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First Case of Infant Botulism Caused by *Clostridium baratii* Type F in California

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In late 2003 a severely hypotonic neonate, just 38 h old at onset of illness, was found to have infant botulism caused by neurotoxigenic *Clostridium baratii* type F. Environmental investigations failed to identify a source of this strain. This is the youngest patient reported to have infant botulism and the fifth instance of infant botulism caused by *C. baratii* type F.

CASE REPORT

In late 2003 a 47-h-old neonate with a 9-h history of poor feeding and lethargy was airlifted from a community hospital in rural northern California to an acute-care tertiary facility in San Francisco, California, where the admitting diagnosis was acute hypotonia and respiratory failure. An inborn error of metabolism was suspected because the urine had an unusually sweet smell, which prompted suspicion of maple syrup urine disease. As a result, the patient underwent three consecutive daily courses of hemodialysis. The day after the third course, she had intermittent movement of her distal extremities but otherwise remained mostly hypotonic. The possibility of infant botulism was initially dismissed because of the patient's young age, fulminant onset of illness, and the quick recovery of her slight distal extremity control.

By hospital day 12 all metabolic test results were normal, thereby leading the attending neurologists to consider the possibility of infant botulism. A stool specimen submitted to our laboratory was emulsified and extracted in gelatin phosphate diluent and centrifuged. The extract was injected into pairs of Swiss-Webster mice in accord with the standard mouse neutralization bioassay for botulinum toxin detection (Table 1) (5). All relevant institutional policies and federal guidelines for the ethical use of laboratory animals were followed. The stool pellet was inoculated into two chopped-meat–glucose–starch broth tubes and onto 4% egg yolk agar, botulinum selective medium (16), and 5% Schaedler sheep blood agar. One broth tube was heat-shocked at 70°C for 15 min. All media were incubated at 35°C in an anaerobe chamber (5).

The bioassay of the stool extract identified a heat-labile toxin that was neutralized only by type F monovalent botulinum antitoxin (Table 1). The directly inoculated stool culture plates revealed heavy growth of lecithinase-positive colonies in almost pure culture on egg yolk agar and slightly beta-hemolytic colonies on sheep blood agar after 24 h of incubation. No growth was observed on botulinum selective medium at 72 h. Nonproteolytic growth was evident at 24 h in both broth culture tubes. Filtrate from a pure culture of the lecithinase-positive organism, like the stool extract, tested positive for botulinum toxin type F. Test results from additional subsequent stool specimens confirmed the finding (Table 1). Biochemical characterization and 16S rRNA sequencing, together with the culture and bioassay results, identified the organism as *Clostridium baratii* type F. The patient received supportive care but was not treated intravenously with botulism immune globulin (human) (commercially known as BabyBIG) because of the delayed referral. Although initially severely paralyzed, the patient quickly regained muscle strength and was released from the hospital on day 19 of illness. The three episodes of hemodialysis for suspected maple syrup urine disease early in the course of illness may have aided in the rapid recovery. Intestinal colonization by *C. baratii* lasted more than 3, but less than 5, weeks (Table 1). *C. baratii* differs from the ubiquitous *C. botulinum* in that an environmental source of toxigenic *C. baratii* has not been identified, so an extensive investigation to identify a possible environmental reservoir of this organism was undertaken. An epidemiological interview was conducted at home with the patient's parents. Construction of a timeline of events from birth to initial hospitalization indicated that the onset of illness had occurred just 38 h after birth, suggesting that the patient's exposure to *C. baratii* may have occurred at the birthing hospital in the immediate perinatal period.

Multiple environmental samples were collected from the patient's home and birthing hospital (Table 2). Also, when the patient's correct diagnosis became known, fecal specimens were collected from both parents (a month after onset of the infant's illness) to evaluate the possibility of subclinical intestinal colonization. Despite an extensive laboratory effort, no *C. baratii* was isolated from the adult fecal or environmental specimens, including household vacuum cleaner dust, a known

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TABLE 1. Laboratory identification of *C. baratii* neurotoxin type F as the causative agent of the case reported here

Specimen no.[a]	Date collected (DOI)[b]	Direct toxin detection by bioassay	EYA stool culture		Isolate identification	
			Lipase	Lecithinase	Biochemical[c]	16S rRNA[d]
1	10/26/03 (16)	Type F	−	+	*C. baratii*	*C. baratii*
2	10/30/03 (20)	Type F	−	+	*C. baratii*	*C. baratii*
3	11/1/03 (22)	None	−	+	*C. baratii*	*C. baratii*
4	11/15/03 (Home[e])	None	−	−	N/A[f]	N/A
5	12/11/03 (Home)	None	−	−	N/A	N/A

[a] In all instances, the specimen type was stool.
[b] DOI, day of illness (starting at onset of symptoms). Dates are given in the form mo/day/yr.
[c] Biochemical identification with api20A, Biomerieux, Hazelwood, MO.
[d] RNA sequence determined with ABI Prism 7000, Applied Biosystems, Foster City, CA.
[e] Specimen collected at home (patient no longer hospitalized).
[f] N/A, not applicable.

source of *C. botulinum* (1, 13, 15, 17, 18). The family car and pickup truck air filters, studied as a means of sampling airborne spores, were also negative. Parenthetically, *C. botulinum* type A was isolated from most of the soil sites sampled (which exemplifies the ubiquity of this organism) (Table 2).

Infant botulism is an acute, symmetric, descending, flaccid paralysis that occurs in infants younger than 12 months of age. The mean (median) age at onset for all California cases from 1976 to 2004 was 3.4 (3.1) months. Definitive laboratory diagnosis identifies *Clostridium botulinum* toxin and/or organisms in fecal specimens following intestinal colonization by swallowed *C. botulinum* spores (2). After absorption, botulinum toxin produced in the intestinal lumen binds to terminal motor neurons, where it prevents acetylcholine release and thereby causes flaccid paralysis (2). Detection and identification of botulinum toxin is accomplished using the mouse neutralization bioassay (5).

With the exception of rare dual-toxin-producing strains, most *C. botulinum* strains produce just one of the seven known botulinum toxin types designated A to G (4, 7, 11). Worldwide, reported infant botulism almost always results from *C. botulinum* strains that produce botulinum toxin type A or type B. However, four cases of infant botulism caused by neurotoxigenic *C. butyricum* type E have been reported from Italy (3, 6). Also, *C. baratii* type F has caused four cases of infant botulism (Table 3) (8, 10, 11, 12, 19, 20, 21). We now report laboratory, environmental, and epidemiological aspects of the fifth instance of infant botulism caused by neurotoxigenic *C. baratii* type F. Clinical particulars of this case are reported elsewhere (14). This is the first such case to occur in California in our 29 years of laboratory surveillance and the youngest (age at onset of disease) infant botulism patient ever recorded.

Rare strains of *C. botulinum* that produce two toxins and the non-botulinum clostridia that produce botulinum toxin (i.e., *C. butyricum* type E and *C. baratii* type F), may be more prevalent than realized (4, 9). The botulism diagnostic laboratory serves a critical role in identifying these seldom-reported strains (4, 9). All previously reported U.S. cases of *C. baratii* type F infant botulism occurred in unusually young patients. We suggest that clinicians include infant botulism caused by *C. baratii* type F in their differential diagnosis if the infant's illness is characterized by the triad that includes the following: (i) rapid onset, (ii) severe paralysis. and (iii) young patient age. The case reported

TABLE 2. Environmental samples collected from California infant botulism patient's home and birth hospital for *C. baratii* culture

Source and sample no.	Description	Culture result
Home		
1	Heater dust #1	Negative
2	Heater dust #2	Negative
3	Heater dust #3	Negative
4	Ceiling fan dust	Negative
5	Indoor plant #1 soil	Negative
6	Indoor plant #2 soil	Negative
7	Indoor plant #3 soil	Negative
8	Armoire dust	Negative
9	Vacuum cleaner bag dust	Negative
10	Front yard soil	Negative[a]
11	Back yard soil	Negative
12	Car air filter[b]	Negative
13	Truck air filter[c]	Negative
14	Bee segments from truck air filter	Negative
Hospital		
15	Soil from front hospital landscaping	Negative[a]
16	Soil from hospital construction site	Negative[a]
17	Soil outside delivery room window	Negative[a]
18	Soil from landscaping near heli-pad[d]	Negative[a]
19	Window track dust from inside delivery room	Negative[e]

[a] Culture positive for *C. botulinum* type A.
[b] Vehicle driven through forest fire smoke that had enveloped home prior to infant's birth.
[c] Truck that traveled to and from patient's birthing hospital.
[d] Helicopter landings approx. 50 ft from delivery room windows were a possible mechanism for creating spore-containing dust aerosols.
[e] Window track dust positive for *Clostridium perfringens*.

TABLE 3. Summary of the California and previously reported *C. baratii* type F infant botulism cases

Case no.	Location (reference[s])	Yr	Age of onset of illness[a] (days)	Length of hospital stay (days)
1	New Mexico, United States (8, 12)	1979	14	66
2	Oregon, United States (19)	1992	9	31
3	Hungary (20)	1994	90	24
4	Ohio, United States (21)	1998	3	21
5	California, United States	2003	<2	19

[a] Mean (median) age of onset of illness for all California cases from 1976 to 2004 was 3.4 (3.1) months.

here is remarkable because it is the first recognition of *C. baratii* type F infant botulism in California, it describes the youngest known patient to have infant botulism caused by *C. baratii* type F, and it involves the youngest known patient ever to have had infant botulism.

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