First Report of an Infant Botulism Case Due to \textit{Clostridium botulinum} Type \textit{Af}

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Most infant botulism cases worldwide are due to botulinum toxin types A and B. Rarely, \textit{Clostridium botulinum} strains that produce two serotypes (Ab, Ba, and Bf) have also been isolated from infant botulism cases. This is the first reported case of infant botulism due to \textit{C. botulinum} type \textit{Af} worldwide.

CASE REPORT

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n 30 January 2011, a 2-month-old boy from San Rafael, Mendoza, Argentina, was brought to a local health care center due to weakness and low-grade fever. The infant was referred to a local hospital with suspected pneumonia. He was previously healthy and exclusively breast fed. Subsequent laboratory results and chest radiography excluded pneumonia. On 2 February, the patient was admitted to the pediatric intensive care unit due to decreased oral intake, dysphagia, hypotonia, bilateral ptosis, and respiratory deficiency. Various diagnoses, including infant botulism, metabolic disorders, and muscular disorders, were considered. The patient received oxygen therapy through a nasal cannula and was fed breast milk by nasogastric intubation. Administration of antibiotics may increase the amount of botulinum neurotoxin (BoNT) in the large intestine, and it is indicated only for the treatment of secondary infections. Therefore, the infant was not treated with antibiotics. Mechanical ventilation was not necessary, and botulinum antitoxin was not administered. At 5 days of hospitalization, the patient had no head control, and sucking and crying were weak. Cranial ultrasound performed on 7 February was normal. On 17 February, the patient was able to move extremities but had no head control. Three days later, the nasal cannula was removed. The patient was transferred to a regular pediatric room on 21 February. At that time, he had reactive isochoric pupils, slight axial hypotonia, and the ability to move extremities, but no head control. On 22 February, the patient started speech therapy to stimulate the facial area. His head control was still weak and he still had generalized hypotonia and weak gag reflex. The patient had improved movement of extremities and started kinesiotherapy on 24 February. On 28 February, his head control, sucking-swallowing coordination, strength, and muscle tone had improved noticeably, and 3 days later, he started oral feeding. The patient was discharged from the hospital on March 5.

Clinical specimens were submitted to Área Microbiología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, for laboratory testing by standard methods (1). A stool sample collected February 3 tested positive for BoNT by mouse bioassay and was neutralized when a mixture of both serotypes was used. During the hospital stay, additional stool samples were regularly collected and cultured. Seven additional stool samples collected between February 26 and March 4 were all positive for BoNT by mouse bioassay. Four isolates obtained from the stool specimens were transferred to the Centers for Disease Control and Prevention for further characterization and were identified as \textit{Clostridium botulinum} type \textit{Af}. The culture supernatant from one of these isolates (CDC66185) was injected intraperitoneally into mice to determine the BoNT/A-to-BoNT/F ratio, which was 200:1. In addition, DNA was extracted from strain CDC66185, and \textit{bont/A} and \textit{bont/F} genes were sequenced using primers previously reported (2, 3, 4). The \textit{bont/A} and \textit{bont/F} gene nucleotide sequences...
were 100% identical to those of the previously reported subtypes A2 and F5, respectively (Fig. 1 and 2) (4, 5).

Infant botulism is caused by growth of *C. botulinum* and production of BoNT in the intestinal tract of infants younger than 1 year of age. Clinical signs of infant botulism include constipation (three or more days without defecation in a previously regular infant), hypotonia, lethargy, difficulty in swallowing, weak cry, pooled oral secretions, general muscle weakness, and loss of head control. Neurological findings include ptosis, ophthalmoplegia, sluggish pupillary reaction to light, flaccid expression, dysphagia, weak gag reflex, and poor anal sphincter tone (6).

There are seven confirmed serotypes of BoNT (A through G), defined by neutralization of toxicity by serotype-specific antibodies. The majority of infant botulism cases worldwide are due to serotypes A and B (51% and 46% of cases, respectively) (7). Most strains of *C. botulinum* produce one toxin serotype, but some produce more than one, i.e., Ab, Af, Ba, and Bf (a capital letter denotes the predominant toxin type). Strains that produce types Ab, Ba, and Bf have been identified in infant botulism cases in Italy, Sweden, the United Kingdom, and the United States (7). To the best of our knowledge, this is the first report of an infant botulism case due to *C. botulinum* type Af worldwide.

The genome sequence of a *C. botulinum* type Af strain (strain Af84) was shown to contain three neurotoxin genes (bont/A2, bont/F4, and bont/F5) (8). However, Raphael et al. reported that six *C. botulinum* type Af strains isolated from soil and clinical specimens harbor bont/A2 and bont/F5 or bont/F4, but not all three (9). Similarly, strain CDC66185 harbors bont/A2 and bont/F5 only. Subtype A2 has been identified in clinical and environmental samples collected in Argentina, France, Italy, Japan, Uganda, and the United States (5, 10, 11, 12). The predicted amino acid sequence of subtype A2 shows 88 to 90% identity with other serotype A subtypes (13). Subtype F5 strains have been isolated in clinical and environmental samples collected in Argentina. The predicted amino acid sequence of subtype F5 is highly divergent compared to other type F subtypes (4).

*C. botulinum* type Af was first isolated from a soil sample in Mendoza, Argentina (14). In a subsequent study of soil samples from that country, *C. botulinum* type Af was identified in 17 of 470 (3.6%) samples (15). In addition, type Af was reported as the cause of a case of food-borne botulism in Mendoza, Argentina, in 1982 (16). Here we report an infant botulism case due to *C. botulinum* type Af. Notably,  *C. botulinum* type Af has been isolated exclusively from soil and botulism cases in Argentina.

**Nucleotide sequence accession numbers.** The bont/A and bont/F gene nucleotide sequences were submitted to GenBank with accession numbers KMI75565 and KMI875566, respectively.

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