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SHORT REPORT
Two simultaneous botulism outbreaks in Barcelona: *Clostridium baratii* and *Clostridium botulinum*

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SUMMARY

Botulism is a severe neuroparalytic disorder that can be potentially life-threatening. In Barcelona, Spain, no outbreaks had been reported in the past 25 years. However, in September 2011, two outbreaks occurred involving two different families. A rare case of *Clostridium baratii* which produced a neurotoxin F outbreak was detected in five family members who had shared lunch, and several days before that another family was affected by *C. botulinum* toxin A which was probably present in homemade pâté.

Key words: Botulism neurotoxin, *Clostridium baratii*, *Clostridium botulinum*, foodborne outbreak.

Botulism is a severe neuroparalytic disease caused by neurotoxins produced by certain members of the anaerobic Gram-positive spore-forming bacteria *Clostridium* [1]. There are seven botulinum neurotoxins, A–G, that are distinguished according to their antigenic properties [2].

In humans, botulism usually results either from the consumption of preformed toxin in food or the *in vivo* elaboration of toxin from infected wounds or intestinal colonization. Foodborne outbreaks, especially those involving commercially prepared food products, are considered a public health emergency due to the severity of illness and the potential for a large number of cases [3]. Toxin production can occur in contaminated food under anaerobic, low-salt, low-sugar, and low-acid conditions.

Type F botulism causes $<1\%$ of botulism cases in the USA [4]. It was first recognized in an outbreak in Denmark in 1958 [5] associated with homemade pâté; the first reported outbreak in the USA occurred in 1966 [6]. Since 1981, 14 cases of adult botulism type F have been reported. In 2002, the first confirmed foodborne case attributed to toxin F production by *C. baratii* was described [4]. Furthermore, there have been no reported of outbreaks [7] since the first description and identification of *C. baratii* in 1979 [8].

On Sunday 18 September 2011, four members of a Chilean family (parents F, M and two daughters D1, D2) and the boyfriend (B) of one of the daughters celebrated their country's national day with a lunch. The next day all five developed gastrointestinal signs (strong abdominal cramps, nausea, vomiting); slurred

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Table 1. *Symptoms and laboratory reesults for outbreaks 1 and 2*

	Outbreak 1					Outbreak 2	
	Father	Mother	Daughter 1	Daughter 2	Boyfriend	Father	Son
Symptoms and sample date							
Symptom onset	19 Sept.	19 Sept.	19 Sept.	19 Sept.	19 Sept.	8 Sept.	11 Sept.
Serum sample	20 Sept.	20 Sept.	20 Sept.	20 Sept.	20 Sept.	15 Sept.	15 Sept.
Stool sample	20 Sept.	20 Sept.	20 Sept.	24 Sept.	27 Sept.	15 Sept.	15 Sept.
ICU admission	21 Sept.	21 Sept.	21 Sept.	20 Sept.	21 Sept.	9 Sept.	14 Sept.
Hospital discharge	10 Oct.	11 Oct.	10 Oct.	17 Oct.	11 Oct.	24 Sept.	22 Sept.
Laboratory results							
Mouse bioassay	+	−	+	−	+	−	−
Stool culture	+	−	−	−	+	+	−
BoNT PCR	+(F)	−	−	−	+(F)	+(A)	−

ICU, Intensive care unit; BoNT PCR, detection of neurotoxin encoding gene F or A neurotoxin.

speech, difficulty in swallowing and generalized muscle weakness requiring medical care appeared within a few hours. They all developed respiratory arrest and required intubation; unresponsive pupils, minimal extra-ocular muscle motion and facial paralysis were also detected. Electromyogram testing suggested pre-synaptic neuromuscular junction impairment. Antitoxins to botulism toxin types A, B and E were administered within 24 h of admission. Nevertheless, paralysis progressed and the patients became quadriplegic with no voluntary muscle function.

Neurological function gradually resolved with clinical improvement after 7 days of hospitalization. The patients were weaned from mechanical ventilation between 10 and 17 days after admission. All of the patients were discharged from the hospital approximately 1 month after intoxication (Table 1).

Serum was obtained prior to antitoxin treatment. Stool samples were obtained from patients and submitted to the Centro Nacional de Microbiología, ISCIII, Majadahonda, Madrid. The mouse bioassay (MBA) was performed on stool samples [9] using a standard method for detecting neurotoxin. Three out of five patients had a positive MBA (F, D1, B; Table 1). Standard culture methods and the multiplex polymerase chain reaction (PCR) method used for A, B, E and F neurotoxin gene detection were also performed [9]. Spore-forming Gram-positive bacilli were detected in the anaerobic stool cultures of two patients (F, B) and identified by 16S rRNA as *C. baratii* with a 99% match to *C. baratii* ATCC43756 (GenBank accession no. X68175). In addition, this strain harboured the BoNT/F gene by PCR and lacked proteolytic activity due to the absence of the *fldB* gene coding the phenyllactate dehydratase enzyme [10].

Food exposures included: *pebre*, a dipping sauce made of fresh vegetables and a commercial green chili sauce, a corn cake made of corn pasta and meat, and individual meat pit pies (baked by an aunt and given to the family on Saturday to share the next day). Samples of the corn cake and *pebre* analysed for the presence of botulism neurotoxin by MBA were negative. No pit pies were available to test.

Another botulism outbreak was reported a week before this incident, which affected two of four members (father and son) of a different family. In this incident, BoNT/A genes were detected by PCR in the mixed anaerobic culture of the stool sample from the father. These cases did not require mechanical ventilation. although no food source was identified by laboratory testing, a homemade olive and tuna pâté was considered to be the most likely source.

Worldwide, reported botulism outbreaks are rare events. We report the first *C. baratii* outbreak since 1979. Samples of the *pebre* and corn cake were tested for toxin activity, but yielded negative results. Unfortunately, no pit pies remained to be tested. The *pebre* and green chili were considered unlikely causes of contamination because of their low pH (<4 as confirmed by laboratory testing). The corn pie was eaten shortly after baking, and the cooking temperature should have destroyed any botulinum toxin present. However, individual pit pies were eaten after re-heating; the original baking could have killed vegetative cells and eliminated much of the oxygen.

Heat-resistant, anaerobic *Clostridium* spores, which can be found in a variety of food products, could have germinated under appropriate conditions and produced the toxin that would not have been destroyed by inadequate reheating.

No botulism outbreaks had been reported in Barcelona in the past 25 years. Yet over the course of 10 days in 2011 two families were seriously affected by foodborne botulism intoxication. No epidemiological link, other than time and space, was found between these two clusters. An increase in botulism outbreaks was detected last year throughout Europe with outbreaks reported from September to November 2011 in France, Scotland and Finland [11]. In light of this situation we believe additional public education regarding the causes and prevention of botulism needs to be effectively and rapidly implemented. Furthermore, testing for botulism neurotoxin F is also strongly recommended when confronted by any botulism outbreak.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Centers for Disease Control and Prevention.** *Botulism in the United States, 1899–1996*. Handbook for epidemiologists, clinicians and laboratory workers. Public Health Service, U.S. Department of Health and Human Services. Centers for Disease Control and Prevention; 1998.
2. **Hill KK, *et al.*** Genetic diversity among botulinum neurotoxin-producing clostridial strains. *Journal of Bacteriology* 2007; **189**: 818–832.
3. **Bell CKA.** *Clostridium botulinum: A Practical Approach to the Organism and its Control in Foods*. Oxford: Blackwell Science, 2000.
4. **Harvey SM**, *et al*. Botulism due to *Clostridium baratii* type F toxin. *Journal of Clinical Microbiology* 2002; **40**: 2260–2262.
5. **Moller V, Scheibel I.** Preliminary report on the isolation of an apparently new type of *Clostridium botulinum*. *Acta Pathologica Microbiologica Scandinavica* 1960; **48**: 80.
6. **Midura TF, *et al.*** Botulinum type F: isolation from venison jerky. *Applied Microbiology* 1972; **24**: 165–167.
7. **Gupta A, *et al.*** Adult botulism type F in the United States, 1981–2002. *Neurology* 2005; **65**: 1694–1700.
8. **Hall JD, *et al.*** Isolation of an organism resembling *Clostridium baratii* which produces type F botulinal toxin from an infant with botulism. *Journal of Clinical Microbiology* 1985; **21**: 654–655.
9. **Lindstrom M, Korkeala H.** Laboratory diagnostics of botulism. *Clinical Microbiology Reviews* 2006; **19**: 298–314.
10. **Dahlsten E, *et al.*** PCR assay for differentiating between group I (proteolytic) and group II (nonproteolytic) strains of *Clostridium botulinum*. *International Journal of Food Microbiology* 2008; **124**: 108–111.
11. **Cowden J.** Food-borne *Clostridium botulinum* intoxication from mass produced foodstuffs in Europe. *Eurosurveillance* 2011; **49**.