the ingestion of bacteria and/or spores of *C. botulinum*. This form has been observed in young children (0-12 months, infant botulism), following ingestion of honey or inhalation of dust contaminated by spores of *C. botulinum*. Intestinal toxaemia botulism is also observed in adults (see § Foodborne human disease).

Wound botulism is caused by inoculation of spores of *C. botulinum* in a wound. Cases of botulism occurring as an adverse side-effect have been reported following injections of botulinum toxin for therapeutic or cosmetic purposes.

Foodborne human disease

Nature of the disease (Table 2)

Botulism is characterised by symmetrical flaccid paralysis, with no effect on the sensory system. Types A, B and E botulism are the most common in humans. The severity of clinical symptoms depends on the quantity of botulinum toxin absorbed and the type of toxin, Type A botulism being the most serious with respiratory failure occurring more rapidly and more severely than with the other types of botulism.

Susceptible population groups[1]**:** any individual is susceptible to developing foodborne botulism following ingestion of the toxin preformed in food.

Infants (< 12 months old), whose intestinal flora is not fully formed or fully functional, are susceptible to intestinal toxaemia/infant botulism, by germination and multiplication of *C. botulinum* in the intestine and local production of toxin. Intestinal toxaemia botulism is also observed in adults having undergone digestive surgery or suffering from intestinal carcinomas, chronic lesions of the intestinal wall, anatomical or functional anomalies of the intestine, chronic inflammation, or after antimicrobial treatment.

Dose-response relationship[2]

Botulinum toxin is currently thought to be the most powerful poison on earth. Type A botulinum toxin is the most active. The lethal dose for an adult male human is estimated to be 100 ng – 1 µg by parenteral route and 70 µg by oral route (1 µg per kg).

The effects depend on the concentration of the toxin or the number of bacteria/spores of *C. botulinum*. The higher the quantity of toxin ingested, the faster the onset and the more severe the illness. In general, the ingestion of only a few grams of food containing botulinum toxin is sufficient to cause botulism. In a newborn child or an infant, the ingestion of from ten to a hundred spores is capable of causing intestinal toxaemia, which can represent as little as a few milligrams of food such as honey or a few particles of dust.

Epidemiology

In France, since 1991, the median number of suspect and confirmed cases declared to the National Institute for Public Health Surveillance (InVS) is 28, ranging from 8 to 44. The annual mean is 26. In the period 2007-2009, 43 cases of botulism were confirmed, 10 of which were severe forms requiring prolonged intensive care (four of these ten were infant botulism). The last two lethal cases were observed in 1997 and 2010.

Most outbreaks are associated with type B toxin. Foods involved in outbreaks of botulism in France include home-made or traditionally-made delicatessen products or preserved fruit and vegetables and, exceptionally, industrial products (two cases in 2008).

Incidence of botulism in other countries is variable and depends on several factors such as dietary habits, or the prevalence of *C. botulinum* in the environment. In the United States, infant botulism is the predominant form while in the United Kingdom wound botulism amongst drug users is a cause of concern.

(1) Susceptible population group: people with a higher than average probability of developing symptoms of the disease, or severe forms of the disease, after exposure to a foodborne hazard [definition used in ANSES data sheets].

(2) Relationship between the dose (the quantity of microbial cells ingested during a meal) and the effect on an individual.

Table 2. **Characteristics of the disease**

Mean incubation period	Main symptoms	Complication
1-10 days 1-3 days (usually)	• Digestive disorders (vomiting, diarrhoea) observed irregularly during the early stages of the disease (30-50%) • Constipation frequent in final stages (20-70%)	Mortality by respiratory failure (5-10%, up to 25%, depending on medical care)
Duration of symptoms	• Paralysis of the accommodation muscles: blurred vision, double vision, mydriasis (70-100%) • Facial paralysis: dry mouth, swallowing and elocution difficulties (80-100%)	
A few days to 8 months	• Most severe forms: paralysis of limbs (from limb weakness to paraplegia) and respiratory muscles (50-80%)	
Target population	**Formes asymptomatiques**	
Cosmopolitan, all ages	None, but possibility of attenuated forms (visual and/or swallowing disorders)	

Role of foods

Main foods to consider

Raw foodstuffs are contaminated by bacteria/spores of neurotoxigenic *Clostridium* from the environment (see § Sources of the hazard). Certain foodstuffs can be contaminated *via* spices or condiments (pepper, garlic, etc.).

Potential germination of the spores and growth of these bacteria, along with their toxinogenesis, are determined by the preparation and storage conditions of foods. The presence of botulinum toxin in low-acid manufactured foods is often due to food processing failure (cooking/sterilisation temperature or storage temperature, insufficient monitoring of pH or aw, leaky packaging). Botulinum toxin is stable in foods for long periods.

Foods at risk for consumers are low-acid preserved foods. The foods most often implicated in botulism outbreaks are home-made preserved products or traditionally-made products such as:

- mortadella, salted and dried raw ham, delicatessen products (sausages, pâté) (Type B toxin);
- canned vegetables (asparagus, green beans, carrots and carrot juice, peppers, olives à la grecque, pumpkins, etc.) and salted cured beef (Type A toxin);
- vacuum packed salted and dried fish, marinated fish, fermented fish or seal meat (Type E toxin).

Honey contaminated by spores of *C. botulinum* is the only food known to transmit infant botulism.

Inactivation treatments in industrial environments

Disinfectants	High Pressure
The spores are sensitive to most disinfectants authorised in the food processing sector, on condition that the recommended procedures for use are followed. The most active chemical agents are chlorine compounds. The spores of *C. botulinum* A, B and E are inactivated at concentrations of 4.5 ppm (m/v) of free chloride (pH 6.5).	The spores of *C. botulinum* are highly resistant to pressure. They can be inactivated by a combination of heat treatment and high-pressure treatment.
	Irradiation
	D_{10} value* for spores (Treatments for frozen foods): • D_{10} value (T°C ≤ -18) = 2.0-4.5 kGy (Group I); • D_{10}-value (T°C ≤ -18) = 1.0-2.0 kGy (Group II),

Effets de la température

The resistance to heat of *C. botulinum* varies both between and within groups. For Groups I and II, an international consensus has been established defining tables to provide an acceptable degree of safety. The proteolytic strains of Group I have the most heat-resistant spores, while the non-proteolytic strains of Group II are the most heat-sensitive.

Table 3. D times[3] and Z-values[4] for spores of *C. botulinum*

C. botulinum Group I	*C. botulinum* Group II	*C. botulinum* Group III	*C. botulinum* Group IV
$D_{121,1°C}$ = 0.21 min	$D_{80°C}$ = 0.6-1.25 min*	$D_{104°C}$ = 0.1-0.9 min*	$D_{104°C}$ = 0.8-1.12 min*
Z =10°C			

* Variable depending on strain. The spores are resistant to freezing

Monitoring in food

There are no specific criteria in European regulations for *C. botulinum* in food. There is no standardised method for the detection of *C. botulinum*. The detection of *C. botulinum* is based on screening for the toxin (*in vivo* test, ELISA, testing for enzyme activity) and revealing the presence of the bacteria by enrichment broth culture followed by detection of toxin and/or genes encoding neurotoxins (mainly by PCR). It is not possible to screen for *C. botulinum* in food routinely as strict security conditions are required for any work on the toxin.

Recommendations to operators

- Sterilization of low-acid canned products: any product with a pH equal to or greater than 4.5 must be subjected to a heat treatment guaranteeing efficient elimination of *C. botulinum* spores (equivalent sterilisation value[6] $F^{10}_{121,1} \geq 3$ min). The time-temperature combinations depend on the nature of the product and of the container.
- Compliance with the recommendations of the UK's ACMSF to prevent the development of *C. botulinum*, especially Group II (psychrotrophs), in low-acid chilled products whether or not they have undergone minimal heat treatment.
- Salt cured meats: NaCl (10%) and nitrites (150 mg/g) are the most effective additives for inhibiting the growth of *C. botulinum*. The water activity (a_w) is also a parameter to be controlled.
- Warning labels concerning the consumption of honey by infants less than 12 months old.

Domestic hygiene

Recommendations to consumers

- Hygiene regarding the preparation of foods to be canned (thorough washing of vegetables, hygiene during the slaughter of animals on farms and the preparation of meat, cleanliness of recipients or packaging).
- Carefully following manufacturers' instructions for sterilisation (temperatures/times, number of containers per steriliser). Simple boiling is not sufficient for sterilising foodstuffs.
- Deformed or swollen cans and those releasing a suspicious odour when opened must not be consumed. Vegetables contaminated by botulinum toxin do not emit any specific odour.
- For home-cured hams, it is indispensable to use the correct concentrations of salt in the brine and the correct time for brining so that the concentrations of NaCl and nitrites required to inhibit the growth of *C. botulinum* can reach the core of the ham.
- For preparations that undergo no, or insufficient, heat treatment, it is indispensable to maintain the cold chain.
- For commercial foodstuffs, instructions for cold storage and use-by dates must be observed.

(3) The D_{10} value is the dose of radiation (in kGy) required to reduce the viability of a population by 90% (1 log10 cycle) under the stated conditions.

(4) D- is the time required to divide by 10 the initial population of a microbiological hazard.

(5) Z is the variation in temperature (°C) corresponding to variation by a factor of 10 of the decimal reduction time.

(6) F_0: time in minutes of a heat treatment applied at the core of a product at the reference temperature of 121.1°C.

References and links

General references

- ACMSF (Advisory Committee on the Microbiological Safety of Food, UK) (1992). Report on vacuum packaging and associated processes. HMSO. London (UK).
- AFSSA (2002). *Rapport sur le botulisme d'origine aviaire et bovine* [Report on avian and bovine botulism].
- EFSA (2005). Opinion of the BIOHAZ panel related to *Clostridium* spp in foodstuffs. The EFSA Journal, 199: 1-65.
- ICMSF (International Commission on Microbiological Specifications for Foods) (1996). *Clostridium botulinum*. In: Roberts T. A, Baird Parker A. C., Tompkin R. B (Eds.) Micro-organisms in Foods 5: Microbiological Specifications of Food Pathogens, pp. 66-111. Blackie Academic and Professional, London.
- Peck M. W. (2009). Biology and genomic analysis of *Clostridium botulinum*. Advances in Microbial Physiology 2009, 55: 183-265.
- Popoff M. R., Carlier J.-P., Poulain B. (2009). Botulisme, In: EMC (Ed.) Maladies Infectieuses, pp. 1-17. Elsevier Masson SAS, Paris.
- Popoff, M. R. (1995). Ecology of neurotoxigenic strains of *Clostridia*. Current Topics in Microbiology and Immunology 195, 1-29.
- Smith, J.P. (1993). *Clostridium botulinum*-Ecology and control in foods. Haushchild H. W. and Dodds K.M. (Eds). Marcel Dekker, New York.
- Szabo E. A. and Gibson A. M. (2003). *Clostridium botulinum*, In: Hocking A. D. (Ed.) Foodborne Microorganisms of Public Health Significance. 6th edition, pp. 505-542. Australian Institute of Food Science and Technology Inc, NSW Branch.

Useful links

- National Reference Centre for Anaerobic Bacteria and Botulism, Institut Pasteur: http://www.pasteur.fr/sante/clre/cadrecnr/anaer-index.html
- http://www.invs.sante.fr/publications/guides_biotox/guide_botulisme.html
- http://www.who.int/mediacentre/factsheets/en/
- http://www.ctcpa.eu