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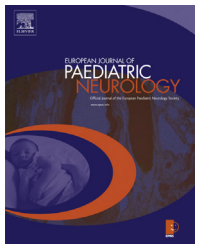

Case study

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*.

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 mater. 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