Letter to the Editor

Molecular and Epidemiological Characterization of Infant Botulism in Beijing, China*

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Laboratory-based pathogen isolation, identification, and toxicity determination were performed on samples from a suspected case of infant botulism. Mice injected with cultures generated from the enema sample and ingested Powered infant formula (PIF) presented typical signs of botulism. Antitoxins to polyvalent botulinum neurotoxins (BoNTs) and monovalent BoNT type B antitoxin had protective effects. *Clostridium botulinum* isolated from the enema and residual PIF samples were positive for type B toxin. Pulsed-field gel electrophoresis (PFGE) revealed that the two strains of *C. botulinum* isolated from the two samples produced indistinguishable pulsotypes. These findings confirmed this case of type B infant botulism associated with the ingestion of PIF contaminated by type B *C. botulinum* spores.

Key words: *Clostridium botulinum*; Infant botulism; Powdered infant formula; China

Botulism is a severe flaccid-paralytic disease caused by the botulinum neurotoxins (BoNTs) produced by *Clostridium botulinum*, as well as some strains of *Clostridium butyricum* and *Clostridium baratii*[1-2]. Human botulism is a rare but life-threatening disease, mainly caused by the ingestion of food contaminated with BoNTs (foodborne botulism). It may also arise following contamination of a wound with *C. botulinum* spores (wound botulism), or in infants by intestinal colonization and subsequent toxin production (infant botulism, IB)[3]. IB often occurs in children under the age of 1 year, which reflects their susceptibility to gut colonization by BoNT-producing clostridia. Positive confirmation of IB is normally established when BoNT and/or isolates of BoNT-producing clostridia are detected in the stool. The aim of this study was to conduct a retrospective epidemiological investigation of the etiology of a suspected case of IB.

BoNT poisoning was suspected in a three-month-old infant. The 11 samples collected for this investigation included a sample of leftover PIF that had been ingested by the infant, a 5-mL enema sample, and nine environmental swabs taken from the infant's family environment (one from the indoor and one from the outdoor window sill, three desks and one table, and each from the inside of the infant's feeding bottle, water cup, and bottle nipple). Both animal and human fecal specimens were processed in accordance with the protocols approved by Ethics Committee of China National Center for Food Safety Risk Assessment (CFSA, permit no. 2014004) and written informed consent from the patient's parent.

C. botulinum was isolated and BoNT was detected according to the China National Food Safety Standard (GB/T4789.12-2003) and the US Food and Drug Administration[4-5]. Presumptive *C. botulinum* colonies were selected for Gram staining, microscopic examination, and API 20A and VITEK2 biochemical test-based identification in accordance with the recommended procedures. In addition, 16S rRNA gene sequencing, detection of the *bont* genes encoding neurotoxin types A, B, E, and F, and BoNT production assays were carried out according to previously published methods[5-6]. PFGE typing was performed on *C. botulinum* and *C. sporogenes* cultured from the samples described above according to a previously published method[7].

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Neither *Clostridium* species nor lethal BoNT were isolated or detected in the pre-culture obtained from the PIF sample. However, the culture supernatants prepared from cooked meat medium (CMM) cultures taken from the environmental swabs (including a desk, window sill, and table) and the enema sample resulted in the death of laboratory mice within 10 min after intraperitoneal injection. Signs of poisoning and the nature of the death of the mice were consistent with type I poisoning by the positive type strain of *C. sporogenes*. Mice injected with the same cultures that had been either boiled at 100 °C for 10 min or trypsinized also died (Table 1). Mice injected intraperitoneally with the CMM- and tripticasopeptone glucose broth (TPGY)-cultured PIF sample and the tripticasopetone glucose broth TPGY-cultured enema sample exhibited type II poisoning symptoms and death. The typical signs of type II poisoning were noted in the first 24 h, and included ruffling of the fur followed in sequence by labored breathing ('wasp waist'), limb weakness, and finally total paralysis, during which the mice gasped for breath before they died. Signs of poisoning prior to death were also observed in mice injected intraperitoneally with trypsin-treated cultures. However, neither symptoms nor death occurred in mice injected intraperitoneally with the above cultures but that had been heated at 100 °C for 10 min (Table 1). The poisoning symptoms and deaths of the mice were consistent with those observed in type A BoNT positive control mice.

Table 1. Death Caused by Inoculation with Prepared Supernatants Recovered from Study Isolates

Sample Source	Medium	Group	No. of Mice	No. of Deaths
PIF	CMM	Untreated	3	3
		Heated	3	0
		Trypsinized	3	3
	TPGY	Untreated	3	3
		Heated	3	0
		Trypsinized	3	3
Enema	CMM	Untreated	3	3[a]
		Heated	3	3[a]
		Trypsinized	3	3[a]
	TPGY	Untreated	3	3
		Heated	3	0
		Trypsinized	3	3
Window sill swab	CMM	Untreated	3	3[a]
		Heated	3	3[a]
		Trypsinized	3	3[a]
Table swab	TPGY	Untreated	3	3[a]
		Heated	3	3[a]
		Trypsinized	3	3[a]
Desk swab	CMM	Untreated	3	3[a]
		Heated	3	3[a]
		Trypsinized	3	3[a]
BoNT positive control	TPGY	Untreated	3	3
		Heated	3	0
		Trypsinized	3	3
C. sporogenes control	CMM	Untreated	3	3[a]
		Heated	3	3[a]
		Trypsinized	3	3[a]
Negative control	CMM	Blank control	3	0
	TPGY	Blank control	3	0
	PBS	Blank control	3	0

Note. [a]These mice died within 5-10 min; unmarked entries indicate that the mice died within 2-6 h with symptoms resembling those of BoNT poisoning. CMM, cooked meat medium. TPGY, triptica sopeptone glucose broth. PBS, phosphate buffer. PIF, powdered infant formula.