Commentary: Where Marco Polo Meets Meckel: Type E Botulism from *Clostridium butyricum*

Ye emperors, kings, dukes, marquises, earls and knights, and all other people desirous of knowing the diversities of . . . mankind . . . read through this book . . . by Marco Polo, a wise and learned citizen of Venice, who states distinctly what things he saw and what things he heard from others. [1]

The most potent poison known, lethal to man in nanogram amounts, is botulinum neurotoxin. Until a generation ago, botulism was recognized almost exclusively as the result of ingesting botulinum neurotoxin in preserved food contaminated by *Clostridium botulinum* [2]. Botulism had rarely occurred from infected wounds in analogy to tetanus [3]. The victims of botulism were usually adults. Of the 7 serologically distinct types of botulinum neurotoxin, nearly all human cases were caused by types A, B, or E. Type E botulism, with rare exception [4], was associated with eating fish or other aquatic animals [5].

See article by Fenicia et al. on pages 1381–7.

This paradigm of botulism as an adult disease caused by tainted food, still the sole concept of botulism for most of the public, was altered by the recognition of infant botulism in California in 1976 [6, 7]. The colon during infancy is distinctively susceptible to colonization by spores of *C. botulinum*. After germination, the bacteria cause an extended neurotoxin that results in acute flaccid paralysis [8]. Food, specifically honey, is an infrequent source of spores causing infant botulism [9]. Most affected infants are presumed to inhale or swallow from their environment the spores that eventually multiply in the colon. Since its recognition, infant botulism has become the most common form of human botulism diagnosed in the Western Hemisphere. Botulism resulting from intestinal colonization has also been diagnosed in a few adults (tabulated in [8]), most of whom had undergone ileojejunal bypass or other major gastrointestinal surgery before contracting botulism.

Understanding of the microbiology of botulism has expanded in parallel to its clinical recognition. The species name *C. botulinum* was given originally to all bacteria that produced botulinum neurotoxin, despite their significant genetic and biochemical heterogeneity [10]. Another characteristic of the species was the ability to produce lipase. In attempting to isolate *C. botulinum* from feces or a wound, microbiologists routinely limited their search to those bacterial colonies radiating the “mother-of-pearl” sheen that results when lipase digests egg yolk agar [2]. The taxonomy of *C. botulinum* was tested after Giménez and Ciccarelli [11], in soil collected from a cultivated field in western Argentina, discovered a clostridium that produces the novel type G botulinum neurotoxin but not lipase. Because of its differences, this bacterium was later renamed from *C. botulinum* type G to *Clostridium argentinense* [12].

In 1979, an Acoma (Native American) baby in New Mexico was the first to be diagnosed with type F infant botulism, initially thought to be caused by *C. botulinum* [13]. On reexamination of the infant’s fecal culture some years later, Hall and colleagues at the Centers for Disease Control [14] segregated a nontoxic, lipase-producing bacterium resembling *C. botulinum* from the true pathogen, an isolate of *Clostridium baratii* that produced type F botulinum neurotoxin but not lipase. At least 3 infants and 6 adults scattered across the United States have been diagnosed with type F botulism resulting from intestinal colonization with *C. baratii* [15, 16, and personal communication, Charles Hatheway, Centers for Disease Control and Prevention, Atlanta, GA). There have been additional cases of type F botulism in the United States in which no pathogen was isolated, but only clostridia that produced lipase were examined for toxicity. Neurotoxic *C. baratii* has been found only in the feces of patients with human botulism; as a clostridium, it probably resides in soil, and its ability to contaminate foods remains unknown.

Shortly after the discovery of neurotoxic *C. baratii* in North America, Aureli et al. at the Italian National Reference Center for Botulism [17] isolated a strain of *Clostridium butyricum* that produced botulinum neurotoxin type E from the feces of 2 babies with infant botulism who lived in Rome. The first infant presented with an acute abdomen. Abundant ascites and a small ileocecal intussusception were found at laparotomy. Although it was not mentioned in the case report, the lead point of the intussusception was a Meckel’s diverticulum (Giovanna Franciosa, personal communication). In extensive testing of soils and foods in and around Rome, including honey fed to both infants, the investigators did not find any clostridia that produced type E botulimum toxin [18].

The province of Manji [south of the Shandong Peninsula in China] is the most magnificent and the richest that is known to the Eastern world. . . . On both sides of the causeway [the Grand Canal] there are very extensive marshy lakes, the waters of which are deep, and may be navigated. [1]

In 1994 at Guanyun in Jiangsu Province of eastern China, botulism killed 3 of 6 residents who became ill after eating a
paste of fermented soybeans and winter melon. The paste was contaminated with \(C. \text{butyricum}\) that produced type E neurotoxin [19, 20]. Since the typical incubation period for foodborne botulism is <2 days, an incubation period of up to 14 days in this outbreak [19] suggests that some patients may have had botulism from intestinal colonization. In the aftermath of the Guanyun outbreak, a team of Chinese and Japanese investigators reviewed clostridia archived from prior outbreaks of type E botulism in China. They identified isolates of neurotoxigenic \(C. \text{butyricum}\) from 2 outbreaks of type E botulism caused by soybean dishes in 1973 and 1983 [21]. These outbreaks occurred ~100 miles west of Guanyun, adjacent to Lake Weishan, which abuts the Grand Canal in Shandong Province. Neurotoxigenic \(C. \text{butyricum}\) was cultured from soil obtained around the home of patients with botulism in Guanyun and in 3 fields surrounding Lake Weishan [21]. These isolates varied in their ability to ferment inulin, which implies the existence of several toxigenic strains.

The kingdom of Guzzerat [Gujrat], which is bounded on the western side by the Indian Sea . . . affords harbour to pirates . . . who, when in their cruises they seize upon a traveling merchant, immediately oblige him to drink a dose of sea-water, which by its operation on the bowels, discovers whether he may not have swallowed pearls or jewels, upon the approach of the enemy, in order to conceal them. [1]

In 1996, 34 students at a boarding school in Gujrat, India, contracted botulism [22]. Three died before they could be hospitalized. The students had eaten a crisp made of gram (legume) flour that was contaminated with neurotoxigenic \(C. \text{butyricum}\). The botulinum toxin type of the isolate was not specified in.
Recent sporadic cases of botulism associated with *Clostridium butyricum* have occurred in patients hospitalized near Venice and the Po River delta [23]. Reproduced with permission of the National Gallery of Art, Washington, DC.

Pursuing, therefore, their intended route, they at length reached . . . finally to Venice, at which place . . . they safely arrived in the year 1295. [1]

In this issue of *Clinical Infectious Diseases*, Fenicia et al. [23] report 3 additional cases of botulism associated with *C. butyricum* that produces type E botulinum neurotoxin. These cases expand upon this laboratory’s remarkable discovery of neurotoxigenic *C. butyricum* in 1984 [17]. All 3 patients were hospitalized within 75 miles of each other near the Po River delta in northern Italy. In addition to a baby near Venice with infant botulism, the authors describe a 9-year-old boy and a 19-year-old woman who were, like the first patient with type E infant botulism in Rome [17], hospitalized with an acute abdomen and bulbar palsy. Surgeons removed an inflamed Meckel’s diverticulum and abundant ascites from both older patients.

The evidence that *C. butyricum* caused botulism in the boy and the young woman is almost conclusive. *C. butyricum* that produced type E botulinum neurotoxin was cultured from the feces of each patient. Unfortunately, type E neurotoxin was not detected in either serum or feces, because, it is suspected, samples were too small or were delayed in shipment. As the authors regret, the Meckel’s diverticula and ascites were not saved for testing. Their struggle to obtain definitive specimens is a recurrent theme in the laboratory diagnosis of botulism throughout the world.

Other than neurotoxigenic *C. butyricum*, what else connects these far-flung locales in northern Italy, eastern China, and western India? The Venetian Marco Polo, who described his journey in the late thirteenth century from Italy across central
Asia and his return homeward along the coast of India (figure 1) [1]. We allude to the voyage of Marco Polo for its astonishing breadth, spanning >5000 miles from the Adriatic Sea to the Yellow Sea, from the Grand Canal of Venice (figure 2) to the Grand Canal of China (figure 3). The discovery of neurotoxigenic \textit{C. butyricum} at the western and eastern extremes of his journey suggests that the species’ habitat includes soils throughout Eurasia, home to most of humanity. (We do not imply that Marco Polo sowed spores of \textit{C. butyricum} during his travels, prefiguring the legendary John Chapman who acquired the nickname “Johnny Appleseed” by broadcasting seed throughout the eastern United States in the early nineteenth century.)

Does an organism currently found only in Eurasia pose any threat in North America? The only type E botulism outbreak in the United States not associated with aquatic foods occurred in San Francisco in 1941 [4]. Botulism was caused by toxic imported Yugoslavian mushrooms, from which no organism was isolated [24]. One of the 3 cases occurred in a child who died after receiving the standard therapy of the day, bivalent AB botulinum antitoxin that did not contain neutralizing antibody against type E neurotoxin. (Similarly, contemporary trivalent antitoxin offers limited protection against type F botulism [25].)

Could these 1941 type E cases in California have been caused by neurotoxigenic \textit{C. butyricum}, now identified as an agent of botulism in Italy (located just across the Adriatic Sea from Yugoslavia)? Routine testing may not have detected lipase-negative \textit{C. butyricum} as a source of neurotoxin. Type E botulism caused by eating mushrooms has also occurred in the former Soviet Union, including the Amur region that borders northern China [26, 27]. Which clostridia caused these outbreaks?

Fermented soybeans were implicated in all 3 Chinese outbreaks of botulism due to \textit{C. butyricum}, whereas another legume product was contaminated in the Indian outbreak. In future cases of type E botulism, any Eurasian fruits or vegetables eaten before illness, particularly legumes and mushrooms, should be examined for neurotoxigenic \textit{C. butyricum}.

\textit{C. baratii} cause type F botulism or other clostridioses outside of North America? A strain of \textit{C. baratii} that produced \textit{Clostridium difficile} toxins A and B was isolated recently from...
an elderly Italian with diarrhea [28]. In the first reported infant botulism case in Hungary, a fecal isolate of *C. baratii* produced a heat-labile toxin that was lethal to mice but was not neutralized by type A, B, C, D, or E botulinum antitoxins [29]. The infant was fed honey repeatedly, but the origin and microbiology of the honey were not described. Perhaps this honey originated in the Americas and contained *C. baratii*. In the current global marketplace, other European infant botulism cases have been associated with eating honey imported from as far away as Argentina [30].

Table 1 summarizes current knowledge of the clostridia that produce botulinum neurotoxins, the types of human botulism that they cause, and their implicated sources, food or soil, in cases of botulism.

The presence of a Meckel’s diverticulum in 2 of the recent Italian type E botulism patients is remarkable. Named for the German anatomist, J. F. Meckel the Younger, a Meckel’s diverticulum is a patent remnant of the omphalomesenteric duct that contains mucosa from the ileum and potentially any other part of the gastrointestinal tract [31]. The proportion of Italians with *C. butyricum*–associated botulism who also had Meckel’s diverticulum, at least 3 (60%) of 5, is >60 times the incidence of Meckel’s diverticulum found in Italy [32]. If a causal relation between Meckel’s diverticulum and botulism from *C. butyricum* is proven, it might be related to the Meckel’s diverticulum providing a favorable site for colonization, either as a blind sac conducive to anaerobic growth or as a source of a specific type of heterotopic tissue, or to the Meckel’s diverticulum serving as a lead point for intussusception, a condition not associated as yet with botulism due to *C. butyricum*. By causing segmental ischemia, intussusception might reduce the local redox potential and thereby promote clostridial germination.

Few constitutional factors that predispose to botulism are known [8]. Therefore, determining whether there is an association between Meckel’s diverticulum and intestinal colonization botulism, regardless of patient age or clostridial species, is important. However, prospective screening for Meckel’s diverticulum would be expensive and insensitive. The noninvasive diagnostic test with the highest yield, radionuclide scanning, relies on the presence of gastric mucosa in the Meckel’s diverticulum. Because gastric acidity can inhibit the germination of clostridial spores, it would not be surprising if clostridia colonized only those Meckel’s diverticula without gastric mucosa, which are thus undetectable by technetium scan.

The tympanic abdomen described in the Italian cases is a novel presentation for botulism and may reflect vigorous gas production by *C. butyricum*. The diagnosis of future cases may be similarly confounded by the presence of an acute abdomen, and paralysis may be mistaken as prostration from abdominal sepsis.

The existence of at least 2 heterogenous species of clostridia with the capability of producing type E botulinum neurotoxin is evidence for the mobility of the neurotoxin gene complex [10]. Nontoxigenic *C. butyricum* is a component of the normal intestinal microflora [33] and is currently used in Japan as a probiotic to prevent gastrointestinal and vaginal infections [34, 35]. Continued oversight will assure that medicinal *C. butyricum* remains nontoxic to its consumers.

Which other bacteria possess the genes for the botulinum neurotoxins? Are there additional neurotoxin types yet undiscovered that cause human botulism? How many cases of botulism go undiagnosed? For a limited time, the medical community may have a unique opportunity to answer these questions. As part of the World Health Organization’s effort to eradicate poliomyelitis, nearly all nations are tallying and obtaining fecal samples from their citizens with acute flaccid paralysis [36]. We suggest a modest expansion of this historic effort to maximize the global surveillance for botulism and other paralytic diseases. Supplementary analysis of fecal samples might broaden existing knowledge about established and novel bacteria that produce botulinum toxin and thus improve the diagnosis, treatment, prevention, and control of botulism. Seven hundred years after Marco Polo returned home, another international voyage awaits explorers.

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Table 1. Clostridia that produce botulinum neurotoxin.

<table>
<thead>
<tr>
<th>Clostridium</th>
<th>Neurotoxin types made</th>
<th>Produces lipase</th>
<th>Causes foodborne botulism</th>
<th>Causes intestinal colonization botulism in Infants</th>
<th>Adults</th>
<th>Clostridium isolated from*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. botulinum</em></td>
<td>A, B, C, D, E, F</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td><em>C. butyricum</em></td>
<td>E</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td><em>C. baratii</em></td>
<td>F</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>?</td>
</tr>
<tr>
<td><em>C. argentinense</em></td>
<td>G</td>
<td>N</td>
<td>?</td>
<td>?</td>
<td>Y</td>
<td>?</td>
</tr>
</tbody>
</table>

NOTE: N, no; Y, yes; ?, unknown.
* In associated botulism cases.

a *C. argentinense* was discovered in soil but to date has not been associated conclusively with botulism in humans or other animals.
References