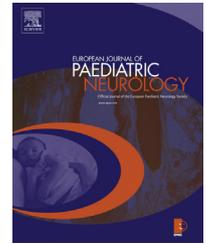




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Case study

Infant botulism: First two confirmed cases in Slovenia and literature review

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ABSTRACT

In Europe, infant botulism is a rare but probably under-diagnosed disease. With the intent to spread the awareness of this potentially life-threatening disease, we present a review of the literature with the emphasis on European epidemiology and a practical approach to diagnosis. We also report the first two confirmed cases of infant botulism in Slovenia and describe our way to the final diagnosis in a clinical setting where all appropriate diagnostic tests and treatment options are not readily available. The second case is particularly interesting, presenting with profound diarrhea following initial constipation, an unlikely symptom for an infant with botulism and possibly caused by *Bacteroides fragilis*.

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1. The first case

A previously healthy 2.5-month-old boy was admitted to the University Children's Hospital in Ljubljana for acute hypotonia, feeding problems and constipation.

He is the first child in the family. The mother was treated with oral diazepam for two weeks in the last trimester of pregnancy due to anxiety. The pregnancy and perinatal history were otherwise unremarkable. The infant's development was normal. He was fed with a combination of breast milk and infant formula, drinking up to 90 ml per feeding. The mother

stored the powdered milk formula in a tin container not the original box. He passed stools every second day.

Infant's mother was treated in her twenties for psychiatric disorder. At the time of admittance, she had a generalized skin yeast infection that was treated topically with a silver-containing cream, prescribed by an alternative medicine practitioner. She also mentioned taking lithium containing medicines, which she later denied. She smoked up to five cigarettes per day.

The family lives in a rural environment and owns dogs and cats. Their neighbor is farming pigs. Infant's mother is

Abbreviations: CB, *Clostridium botulinum*; IB, infant botulism.

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currently unemployed, while the father is working in a meat processing facility.

1.1. The disease course

The infant's first symptom was constipation lasting for 4 days. He was checked at the local hospital and was discharged home with no clinical or laboratory abnormalities found.

Over the next three days he became very weak and appeared sleepy. His feeding ability reduced to 20 ml per meal. He was readmitted to the local hospital 8 days after the onset of constipation. All laboratory results (full blood count, electrolytes, glucose, blood gas analysis, metabolic markers, liver function tests, cerebrospinal fluid, urine) were normal. After a bowel enema, he excreted a small amount of stool that contained unfamiliar white matter. Toxicological analysis revealed it was a piece of soap that the parents used (but not previously mentioned) in the hope of stimulating stools. Unfortunately, at this point the possibility of infant botulism (IB) was not yet considered and the stool was not examined for the presence of *Clostridium botulinum* (CB).

Hypotonia progressively worsened, with the development of ptosis and absent spontaneous movement. The child was transferred to the department of pediatric neurology at the University Children's Hospital in Ljubljana. In spite of severe hypotonia, social contact with the child remained normal. There was no loss in peripheral myotatic or bulbar (gag, swallow and corneal) reflexes. As the vital signs remained normal, there was no need for intensive care treatment. He received a topic antifungal drug for mouth sores.

Laboratory tests were repeated and were again normal as well as other examinations: electrocardiogram, continuous monitoring of cardio-respiratory function, electroencephalography, abdominal and head US, and abdominal X-ray. Standard electromyography did not reveal any abnormalities. Single-fiber EMG (SFEMG) was also requested, but was not performed. Our working diagnoses narrowed to intoxication (accidental or within the Munchausen by proxy syndrome) and IB. The toxicological analysis of blood was normal (including lithium and silver levels) and the psychological assessment didn't indicate any parental hostile intentions. Due to infant's lasting constipation even after several enemas it was not possible to prove or exclude IB.

Over the next few days the infant's clinical condition started to spontaneously improve. He started drinking up to 60 ml per feeding and was thus able to cover 2/3 of his daily energetic needs. However the hypotonia, ptosis and general muscle weakness remained unchanged. We considered botulinum antitoxin administration and contacted the California Health Institute, the only facility in the world producing and worldwide distributing human antibodies against botulinum toxins (BabyBIG®). However, as the patient's clinical condition was not life threatening and the cost of this treatment option was relatively high the drug was not given.

On the 17th day of the disease, the infant finally passed stools. Faecal sample sent for analysis to the Institute of Microbiology and Immunology, Ljubljana confirmed the presence of CB in culture. Neurotoxin gen B was detected in isolated strain by multiplex polymerase chain reaction (PCR)

assay for the detection of CB types A, B, E and F at the Statens Serum Institut, Copenhagen, Denmark. The mouse lethality assay for the detection of botulinum toxin in patient's serum was negative. The improperly stored milk formula contained CB/*sporogenes*, lacking the toxigenic genes, as shown with PCR assay at the same laboratory. The source of infection therefore remained unidentified.

Since the gut microbiota seems to play a crucial role in the pathogenesis of IB, we considered alternative treatment options, like fecal microbial transplantation, but we abandoned the idea due to lack of experience in pediatric population and because of uncooperative parents. We decided to give the patient a probiotic on the "can't harm, can benefit" basis and chose the probiotic strain of *Lactobacillus reuteri* DSM 17938 in the therapeutic dose of 1×10^9 bacteria daily. Vitamin B complex and coenzyme Q10 preparations were also administered.

On day 21 of the disease the infant was discharged from the hospital, although hypotonia and ptosis had not yet disappeared completely. We reexamined the child again 9 weeks after the first signs of the disease when no remaining neurological deficits were found. At that time CB was still present in stools.

2. The second case

The second case of IB in Slovenia (interestingly, identified only one month after the first case) is a 6 months old girl. She was admitted to University Children's Hospital in Ljubljana because of acute hypotonia, excessive tiredness and constipation.

The pregnancy and perinatal history were unremarkable as was her early development. She was solely breastfed until a day before admission when her mother fed here some cooked carrots. She regularly passed stools a few times daily. Her older sister ate honey every morning, but it was never given to the infant girl (honey was later examined and did not contain CB bacteria).

The family lives in a rural area. The mother is a teacher; the father works in construction business.

2.1. The disease course

The infant was admitted with a history of hypotonia and constipation lasting for four days. Spontaneous movement was very poor, but she smiled when she was talked to. Ptosis was present. With physiological muscle strength graded 2/5 she was unable to sit by herself or control her head if supported in the sitting position. Proprioceptive and bulbar reflexes were diminished. She had difficulties breastfeeding (Fig. 1).

All laboratory results (full blood count, electrolytes, glucose, blood gas analysis, metabolic markers, liver function tests, lumbar puncture, urine, toxicological analysis) were normal. SFEMG showed severe dysfunction at the level of neuromuscular junction.

After enema, we were able to get a small sample of stool that we immediately sent for microbiological analysis. The isolate cultivated from the sample was identified as



Fig. 1 – A 6 months old baby girl with infant botulism (case 2). Bottom left: frog-leg sign of acute muscular hypotonia, which is the most common presenting sign. Top left: floppiness is accompanied by ptosis, feeding problems (the necessity of gastric tube feeding) and excessive drooling. In spite of these problems, the girl was alert and in a good mood. Right: after the onset of the disease, the girl could not sit independently any more and could not control her head if supported in the sitting position.

Clostridium botulinum by 16S rDNA PCR and 4 days after admission IB was confirmed. Neurotoxin genes were not detected by specific multiplex polymerase chain reaction assay. The mouse bioassay done on the patient's serum was negative.

Five days after admission the infant's hypotonia worsened, swallowing problems occurred and nasogastric tube was introduced. Continuous monitoring of cardio-respiratory function didn't reveal any breathing problems. We considered the use of antibodies against botulinum toxins (Baby-BIG[®]), but the infant remained vitally stable and over the next days eventually started to improve spontaneously. Probiotic strain of *Lactobacillus reuteri* DSM 17938 was used in the therapeutic dose of 1×10^9 bacteria daily.

Interestingly, she developed severe watery, self-limited diarrhea from days 4 to 6 after admission, which correlated with her general worsening. The stool culture grew *Bacteroides fragilis* (*B. fragilis*). The presence of *B. fragilis* enterotoxin was not confirmed. All tests for other pathogenic gut bacteria and viruses were negative.

On 28th disease day the girl was able to feed safely and sufficiently on her own and was dismissed from the hospital with remaining signs of moderately lowered muscle strength and tone, moderate ptosis and lack of complete head control.

We reexamined the child 8 weeks after onset of symptoms and no remaining signs of the disease were found. Her stools tested negative for CB.

3. Infant botulism – a review based on clinical considerations

3.1. Etiology

The causative organisms are anaerobic, gram-positive, neurotoxic clostridia, mainly CB (rarely *Clostridium baratii* or *Clostridium butyricum*), that produce seven known neurotoxins, among which A and B are most commonly related to the disease in infants.^{1,2}

3.2. Epidemiology

Infant botulism is the most common form of botulism in humans worldwide, being far more common than food or wound botulism.³

The highest number of cases have been reported from US, ranging from 80 to 110 cases annually.⁴ In Europe, between years 1976 to date, 96 cases have been recognized in 13 different countries, most in Italy, Spain and UK.^{2,4–6} Two cases described in this paper are the first two diagnosed and reported in Slovenia.

Ninety percent of cases of IB occur in infants younger than 6 months.⁷ The youngest described patients were only 38 and 54 h old.^{8,9}

CB spores are abundantly present in the environment.^{10–15} Honey, worldwide contaminated with CB spores in 6–10%,

still remains the only recognized and avoidable food source proven to be directly associated with IB. It is therefore advised not to feed honey to babies before the age of one year.^{13,15–17}

IB is considered underestimated. The reasons include insufficient physician awareness, the inaccessibility of optimal laboratory tests, mild infections that don't call for medical attention and the fulminant form of IB that could be related to sudden infant death syndrome cases.^{18,19}

3.3. Pathogenesis

The disease is caused by the ingestion of neurotoxic clostridial spores that germinate in the infant's gut. The minimal infective dose has been estimated to be as low as 10–100 spores.^{20–22} The neurotoxin is absorbed into the bloodstream and inhibits the release of the acetylcholine from presynaptic motor nerve terminals of neuromuscular junction, causing a typical clinical picture of acute flaccid paralysis. Autonomic nervous system can also be affected.

Gut microbiota highly influences the susceptibility for CB colonization.^{20,21} Breast milk with certain strains of probiotic bacteria and other molecules (IgA, lactoferrin, lysozyme) is considered protective.^{23–26} Weaning seems to be a critical period in which infants are more prone to develop IB. Formula-fed infants are usually significantly younger at the onset of IB and often experience a more rapidly progressive and severe course.^{27,28}

Other common risk factors for IB are constipation and stool frequency of less than once per day, exposure to dust or soil with high spore density (construction sites, risky parents' occupation), and Meckel's diverticulum.^{29,30}

3.4. Clinical picture

The first sign of IB is usually constipation, followed by poor feeding and general weakness with poor head control. IB typically presents with descendant flaccid paralysis, first affecting cranial nerves and showing signs of expressionless face, ptosis and weak crying. Suck, gag, swallow and corneal reflexes can be weak or absent. Peripheral myotatic reflexes can be absent later in the disease course.

Altered mental status is always a sign of secondary complications of IB such as dehydration, hypoxemia or respiratory failure. Fever in IB patient suggests a secondary bacterial infection. Electrolytic disturbances due to syndrome of inappropriate antidiuretic hormone secretion have also been described.³¹

Contrary to the usual constipation, diarrhea might be the presenting gastrointestinal symptom in some IB patients with *Clostridium butyricum* toxin type E infection or *Clostridium difficile* (*C. difficile*) co-infection.^{32–34} Our second case presented with profound diarrhea following initial constipation. Besides CB, *B. fragilis* was the only stool isolate in our patient, showing rapid and extensive growth, which unusual for a carrier state.^{35–37}

IB symptoms are usually at their worst 1–2 weeks after the onset. The recovery is slow, but usually complete. Patients can be safely discharged from the hospital when signs and symptoms of IB are regressing and suck, gag and swallow reflexes enable appropriate feeding and breathing.

No thorough study on the length of CB stool colonization exists. Some small trials suggest that CB and neurotoxin can

be excreted in stools for months following IB.^{38,39} The relapses are unlikely.⁴⁰

3.5. Diagnosis

The presence of neurotoxic strain of CB and/or neurotoxin in stools confirms the diagnosis. Extensive studies have demonstrated that CB is not part of the normal comensal flora of infants or adults.^{41–43} One study suggests the possibility of asymptomatic carrier state in the areas with high environmental load of CB spores.²⁹

The identification of bacteria usually takes at least 5 days.⁶ PCR methods are more rapid but can detect also the non-expressing toxin genes.^{44,45} The detection of neurotoxin in blood or stool is based on animal bioassay methods, which are often negative due to low levels of circulating neurotoxin.⁴⁶

EMG may support the diagnosis of IB, but typical EMG signs usually take 7–10 days to develop.⁴⁷ Hypermagnesemia is the only entity in infants that mimics IB in EMG features.⁴⁸ The stimulation SFEMG is more sensitive and specific when compared to conventional EMG and typically shows a rate-dependent improvement of jitter.⁴⁹

3.6. Treatment

In a patient with suspected IB, treatment should not be delayed even if the presence of neurotoxic CB in stool is not yet confirmed.

Supportive therapy is the mainstay of treatment as it reduces the risk of serious secondary complications and long-term sequelae.⁷ Careful cardiorespiratory monitoring is crucial in the early phase. If the gag and swallow reflexes are impaired, tilting the bed to 30-degrees can protect the airways, but in serious cases intubation is required. When suck reflex is weak the introduction of nasogastric tube is recommended. If available, mother's milk is the food of choice. Parenteral feeding is discouraged due to risk of secondary bacterial infections.⁵⁰

Since 2003, human botulinum antitoxin (BabyBIG[®]) is the therapy of choice.⁵¹ The specific human immunoglobulins are safe, effective and remain in neutralizing amounts in the circulation for about 6 months, allowing regeneration of nerve endings and making eventual use of antibiotics safer.^{52,53} Due to its high cost their use is generally limited to IB patients with severe, life threatening disease.⁵⁴ Equine botulinum antitoxin has been used in the past, but is no longer available due to safety concern in pediatric population.^{55,56}

Antibiotic treatment of secondary bacterial infections should be implemented carefully throughout the course of IB and also in the following months due to persistence of CB in infant's gut. CB is sensitive to a wide range of antibiotics, which can cause a surge of released neurotoxin and sudden aggravation of IB symptoms or even death.⁴⁷

Although there are no written reports of the benefits of probiotic use in IB, their potential to favorably balance gut flora might be beneficial.⁵⁷ Their use is safe in infancy and even in neonates.^{58–60} The probiotic strains of *Lactobacillus rhamnosus* GG, *Lactobacillus casei* DN114 G01 and *Saccharomyces boulardii* showed beneficial effects in treatment and prevention of *C. difficile* infections and could potentially alleviate symptoms of IB as well.^{58,61}

Fecal microbiota transplantation has been used for various indications for over 50 years.⁶² In adults it has been successfully used as a treatment for resistant *C. difficile* infections with about 90% of reported patients being cured.^{63,64} A case of a 2-year old patient with safe and effective microbiota transplantation for resistant *C. difficile* infection was reported.⁶⁵

4. Conclusion

IB is a potentially life-threatening disease. It is rare, but probably under-diagnosed due to low awareness of the disease among physicians. The typical presentation is one of acute descending hypotonia with or without bulbar signs, accompanied with constipation and poor feeding. Detection of clostridium bacteria and/or neurotoxin in the stools confirms the clinical diagnosis. Besides supportive measures, effective and safe treatment with human botulinum antitoxin is available and should be administered early in the disease course, particularly when dealing with a life threatening form of the disease.

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