

# Infant botulism

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**Summary.** Infant botulism is a rare disease that affects infant less than 12 months of age. The illness results from absorption of botulinum toxin produced *in situ* by neurotoxicogenic clostridia that can temporarily colonize the intestinal tract of infants. To date, all inhabited continents except Africa have reported cases of infant botulism. Recognition of cases seem directly related to physician awareness and clinical suspicion. This review summarizes microbiological, clinical and epidemiological features of infant botulism.

*Key words:* infant botulism, botulism, botulinum toxin, botulinum antitoxin, rare diseases.

**Riassunto** (*Botulismo infantile*). Il botulismo infantile è una malattia rara che colpisce i neonati al di sotto di 12 mesi di età. La sindrome è conseguente l'assorbimento di tossina botulinica prodotta *in situ* da clostridi neurotossigeni che possono colonizzare temporaneamente il tratto intestinale dei neonati. Casi di botulismo infantile sono stati riportati in tutti i continenti eccetto l'Africa. Il riconoscimento dei casi sembra direttamente correlato alla informazione ed al sospetto clinico da parte del medico. Questa rassegna sintetizza gli aspetti microbiologici, clinici ed epidemiologici del botulismo infantile.

*Parole chiave:* botulismo infantile, botulismo, tossina botulinica, antitossina botulinica, malattie rare.

## INTRODUCTION

Botulism is a neuroparalytic disease that affects humans and other animal species, and caused by neurotoxins produced by organisms of the genus *Clostridium*. Botulinum neurotoxin (BoNT) blocks evoked quantal acetylcholine release from motor ending at all peripheral cholinergic synapses, producing a profound but transient muscle flaccid paralysis [1].

Four forms of naturally occurring botulism are to date recognized:

- 1) *foodborne botulism*, results from the ingestion of food containing preformed toxin;
- 2) *wound botulism*, follows growth and toxin production *in vivo* at the site of a wound, particularly infections in intravenous drug users;
- 3) *infant botulism*, caused by the absorption of the BoNT produced by neurotoxicogenic clostridia that temporarily colonize the intestinal tract of infants under one year of age;
- 4) *adult intestinal colonization*, an infant-type botulism that very rarely affects adults and older children.

The two latter forms are also referred as intestinal toxemia botulism [2].

Other forms of human botulism are recently considered, as inhalation botulism (man-made form) resulting from an accidental or a deliberate release of aerosolized BoNT, and iatrogenic botulism as a complication in the treatment with BoNT for therapeutic or cosmetic use [3].

Infant botulism is a "rare disease", recognized as a distinct clinical entity in 1976 in USA [4, 5]. Infants ingest botulinum spores that germinate in the intestinal tract and produce toxin in it. To date infant botulism has been documented in 26 countries representing 4 continents. USA, Argentina, Australia, Italy, Canada, and Japan, reported the largest number of cases in this order.

The etiological agent of the botulism is the BoNT produced by the *Clostridium botulinum*, but particularly for infant botulism, unusual strains of *Clostridium baratii* and *Clostridium butyricum* producing BoNT have also been recognized [6].

## INFECTIOUS AGENTS

BoNT-producing clostridia are gram-positive anaerobe, ubiquitous in nature and commonly present in soil, household dust, and agricultural products mainly as spores.

Currently, the genetically and phenotypically distinct clostridial species, *C. botulinum*, *C. baratii*, and *C. butyricum* are all recognized as causative agents of botulism. The taxonomic status of BoNT-producing clostridia, based on phenotype, was reviewed by Hatheway [7]. They are divided into 6 phylogenetically and phenotypically distinct groups: 4 *C. botulinum* groups, *C. butyricum* and *C. baratii*.

The BoNTs produced by various organisms can be differentiated serologically by specific neutraliza-

tion into seven toxin types: A, B, C, D, E, F and G. Organism producing BoNT type G was firstly isolated from soil in Argentina [8] and subsequently in clinical samples from humans died for SIDS in Switzerland [9].

For *C. botulinum* producing BoNT type G (group 4), the new specie of *Clostridium argentinense* was also proposed [10].

Regardless of serological type, the BoNTs are structurally and pharmacologically similar, causing the typical flaccid paralysis of botulism in susceptible animal species.

Human cases are caused mainly by BoNT type A, B, E and rarely F. Only sporadic and uncertain cases by type C and type D have been reported [11]. Most strains of *C. botulinum* produce a single toxin, but rare strains producing two types of toxin are also isolated. In particular, type Ba, Ab, Bf and Af (capital letter denotes predominant toxin type) [7].

Infant botulism is related to *C. botulinum* type A, B, E, Ab, Ba, Bf and in only one case to *C. botulinum* type C [12]. After 1985, novel neurotoxicogenic strains of *C. baratii* and *C. butyricum* that cause clinical picture of infant botulism and adult intestinal botulism were isolated, and considered in the classification of neurotoxicogenic clostridia [13, 14]. These strains were isolated in America, Europe and Asia.

Regarding physiological characteristics, the strains isolated from cases of intestinal colonization, grow optimally at or close to body temperature. An exception is the recent isolation of a strain of psychrotrophic *C. botulinum* type E in a case of infant botulism in USA [15]. This strain should have a competitive disadvantage in the human gut.

From 1980 to date, *C. baratii* producing BoNT type F has been isolated in 5 infant botulism cases in USA [16] and one case of infant botulism in Hungary [17]. All cases of botulism by type F toxin reported in USA to CDC from 1979 to 2005, were intestinal botulism cases in infants or adults due to *C. baratii*, and the unique case in which a food was implicated, was also suspected due to an intestinal colonization [16, 18, 19].

The first infant botulism case diagnosed in Italy in 1984, was related to a novel strain of *C. butyricum* producing BoNT type E [14]. From 1984 to 2001, other three cases of infant botulism and two of intestinal colonization in young people were related to this strain in Italy [20-23]. Infant botulism by *C. butyricum* type E was also described in Japan in 2005 [24] and in USA in 2006 [25]. Neurotoxicogenic *C. butyricum* was also isolated in three outbreaks of food-borne botulism in China [26], India [27] and Italy [28]. In the Chinese outbreak, the atypically long period of incubation suggests that some patients may have had botulism from intestinal colonization [23]. Neurotoxicogenic *C. butyricum* was cultured from the soil obtained around the home of the patients with botulism, in a region of China [29]. On the contrary, in Italy, despite an extensive testing of environmental and food samples during the cases investigation,

it was not possible to find any clostridia producing type E toxin.

Considering that neurotoxicogenic *C. baratii* has never been isolated from the environment, as *C. butyricum* in Italy, the human gut should be considered as an "indicator" for the environmental distribution of these organisms.

## SOURCE OF SPORES

The geographical distribution of BoNT-producing clostridia in terrestrial and aquatic environments has been extensively studied in continental United States, Alaska and Hawaii, Canada, Europe, Asia, Central and South America, Africa, Indonesia, Australia and New Zealand [30, 31]. The incidence and toxin types for infant botulism reflect density and geography of the spores in the environment.

Spores of BoNT-producing clostridia are widespread in the environment and can be found in the dust inside or outside the house, and in the soil.

Infants are repeatedly exposed and inhale environmental spores, carried by microscopic dust particles.

Disturbing the soil, as in a building site, soil disruption from farming, or staying in a windy site can constitute risk factors [32]. According to USA epidemiological data, in the regions where fathers' occupations frequently involved contact with soil, *C. botulinum* of the same toxin type was recovered in the infant feces and from their work shoes and worksite [33]. In other countries, the majority of cases was in inner cities and probably related to dust produced by works for home renovation [21, 33].

As California experience, infant botulism is diagnosed in every month of the year, with a peak admission during the winter months. The evidence of increasing admission in the Los Angeles area after the Northridge earthquake in January 1994, was also correlated to the possible release of spores into the air [34]. On the contrary, seasonal distribution cases in spring and summer were reported in Utah. Dry climatic conditions, windy environment, and residence in areas with new homes, building or works in the home are ideal factors for propagation and dust-borne dispersion of spores [32, 35].

According to the case reports, honey is the only food proven to be associated with infant botulism [36]. Contaminated honey has been associated with a small number of cases in different countries. The levels of *C. botulinum* spores, in honey associated with infant botulism, were approximately  $10^4$  per kg [37, 38]. According to several baseline surveys, spores of *C. botulinum* have been detected in honey samples collected at retail with the prevalence varying from 2% to 24% [39]. Usually the level was very low, so a statistically-based sampling plan would require huge numbers of tests to be made.

In USA, the close correlation between infant botulism and honey consumption is recently controversial, since the cases with history of honey exposure

have decreased during 30 years. This significant decline could be correlated to an enhanced ability of physicians to recognize the illness in the absence of a history of honey exposure [12].

A single case of infant botulism with a possible link to formula milk powder, was also reported in UK [40, 41].

In Argentina, where outside of USA most cases of infant botulism were reported, herbs infusion (*e.g.* chamomile, anise, mint, and linden) are commonly given to infants at home to treat intestinal colic or simply as beverage. This practice is also common in other mediterranean european countries, as Italy and Spain.

A survey to evaluate the presence of spores of BoNT-producing clostridia was performed in Argentina on samples of chamomile (*Matricaria* spp.) [42] and linden flowers (*Tilia* spp.) [Maria I Bianco, Carolina Luquez, Rafael A. Fernandez, written communication, 2008]. The spore load, calculated by MPN method, was 0.3 spore/g in chamomile and linden flowers. Although this value is smaller than that detected in honey, infants may be more frequently given herb infusions than honey. So, repetitive ingestion of low number of spores could accumulate the minimum infective dose necessary for infant botulism and should be considered as a risk factor for infant botulism like honey.

Recently two cases of infant botulism, diagnosed in Spain, were suspected to be correlated to an infusion of chamomile. A national alert was performed and samples of the commercial product were recalled from the market [43].

#### PATHOGENESIS AND PATHOPHYSIOLOGY

BoNT is the etiological agent that causes all different forms of botulism.

In the effort to explain different steps of action, oral poisoning can be viewed as a model. Before their release *via* bacterial autolysis, BoNTs are present as progenitor toxins, composed of a complex that contains a 150-kDa relatively inactive single-chain protein, and a variable number of nontoxic proteins including a single nonhemagglutinin (NTNH) and three hemagglutinins (HA). The complex pass through the stomach and dissociate from the progenitor toxins in its site of absorption, which is the upper small intestine. The single chain polypeptides become fully active by a specific proteolytic cleavage, carried out by bacterial and tissue proteinases, that can generate di-chain toxins formed by two subunits; a 100-kDa heavy chain (H) and a 50-kDa light chain (L), linked by a single disulphide bond.

In the absorption step, the heavy chain of the toxin binds to the receptor on the apical surface of gut epithelial cells [44]. The toxin is then carried across the cells by active transcytosis with transport endosomes, and released into the general circulation.

A small fraction of the active BoNT escapes the gastrointestinal system through intestinal wall and, by the general circulation (lymph and blood), reaches all peripheral cholinergic nerve endings, clinically the most important of which is the neuromuscular junction.

In terms of the mechanism of action, the toxin must proceed through a complex sequence of events [45].

BoNTs are metalloproteinase and their structural organization and mode of action are recently well established in view of the therapeutic use of the toxins.

The H-chain of toxin is responsible for the specific presynaptic binding through specific receptors, leading to the subsequent internalization of the L-chain by translocation into the terminal nerve and terminal cytosol. The L-chain acts as zinc-dependent endopeptidase and it is responsible for the intracellular activity. The L-chain binds irreversibly to unmyelinated terminal nerve endings and it blocks the release of neurotransmitter acetylcholine, producing flaccid paralysis. Adrenergic systems are also affected, but the dose required to inhibit the release of noradrenaline is very higher than that needed to inhibit the release of acetylcholine [46].

Unlike ingestion of preformed toxin in the foodborne botulism, intestinal toxemia botulism results from toxin produced by spores germinate in the anaerobic environment of the colon. The spores can reach the intestinal tract by inhalation, or via food. The rarity of the disease, attests to the essential role of host factors, as intestinal microbiota, in the developing clinical illness in the consequence of spores ingestion.

Although different species of clostridia are at present recognized as agents of the intestinal colonization, all the studies to establish the intestinal pathophysiology of infant botulism are carried out considering *C. botulinum* specie.

Susceptibility to intestinal colonization by *C. botulinum* spores was demonstrated using a mouse model system [47]. In infant mice colonization peaks between 8 and 11 days of age, while the intestinal microbiota of adult animals normally prevents it [48].

In chickens and mice, the cecum has been identified as the initial site of spore germination [49]. The possibility to study an infant, died due to infant botulism in 1987 permitted bacteriologic studies on the entire length of the gastrointestinal tract [50]. *C. botulinum* and its toxin were found in the content of the cecum, transverse colon and recto-sigmoid colon, but were not found at higher levels in the intestinal tract. It was hypothesized that toxin production in the cecum might initially paralyze the ileocecal valve permitting the reflux of toxin into small intestine, where absorption is more efficient [51].

Soon after its recognition in 1976, epidemiological, clinical and laboratory aspect of infant botulism was well described [52].

Age is the only recognized predisposing factor for infant botulism, indeed, most of the reported cases have occurred in the patients less than 6 months old and all in the infants less than 1 year old. Infant botulism appears to be related to a transient failure of the intestinal microbiota that competitively inhibit outgrowth of spores.

By epidemiological considerations, the susceptibility of humans to *C. botulinum* colonization is at its peak between 2 and 4 months of age. By the end of the first week of life, the human intestinal microbiota is complex and the type of microorganisms present, depends on the type of feeding received [53].

Diet is probably the most important factor that might influence the composition of the normal flora, but a clear cause-effect relationship between the type of diet (breast-feeding, formula-feeding, consumption of solid foods) and infant botulism cannot be drawn. Studies on intestinal microbiota in neonates were recently reviewed [54]. The formula-fed infants show a more putrefactive intestinal flora, including predominately *Bacteroides* sp. that can inhibit *in vitro* *C. botulinum*. The fermentative flora of breast-fed infants, with predominant presence of *Bifidobacterium* sp., relatively high level of iron-free lactoferrin and lower pH, can also inhibit many microorganisms including *C. botulinum*. Infant botulism cases involved either breast-fed or formula-fed infants. Onset of the disease occurs at a significantly younger age with a more rapid and severe course in formula-fed infants, probably for lack of immune factors (SIgA, lactoferrin, lysozyme) contained in human milk. For breast-feeding the period of greatest risk appears to be at weaning when the introduction of first non-human milk substances can create a dramatic perturbation of intestinal microbiota.

It may be during this transitional period that risk of colonization by *C. botulinum* exists. Moreover, the introduction of solid foods, as powdered baby foods, can allow constipation that may create, decreasing intestinal motility, a favorable condition for spore germination, outgrowth and toxin production [55, 56].

The decreased intestinal motility (<1 bowel movement/day) of physiological nature is also considered as an additional predisposing factor for the intestinal colonization of the ingested spores.

Regarding neurotoxicogenic strains of *C. baratii* and *C. butyricum*, no experiments in animal model or human are performed to establish their mode of multiplication in the gut.

In the patients with infant botulism and adult intestinal colonization by *C. butyricum* type E, the presence of an intussusceptions and the Meckel's diverticulum were also considered as a probable favorable niche for colonization of this microorganism [22]. The Meckel's diverticulum is a blind sac, conducive to anaerobic growth, containing a specific type of heterotypic tissue and can serve as a lead point for intussusceptions [23]. This condition has not yet associated with botulism due to *C. botulinum*.

**CLINICAL MANIFESTATIONS**

All forms of botulism result in a pure motor paralysis, because only peripheral cholinergic synapses are affected and clinical manifestations of disease are due to progressive neuromuscular blockade. The classical clinical manifestation of botulism consist of (i) a symmetrical, descending, flaccid paralysis, firstly involving bulbar musculature, (ii) a clear sensorium, and (iii) the absence of fever [2]. Sensory nerves are not affected. As head, face, and throat musculature receive more blood flow and are involved in a more fine and complex functions than trunk musculature, bulbar palsies manifest early in the clinical course of the disease. In infants patients initial symptoms and signs prior to hospitalization are generally noticed by parents (Table 1/A). Physical examination of infant at the time of hospitalization usually reports typical manifestation of descending paralysis (Table 1/B).

Presynaptic autonomic nerves are also affected with autonomic signs (Table 1/C).

Typically, the first evidence of illness is constipation (defined as 3 or more days without bowel movement in an infant previously defecating at least every other day), followed by slow progressive course, culminating in a general muscular weakness rather than paralysis. Peak of paresis and paralysis generally occur within 1 or 2 weeks. Constipation is both, the most frequently initial manifestation of the disease, and a risk factor for infant botulism, but its role is unclear. It may reflect gastrointestinal paralysis and

**Table 1 | Clinical manifestations of infant botulism**

A) Initial manifestations generally referred by parents	B) Evident clinical manifestation of descending paralysis	C) Signs of autonomic nerves paralysis
Constipation	Ptosis	Decreased tearing and salivation
Apparent sleepiness	Mydriasis	Bladder atony
Poor feeding	Diminished gag and suck	Intestinal dismobility
Expressionless face	Loss of head control	Fluctuating blood pressure
Weak or high pitched cry	Loose protective reflexes of airways	Fluctuating heart rate
Drooling	Hypotonia	
	Respiratory paralysis	

on the other hand, it may play a role in decreasing intestinal mobility, permitting favorable conditions for germination of spores and production of toxin.

Typically, infants maintain normal alertness and responsiveness to the environment and a characteristic dichotomy between the normal physical and abnormal neurological findings generally occurs. Secondary complications of hypoxemia, dehydration or incipient respiratory failure can result in an altered mental status.

The incubation period for infant botulism was thought to be at least 3 days [57].

The recent infant botulism case, in which severe hypotonia was manifested in just a 38 hr old neonate, demonstrated that a very rapid colonization and toxin production of the microorganism is possible [16]. This is consistent with a mouse model that demonstrated less than 1 day between ingestion of spores and “*in vivo*” toxinogenesis [47].

In severe cases of infant botulism, respiratory difficulties begin as a late sign of disease, quickly leading to respiratory arrest.

A link between the fulminant type of infant botulism and sudden infant death syndrome (SIDS) was hypothesized although death is not a direct action of the BoNT itself [58]. In California [58], Switzerland [59], Italy [60], Germany [61], and Finland [62] *C. botulinum* organisms were detected in necroscopy specimens, from SIDS cases. On the contrary, in intestinal specimens from 248 SIDS cases in Australia, no positive samples have been found [63]. Probably a small number of SIDS cases can be related to a fulminant form of infant botulism [57].

Moreover, different serotypes of toxin cause neuromuscular paralysis of significantly different duration [64, 65]. Global data on infant botulism report that young infants, formula-fed infants, and those with type A toxin, have more rapid and severe disease [6], and pharmacokinetic studies suggest that the neuromuscular recovery from type E and F may occur more quickly than from type A and B [64].

In an extensive study carried out at the CDC on 336 patients, suspected to be affected by infant botulism [66], the presence of toxin was demonstrated in 9/67 (13.4%) of available serum samples but with difference in 8/22 (36.4%) of the type A cases and only in 1/43 (2.3%) of the type B cases. The possible reason for this difference might be the greater absorbability of type A into the bloodstream or more rapid disappearance of type B from the circulation.

The syndrome of infant botulism is typically a monophasic disease, with a progression of signs during the first 1-2 weeks and the stabilization for 2-3 weeks, before recovery starts.

In the classical course of the disease, most patients fully recover.

Recovery in botulism occurs through regeneration of terminal and sub terminal un-myelinated nerve twigs. Investigations in animal model demonstrated that injection of BoNT type A produced a remodeling of the motor endings characterized

by profuse nerve terminal, increasing in number, length and complexity during muscle paralysis that continues for a long period, even after the recovery of neuromuscular transmission. Once neuromuscular transmission is restored, movement resumes. Without complications, no long term consequences and lasting neuromuscular effects of infant botulism have been generally described. The few reported sequelae in infants who have had infant botulism were correlated to very serious cases in which respiratory arrest resulted in hypoxic brain injury [67].

The Japanese case due to *C. butyricum* type E shown motor development delayed, and he walked unsteadily 12 months after discharge [24]. Because cases of this type of infant botulism are infrequent, it is not possible to definitely attribute this clinical characteristic to the uncommon agent of the disease.

Poor feeding after some months, probably correlate to a sense of repletion, in an Ab case in Italy was also reported.

Recently, long-term outcomes, describing 217 adult foodborne botulism cases in the Republic of Georgia, are reported [68]. Several years after acute disease, individuals who had had botulism are considered in this study and substantially reported a significant health, functional, and psychosocial limitations. They also reported generalized weakness, dizziness, dry mouth, difficulty in lifting things, and difficulty in breathing.

The reason of lack of reporting of sequelae in infants, may be due to the difficulty to check signs as weakness and fatigue in infants, or absence of them may be correlated with the amount of BoNT absorbed from the gut, and variation in the regenerative capacity of adult and infantile motor nerve endings [2].

Regarding relapse of infant botulism, literature data report only few cases. In California no child, diagnosed as infant botulism, had experience a second episode of illness. The few cases firstly reported as relapses have been found, in retrospect to be caused by a premature discharge or the onset of a complication [6].

Most cases of relapses are reported in the Children's Hospital of Philadelphia. The 5% of 63 confirmed infant botulism cases had a recurrence of characteristic signs and symptoms [69, 70].

In Italy, 2 of 29 patients were readmitted to the hospital after two and 80 days [21] respectively, and suffered clinical relapse after an initial full recovery. These patients were admitted in the same hospital, in 1998 and 2001, suffered from botulism by *C. butyricum* type E, and one of them suffered also from *Clostridium difficile* colitis [71]. For these cases, only after some years this biphasic course was referred to the Centro Nazionale di Riferimento per il Botulismo (CNRB).

The real interpretation of the relapse of infant botulism is not established. Different potential mechanisms were examined; a re-infection with botulinum spores, impairment of immunological

response to toxin, new infection by another strain of neurotoxic microorganism [69]. These all hypotheses seem unlikely and there are no historical, clinical or electrophysiological predictors of relapse. Premature discharge appears to be more related to a second presentation, at least in the cases with few days of interval. Finally, decision of patient discharge should be carefully based on the clinical features and should be taken when gag, suck, and swallow are sufficiently strong to ensure adequacy of oral intake and to protect the airway against accidental aspiration. The check of these parameters should be performed not only in the morning, but during the day, particularly in the evening, when the signs of weakness are more evident.

The repetition of signs is also important. The test to verify dilatation of pupil should be repeated for at least two minutes, because the pupil can only initially react. Discharge usually followed soon after the demonstrated ability to feed orally, but few patients were discharged with nasogastric tube.

Standardized criteria for discharge were also determined [72]: no further need for inpatient care for infant botulism or its complications, no need for mechanical ventilation or supplemental oxygen for at least three days, no worsening of paralysis in the previous three days and a demonstrated improvement in motor and bulbar function, and three days of intake by tube feeding of 25 percent or less of maintenance volume and calories, with the remainder consumed by mouth.

### FOLLOW-UP

After resolution of neurological symptomatology, the patient can excrete neurotoxic clostridia and toxin in the feces for weeks or months. The presence of *C. botulinum* and toxin in fecal samples of a girl affected by intestinal colonization of *C. botulinum* type Ab on hospital day 122, demonstrated the necessity of follow up for botulism patients for at least one year after the illness, because the colonized *C. botulinum* might continuously produce the toxin or the spores [73]. This occurrence in infant botulism can constitute a risk and preclude close contact between recuperating patients and healthy infants [6].

### COMPLICATIONS

A wide variety of complications were noted during the hospitalization. These can be classified as general complications or due to the intubation [74]. The general complications include respiratory arrest with resultant hypoxic encephalopathy and irreversible brain damage or death [32, 67], cardiac arrest [75], syndrome of inappropriate secretion of antidiuretic hormone [76] with hyponatremia, serum hyposmolality and urinary hyperosmolality, acute otitis media related to Eustachian tube dysfunction or to the presence of nasogastric tube, urinary tract infection owing to indwelling bladder catheter,

septicemia associated with intravascular catheters, autonomic instability, and pneumonia. Respiratory complications reported in intubated patients [74] are recurrent atelectasis, tracheitis, tracheal granuloma plugged endotracheal tube, unintended extubation, and subglottic stenosis tracheomalacia.

The Italian cases due to *C. butyricum* type E [21] manifested with acute gastroenteritis probably related to type E toxin [77] or *C. difficile* co-infection [71]. In one case *C. difficile* was found to be the cause of gastroenteritis. This prompted us to hypothesize that it had been responsible for this symptomatology in all the cases, particularly in light of the fact that, especially in newborns, *C. difficile* can exist in the intestine without causing symptoms. This hypothesis is consistent with the data from USA, where the gastroenteric symptomatology in several cases of infant botulism was attributed to *C. difficile*, even though the patients were infected with *C. botulinum* [71, 78-80].

Acquired *C. difficile* colitis owing to colonic stasis with manifestations of necrotizing enterocolitis and toxic megacolon, may occur more frequently than is generally reported.

The use of broad-spectrum or clostridiocidal antibiotics to treat co-infections or for prophylactic purpose was also reported as possible cause of worsening of paralysis [21, 78].

### DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Infant botulism displays a spectrum in its clinical severity, and may be difficult to recognize in its early stage [2]. A high index of suspicion and early diagnosis are essential for a prompt intervention and for the optimal management.

Early consideration of infant botulism in the differential diagnosis of the hypotonic infant will facilitate rapid diagnosis, minimize complications, guide supportive care, and allows the possible treatment with botulism immunoglobulin. Even today, suspected sepsis remains the most frequent misdiagnosis at the hospital admission in USA [2]. A careful history and neurological examination remain the best bases for distinguishing infant botulism from its clinical mimics. Laboratory tests of blood and cerebrospinal fluid are not revealing.

The presence of multiple cranial nerve palsies is an essential part of identifying infant botulism, and in this context, a feeble cry, poor suck, weak gag, difficulty in swallowing (drooling), and expressionless face should be viewed to be bulbar in origin. Ptosis may not be evident unless the patient is held in a sitting position (often requiring head support because of neck weakness), while disconjugate gaze and fatigability of the pupillary light reflex may require sustained examination [2]. Fatigability with repetitive muscle activity (*e.g.*, feeding, breathing, pupillary constriction), is the clinical hallmark of botulism.

For mydriasis, repetition of the test for two minutes is essential, because initially the pupil can react and only subsequently it is possible to appreciate the paralysis of the muscle. Absence of fever and alert senses are also distinctive in infant botulism.

Constipation is frequently the first manifestation of infant botulism, but absence of constipation does not exclude the disease suspicion in the infant presenting with hypotonia. In patients who normally have a bowel movement every few days, the parents may not notice the change rapidly, so constipation may not be early recognized [81]. In other cases, the initial diarrhea and gastroenteric symptomatology caused by *C. difficile* can confuse the clinical picture [21, 79].

Additionally, electromyography (EMG) studies can support an early diagnosis. EMG shows in most laboratory-confirmed infant botulism cases a characteristic, but not diagnostic pattern of brief-duration, small-amplitude, overly abundant motor-unit action potentials termed as BSAPs [82]. However, EMG may support the diagnosis of infant botulism if positive, but absence of BSAPs does not exclude the possibility [56]. In some cases negative EMG at the first examination and consistent with the diagnosis of infant botulism only after several days were reported [32, 74]. A standardized EMG was also proposed [83]. Diagnostic triad for infant botulism consists of findings of (i) low compound muscle action potential amplitude in combination with (ii) tetanic facilitation or post-tetanic facilitation and (iii) absence of post-tetanic exhaustion. The results are displayed only in hypermagnesemia. It is also suggested that the single-fiber EMG is more sensitive and specific neurophysiological test as compared to conventional EMG for evaluating neuromuscular transmission, for the botulism diagnosis [84]. However, it can be considered that EMG is invasive, expensive and painful [2], and in some confirmed cases did not exhibit the characteristic features demonstrating the “pitfall” in the diagnosis. It’s very important to consider also the atypical cases described in literature [56].

Catastrophic presentation may also confuse the typical picture and delay diagnosis of infant botulism [78]. Apparently in normal EMG, no constipation and atypical patient’s age does not exclude the possibility of *C. botulinum* infection. The differential diagnosis includes sepsis, intoxication, dehydration, electrolyte imbalance, encephalitis, myasthenia gravis, spinal muscular atrophy (SMA), and polyneuropathies such as Guillain-Barré syndrome, but these different diagnostic possibilities can be discarded on clinical grounds [85]. SMA type I and metabolic disorders are the two most common diagnoses that mimic infant botulism [86]. Patients with SMA type I have a longer history of generalized weakness than do patients with infant botulism, in whom the weakness is subacute to acute in onset. Also, patients with infant botulism typically have ophthalmoplegia and decreased anal sphincter tone, whereas SMA type I typically spares the extraocular

muscles and sphincters. Metabolic disorders are best diagnosed by the appropriate laboratory studies.

Although cranial nerve palsies occur in both infant botulism and in the Miller Fisher variant of Guillain-Barré syndrome, these diagnoses may be distinguished by CSF analysis, nerve conduction studies, and EMG. The age of the patient may also aid in differentiation; 95% of laboratory-confirmed cases of infant botulism occur in the patients who are less than 6 months old, whereas Guillain-Barré syndrome typically occurs in older patients.

Infant botulism should be considered in the differential diagnosis of any infant less than 1 year of age, with acute hypotonia.

Therefore, confirmation of the clinical diagnosis requires demonstration of BoNT and/or BoNT-producing clostridia in the fecal specimens from the patient. Moreover, the persistence of BoNT and/or the neurotoxicogenic microorganism in the feces of affected infants is consistent with intestinal colonization and toxemia, typical of infant botulism [87].

A definitive diagnosis of infant botulism can be made with laboratory confirmation in suspected cases.

## LABORATORY CONFIRMATION

Laboratory criteria for diagnosis of infant botulism cases include the detection of BoNT in stool or serum, or the isolation of *C. botulinum* from stool [88]. More recently, other BoNT-producing clostridia, such as *C. butyricum* and *C. baratii*, are considered in the criteria for the laboratory diagnosis [19, 21].

The difficulties in obtaining definitive specimens is a re-occurring problem in laboratory diagnosis of botulism throughout the world, particularly in infant cases.

Passed stool is the preferred specimen for culture and BoNT investigation, but in most cases due to the patient constipation, an enema with sterile, non-bacteriostatic water may be required for adequate sample collection. A fecal swab is sufficient for organism detection however an enema sample should be obtained for the BoNT investigation.

Detection of BoNTs and BoNT-producing clostridia in biological, environmental, and food samples are based on microbiological methods that includes the mouse bioassay for BoNT identification. The standard method [89] for organism detection, based on selection of lipase-positive colonies, is partially modified considering that all neurotoxicogenic clostridia, different from *C. botulinum* (*C. baratii*, *C. butyricum* and *C. argentinense*), are lipase negative. In the isolation step of the neurotoxicogenic strain on egg yolk agar, lipase-negative colonies, in addition to lipase-positive colonies, have to be considered.

BoNT detection requires only 24-48 h, while the organism identification needs at least 5 days.

Recently, a conventional multiplex PCR [90] and a Real Time PCR [91] for the detection of BoNT type A, B, E, and F -producing clostridia has been developed. These methods are more rapid and reduce the

use of animals. On the other hand, the use of PCR is confounded by extensive sharing of unexpressed toxin genes across the species. Some reference centers, such as the CNRB in Italy, utilize the standard method that includes the mouse test, together with PCR methods, in laboratory confirmation of diagnosis.

Regarding the results of laboratory investigations, different data were reported.

In USA the CDC has reported analysis results of serum and feces in 336 patients [65]. Research of *C. botulinum* was positive in 113 of 336 (33.6%) fecal samples of confirmed cases. The testing of BoNTs was positive in 98 of 111 (88.3%) fecal samples, and only in 9 of 67 (13.4%) serum samples.

In Argentina [92] in 146 cases of infant botulism, *C. botulinum* was isolated in 100% of fecal samples and all strains belonged to type A. BoNT type A was detected in 146 (100%) of fecal samples and in the serum from 29 (63.0%) of 46 patients.

In Italy in 29 cases of infant botulism, neurotoxic clostridia were detected in 29 (100%) fecal samples of confirmed cases (Table 3). In 4 cases, *C. butyricum* producing type E toxin was isolated. BoNT was detected in 2 of 12 (16.6%) serum samples and in 14 of 19 (73.7%) fecal samples (data from CNRB).

Result of toxin detection in serum is frequently negative and not conclusive, because of low levels of circulating toxin. While a positive serum test may help the diagnosis, a negative serum test does not exclude the possibility of infant botulism.

Presence of *C. botulinum* in stool of healthy control infants and in control infants with neurological but not-botulism disease shows the possibility of "asymptomatic carriers" of the organism [32]. This occurrence suggests that a diagnosis of infant botulism must be based on historical documentation and physical confirmation of progressive bulbar paralysis, with a subsequent complete resolution of symptoms and findings over a period of several months.

## TREATMENT

Until 2003, the treatment for infant botulism was mainly supportive, consisting of respiratory, nutritional and good nursing care.

Intubation should be done prophylactically [2]. Intubation for the purpose of airway protection should also be considered also when a loss of marked impairment of the ability to gag, cough, and swallow is observed that indicate a loss of reserve muscle strength [74]. In fact, wait for more evidence of apnea, hypercarbia and hypoxemia may lead to respiratory arrest with resultant hypoxic encephalopathy. It is important to consider that neuromuscular junction has a large margin of safety, so respiratory function may not be impaired until more than 90-95% of the receptors of diaphragmatic muscle are blocked.

The need of nutritional support can require nasogastric tube. This also assists in the resumption of peristalsis that can also support the elimination of

neurotoxic microorganism from colonic flora. Intravenous feeding is discouraged, because of the risk of infection [2]. Antibiotic treatment is not recommended for infant botulism and will not affect the course of illness. Clostridiocidal ones may lyse vegetative cells of BoNT-producing clostridia increasing the amount of free toxin in the constipated bowel [2]. Moreover, broad spectrum antibiotics generally used to treat suspected sepsis may alter the intestinal microecology, by eliminating the normal flora and indirectly permit overgrowth by toxigenic agent [93]. Aminoglycosides may potentiate neuromuscular weakness and a nondepolarizing type of neuromuscular block [94].

Clinical experience also supported this improvement of paralysis after the use of antibiotics for suspected sepsis, to treat concomitant infections or as postsurgical therapy [2, 95, 96].

Antibiotic use in infants should be reserved for the treatment of secondary infection, preferring non-clostridiocidal type.

Therapeutic enemas, intended to reduce the amount of intestinal toxin, are unlikely to be effective, because the presence of organism is equivalent in number ( $10^8/g$ ) throughout the length of the human colon.

Regarding specific antitoxin treatment, equine antitoxin has been rarely used in infant botulism. In fact heterologous antibodies were known to have substantial serious adverse effects, a short (5 to 7 days) half life, and can represent a risk of inducing lifelong hypersensitivity to equine antigens.

In United States a big effort was made from California Department of Health Services (CDHS) through the Infant Botulism Treatment and Prevention Program (IBTPP) to create and test a specific orphan drug to treat infant botulism. Two clinical trials of human-derived botulinum antitoxin (BIG-IV), showed a significant reduction in hospital stay and in the rate of intubation. In 2003, BIG-IV was licensed in USA by the FDA to the CDHS as BabyBIG [72]. Since 2005, BIG-IV is available to treat patients with infant botulism hospitalized outside of the USA.

BIG-IV neutralizes free toxin and it is indicated for the specific treatment of patients below one year of age with infant botulism, caused by toxin type A or B. The titer of antibodies in the reconstituted product is at least 15 IU/mL against botulinum toxin A and at least 4 IU/mL against botulinum toxin B. Treatment with BIG-IV should be initiated promptly, and should not be delayed for laboratory confirmation of diagnosis, because prompt treatment ends further progression of the illness. BIG-IV immediately neutralizes all circulating botulinum toxin and remains in neutralizing amounts in the circulation for about 6 months, thereby allowing regeneration of nerve endings to proceed. Early treatment with BIG-IV within 3 days of admission, shortens hospital stay by about 1 week, more than later treatment does at 4 to 7 days [86].

Although [72] current recommendations are that it should not be administered if the onset of symptoms of infant botulism is more than 10 days before

**Table 2** | Global occurrence of infant botulism

Country	Time period	N. cases	Type A	Type B	Type E	Other Types	NR/ND°
Unites States	1976-2006	2419	1079	1310	1	27°°	2
Argentina	1982-2006	410	409	1			
Australia	1978-2006	32	12	15		1(AB)	4
Italy	1984-2008	29	4	20	4*	1 (Ab)	
Canada	1979-2006	27	22	5			
Japan	1986-2007	24	16	3	1	1 (C)	3
Spain	1985-2007	11	2	3			6
United Kingdom	1978-2007	8	2	5		1 (Bf)	
France	1983-2006	4	1	2		1 (AB)	
Germany	1981-2000	4	2				2
Norway	1997-1999	4	4				
Chile	1984-1995	3	2				1
Sweden	1985-2006	3	2			1(Bf)	
The Netherlands	2000-2005	3	1	2			
China	1986-1989	2		1			1
Denmark	1995-2000	2					2
Hungary	1995-2002	2				1(F)**	1
Israel	1994-2006	2		2			
Czech Rep.	1979	1		1			
Finland	2002	1		1			
Greece	2007	1	1				
Kuwait	2005	1					1
Mexico	2001	1	1				
Switzerland	1987	1	1				
Taiwan	1987	1		1			
Venezuela	2000	1		1			
Yemen	1989	1	1				

°NR/ND: Toxin Type Not Reported or Not Determined

°°1 type AB, 14 type Ba, 4type Bf, 2 type F, 6 C. baratii type F

\* C. butyricum type E

\*\* C. baratii type F

diagnosis, it can be also considered that spores can be present in feces for a long time after resolution of signs and symptoms and there are also reported very rare and not well explained cases of relapse of paralysis. BIG-IV has a half-life of approximately 28 days *in vivo*, and a single infusion neutralize for at least six months the circulating toxin.

Moreover, the circulating antibodies can reduce the risks due to antibiotic treatment of other co-infections.

Regarding drug interactions, antibodies present in immune globulin preparations may interfere with the immune response to live virus vaccines such as polio, measles, mumps, and rubella; therefore, vaccination with live virus vaccines should be deferred approximately for five months after administration of BIG-IV. If such vaccinations were given shortly before or after BIG-IV administration, a re-vaccination may be necessary.

## PREVENTION

The competitive microbiota seem the most protective barrier to outgrowth and toxinogenesis of neurotoxic clostridia in the infant gut.

The sources of spore are mainly the environmental dust inhalation and swallowing by the infant. This is obviously unavoidable, considering the easy availability in many parts of the world. The one identified, avoidable source of botulinum spores is honey, even if correlated to a small number of cases. Nevertheless in USA [6], all major pediatric and public health agencies agree that honey should be not fed to infants less than 12 months of age. The WHO also joined in this recommendation [97] and more recently in Europe [39] “it is recommended that effective and targeted information regarding the risks of infant botulism due to the consumption of honey should be provided, e.g. *via* leaflets, labeling and *via* advice to health-care professionals”.

In any case, other agricultural product, as medicinal plants, should be considered as vehicle of spores as honey.

Recently, interest on strategies aimed at manipulating bacterial colonization in infant gut using probiotics and prebiotics, is improving. However, only few of the benefits have been confirmed in well-conducted randomized controlled trials. Moreover, in its position paper in 2004 [98], the European Society for Pediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition concluded that in addition to limited data on safety and clini-

cal effects, there is a lack of published evidence of the long-term clinical benefits of using formulas, supplemented with probiotic bacteria. Moreover, the prophylactic introduction of exogenous microorganisms in the infant gut, in a period in which the delicate balance of indigenous normal microbiota is developing, seems questionable.

## EPIDEMIOLOGY

Infant botulism case is defined as clinically compatible, and laboratory-confirmed, occurred in a child aged less than 1 year and that was not caused by the ingestion of preformed toxin in food. Three confirmed and 1 suspected case of foodborne botulism in infants have reported in USA [99], Hungary [100], Italy [2005, unpublished data from CNRB] and Denmark [101].

Since its first recognition in 1976, infant botulism has become the most common form of human botulism diagnosed in the Western hemisphere.

A total of more than 3000 cases of infant botulism were globally identified from 1976 to 2007, but reports of cases have been distributed unevenly (Table 2) [12, 43, 62, 75, 102-105]. In the USA and Argentina infant botulism is the most frequent form of botulism with around 100 and 26 cases per year respectively. Australia, Italy, and Canada reported the next highest number of cases.

Almost all recognized cases, because of their severity, need hospitalization.

In USA, a range of 80-110 cases are annually recorded with a total of 2419 cases from 1976 to 2006, mostly recognized in California. Data from USA exclude SIDS cases, in which *C. botulinum* or toxins were found in the intestine only *post mortem*. Cases caused by type B toxin were most commonly reported, particularly in East coast (Pennsylvania), followed by type A toxin that is more diffused in West coast (California). In the report of 8 cases caused by *C. baratii* producing type F toxin, despite of extensive investigation, an environmental source of this organism has not been identified [16]. These cases are also characterized by an unusually young patient age (the youngest was a 38 h old neonate), a rapid onset and progression with respiratory failure and severe paralysis.

In Europe, from 1978 to 2007, a total of 73 cases have been diagnosed from 14 countries. Type B toxin is the most frequent serotype, and Italy has reported most cases among European countries (Table 3). In Italy from 1984 to 2008, 29 infant botulism cases diagnosed by the CNRB laboratory were identified [21, 102]. Table 3 reports the year of the diagnosis, the type of toxin detected in sera or fecal samples, and the microorganism involved.

Four years after licensure, 333 patients were treated in USA with BIG-IV. Internationally a

total of 10 cases were treated after 2005, these include 6 cases in Canada, one in Spain, one in England and one in the United Arab Emirates [43, 106].

## CONCLUSION

Infant botulism is a rare but also and under recognized disease. Recognition depends on awareness of infant botulism by front-line pediatricians and specialist (e.g. in neonatology, intensive care unit, emergency department). An early clinical suspicion is essential for a prompt diagnosis to a rapid treatment and to avoid un-useful and unadvisable therapies. The diagnosis of botulism should be suspected in young infants with the acute onset of weak suck, ptosis, generalized hypotonia, and constipation.

Detection of BoNT and/or identification of *C. botulinum* spores in stool samples, support clinical diagnosis. Also, other BoNT-producing clostridia should be considered as agents of the disease and included in laboratory criteria for diagnosis.

**Table 3** | Infant botulism in Italy 1984-2008

Case No.	Year	BoNT in serum	BoNT in feces	Spores in feces
1	1984	ND	BoNT/E	Type E°
2	1985	BoNT/E	BoNT/E	Type E°
3	1986	Negative	ND	Type B
4	1987	ND	ND	Type B
5	1988	Negative	BoNT/A	Type A
6	1989	Negative	BoNT/B	Type B
7	1991	ND	ND	Type B
8	1995	Negative	Negative	Type B
9	1996	ND	ND	Type B
10	1996	Negative	BoNT/B	Type B
11	1997	Negative	Negative	Type B
12	1998	Negative	BoNT/E	Type E°
13	1998	ND	BoNT/B	Type B
14	2000	ND	ND	Type B
15	2000	BoNT/B	ND	Type B
16	2000	ND	ND	Type B
17	2001	Negative	BoNT/A	Type A
18	2001	ND	Negative	Type E°
19	2003	Negative	Negative	Type A
20	2003	ND	BoNT/B	Type B
21	2004	Negative	BoNT/B)	Type B
22	2004	ND	BoNT/B	Type B
23	2005	ND	ND	Type B
24	2006	ND	ND	Type B
25	2006	ND	ND	Type Ab
26	2006	ND	BoNT/B	Type B
27	2006	ND	BoNT/A	Type A
28	2007	ND	BoNT/B	Type B
29	2008	ND	Negative	Type B

ND = Not Determined  
° *C. butyricum*

The main treatment of infant botulism consists of meticulous supportive care with immediate access to an intensive care unit and to mechanical ventilation. BIG-IV (Baby BIG) has changed the possibility of therapy for infant botulism today. Urgent treatment with Baby BIG at the time of clinical diagnosis is the current standard of care. It is available from the CDHS-Infant Botulism Treatment and Prevention Program ([www.infantbotulism.org](http://www.infantbotulism.org)).

Although environmental microscopic dust seem

the most source of *C. botulinum* spores, ingestion of honey remains the only identified avoidable risk factor for acquiring infant botulism.

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