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Update: Infant Botulism

THADDEUS F. MIDURA*

Microbial Diseases Laboratory, California Department of Health Services, Berkeley, California

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INTRODUCTION

Botulism is a severe neurological disease, characterized by a flaccid paralytic illness affecting humans and a variety of lower animals, which is caused by botulinum neurotoxin. The accepted concept of the pathogenesis of botulism has been that of an intoxication due to the ingestion of a preformed toxin in an improperly preserved food and, rarely, in vivo toxin production resulting in illness from a wound infection. Infants too young to eat foods that may contain toxin generally have been thought to be safe from this disease. In 1976, botulism in infants became appreciated as a distinct clinical entity when two cases that occurred within 4 months of each other were described by Pickett et al. (59). Laboratory and epidemiologic studies have shown that infant botulism results from the ingestion of botulinum toxin-producing organisms that colonize the intestine, with subsequent multiplication and toxin production. Almost 20 years have passed, and now infant botulism has become the most common form of botulism confirmed in the United States. Nationally, there have been more than 1,200 reported cases of infant botulism for the years 1976 to 1994.

* Mailing address: California Dept. of Health Services, 2151 Berkeley Way, Berkeley, CA 94704-1011. Phone: (510) 540-2249. Fax: (510) 540-2374.

FORMS OF BOTULISM

Three forms of botulism are recognized today as distinct clinical entities (29). Food-borne (classical) botulism is caused by the ingestion of preformed toxin in a food. Wound botulism is caused by growth of the organism and toxigenesis at the site of a wound, including infections in intravenous drug users (13). Infant botulism is caused by the absorption of toxin produced by toxigenic organisms that colonize the intestinal tracts of infants under 1 year of age (45).

On several occasions, another form of botulism similar to infant botulism and as yet unclassified has been associated with gastrointestinal infections in adults after broad-spectrum antibiotic treatment and either intestinal surgery or inflammatory bowel disease (14, 43).

ETIOLOGIC AGENTS OF INFANT BOTULISM

Clostridium botulinum

Clostridium botulinum is the taxonomic designation given to a group of anaerobic, gram-positive, sporeforming bacteria that produce a characteristic neurotoxin. The vegetative cells of *C. botulinum* are straight to slightly curved motile rods with oval subterminal spores.

Seven serologically differentiable types of botulinum neurotoxin are recognized. The seven toxigenic types have been assigned the letters A through G and often serve as convenient

clinical and epidemiological markers (32, 64). The designation of the botulinum toxin is identical to the designation of the producing strain. Rarely, some *C. botulinum* strains produce the major type-specific toxin as well as a minor toxin of another type, i.e., Ab, Af, Ba, and Bf (64). Such strains are identified by mouse bioassay testing to confirm that an isolate is a pure culture that produces both toxins.

Toxin types *C. botulinum* A, B, E, and F are well-established causes of human botulism, while C and D primarily cause illness in other animals. Type G has not been fully established as a cause of either human or animal disease.

Most cases of infant botulism are due to *C. botulinum* toxin type A or B. Several isolates from cases of infant botulism designated as type B also produced some F toxin, as determined by definitively identifying the *C. botulinum* organism and toxins by the mouse bioassay test (31). Recently, the first case of infant botulism to be caused by *C. botulinum* type C was reported in Japan (57).

Other Botulinum Toxin-Producing Clostridia

The first two cases of infant botulism that were described in Italy were also the first cases worldwide to have been caused by type E botulinum toxin (8). Interestingly, the organism that produced the type E botulinal toxin was identified by laboratory examinations as *Clostridium butyricum*, not *C. botulinum* (8, 26, 42).

An infant botulism case caused by botulinum toxin type F was reported from New Mexico (35). The organism that produced the neurotoxin was identified as *Clostridium baratii*, not *C. botulinum* (27). What other new botulinum toxin-producing organisms may be awaiting discovery?

CLINICAL ASPECTS

Clinical Symptoms

Infant botulism has a broad spectrum of clinical symptoms. The main clinical features are constipation (often the first sign and defined as 3 or more days without defecation), listlessness, lethargy, difficulty in sucking and swallowing, weak cry, pooled oral secretions, hypotonia, general muscle weakness, and loss of head control. The baby often appears "floppy." Neurologic findings can include ptosis, ophthalmoplegia, sluggish pupillary reaction to light, flaccid expression, dysphagia, weak gag reflex, and poor anal sphincter tone (2, 3, 5). Infant botulism has a spectrum of clinical severity that ranges from a mild infection to fulminant, even fatal illness. The prognosis is excellent when the onset of illness is sufficiently gradual to permit hospitalization. The incubation period for infants is estimated to be from 3 to 30 days (3).

Diseases and Conditions Confused with Infant Botulism

Infant botulism may be difficult to recognize in its early stage. Diagnoses considered for patients whose illnesses were subsequently confirmed as infant botulism include sepsis, pneumonia, hypotonia of unknown etiology, failure to thrive, myasthenia gravis, poliomyelitis, Guillain-Barré syndrome, brain stem encephalitis, meningitis, hypothyroidism, and disorders of amino acid metabolism. Even today, suspected sepsis remains the most frequent admission diagnosis (1–3, 5).

Management of Hospitalized Patients

At present, treatment involves meticulous supportive and respiratory care. Most infants require nasogastric or nasojejunal feeding, and some need mechanical ventilation. Antibiotic therapy is not part of the treatment of uncomplicated infant botulism because the toxin can be released into the intestinal lumen upon vegetative cell death and lysis. Management of patients generally has not involved the use of botulinum antitoxin. The currently available botulinum antitoxin is a horse serum-derived product that has side effects of serum sickness, anaphylaxis, and potential life-long sensitization to equine proteins (3). Because of the favorable outcome in infant botulism without the use of antitoxin and the risks inherent in administering equine antitoxin, its use is not recommended. A clinical trial of botulinum immune globulin, a human-derived botulinum antitoxin, for the treatment of infant botulism is being conducted by the California State Department of Health Services and funded by the federal Food and Drug Administration (25). The therapeutic rationale is to inactivate any toxin in the circulation and extracellular fluid before the toxin can bind to nerve endings, including all toxin subsequently absorbed from its site of production in the large intestine. The study is planned to enroll approximately 120 patients. The recovery of the nerve function in the infant's muscle fibers requires regeneration of terminal motor neurons and the formation of new motor end plates.

Hospitalization Costs

According to Arnon (1–3), infant botulism is a costly illness. The hospital stay for infant botulism cases averages about 1 month but differs by toxin type, with type B cases being hospitalized a mean of 3.8 weeks and type A cases being hospitalized a mean of 5.4 weeks. Illness caused by type A toxin is more severe than that caused by type B toxin. Mean 1990 hospital costs in California exceeded $80,000 per case, and the patient with the most protracted illness was hospitalized for 10 months in 1988 at a cost of more than $635,000.

Link to Sudden Infant Death Syndrome

A link between the fulminant type of infant botulism and sudden infant death syndrome (SIDS) was noted in California because of a similarity between the sudden respiratory arrest of an infant botulism patient and SIDS. Necropsy specimens from SIDS cases were tested for the presence of *C. botulinum* organisms and toxins (4, 6). *C. botulinum* organisms were found in 10 of 211 SIDS cases (4.9%), and botulinum toxin was detected in 2 specimens of the 10 culture-positive cases. In 1983, *C. botulinum* type A was detected in a case of SIDS that occurred in Canada (33). In a study of necropsy specimens in Switzerland, *C. botulinum* toxin types A, B, C, F, and G were reported to have been isolated from 9 of 59 SIDS cases (65). In some of the specimens, the toxin types were very unusual, in that type C and type G were detected. In a study conducted in Australia over a 10-year period from 1981 to 1990, both small and large intestine specimens from 248 SIDS cases were cultured specifically for *C. botulinum*. However, because no specimens were positive, the investigators concluded that botulism was not a significant factor in the cause of sudden death (11). Although infant botulism was not associated with SIDS in Southern Australia, it can explain a small number of SIDS cases in North America and Europe.

LABORATORY CONFIRMATION OF BOTULISM

Collection of Specimens

Suitable clinical specimens for botulinum toxin detection are stools, serum, enema fluid, stomach contents, and autopsy sections of the small and large intestine.

In infant botulism, the specimens of choice are stools and enema fluids, as they are likely to be the only specimens containing viable *C. botulinum* or its toxin. Serum specimens from infant cases are only rarely toxin positive. Stool and serum specimens should be collected as soon as the diagnosis of infant botulism is suspected (28, 44).

A passed stool specimen is preferred for botulinum toxin detection and for isolation of *C. botulinum*. If a passed stool is not available, a specimen obtained after an enema with sterile, nonbacteriostatic water can be used. The volume of water used should be limited so that toxin in the stools is not diluted unnecessarily. If possible, 25 g or ml of stool or enema effluent should be collected in a sterile container. Even if it is small or colorless, the specimen initially obtained should be submitted for testing. Stool specimen collection should be continued until definitive identification can be made or ruled out. The specimen should be kept refrigerated until shipment.

Serum specimens for the botulinum toxin assay may be collected from suspected cases during the acute and convalescent stages of the disease. Two milliliters or more of serum should be collected in a sterile tube, and the specimen should be refrigerated until shipment.

Other samples that may be collected during an epidemiologic investigation and considered for laboratory testing include foods ingested by the infant and any other suspect item that could have served as a source of *C. botulinum*: for example, open containers of honey and house dust (44).

Shipment of Specimens to the Laboratory

All specimen containers should be properly labeled to indicate the patient's name, type of specimen, and date of collection. Since some medications, such as pyridiostigmine, can interfere with botulinum toxin testing, a list of medications that the infant is receiving should also be submitted. All specimens should be transported to the laboratory in insulated containers with cold packs to maintain a temperature of 4°C. During an infant botulism investigation, the State Health Department or the Centers for Disease Control and Prevention (CDC) can arrange for epidemiological investigations and for specimen testing at qualified laboratories using botulism antitoxin reagents received from CDC. The submitting laboratory must comply with all federal labeling and packaging regulations.

Laboratory Testing of Specimens

The diagnosis of infant botulism is established by the identification of botulinum toxin and organisms in the stool specimens. The detection of botulinum toxin in serum is useful in cases of food-borne and wound botulism. However, serum specimens from infant cases are rarely positive for circulating botulinum toxin (30, 58). Stools from ill infants have contained high toxin levels and up to 10^8 organisms per g (42, 58, 76). *C. botulinum* toxin and the organism can continue to be excreted long after the onset of symptoms and the recovery of the infant, but eventually, the infant is cleared of *C. botulinum* (47). The mouse bioassay is used to test for the presence of toxin in stools and serum (i.e., to demonstrate the presence of a substance that is toxic to mice, inactivated by heating to a boiling waterbath temperature for 10 min [not serum specimens], and neutralized by a specific monovalent botulinum antitoxin in a mouse toxin neutralization test) (20). Monovalent antitoxin preparations are obtained from CDC. Although several serological methods for toxin detection, such as enzyme-linked immunosorbent assay and PCR, have been reported (18, 56, 60, 71), reagents for those procedures generally are not available to other interested laboratories, and no interlaboratory evaluation of these tests for diagnostic investigation has been done. Therefore, the mouse bioassay, for which common neutralization reagents are available, remains the accepted procedure for definitive diagnostic purposes. No diagnostic technique to identify botulinum toxin bound at the neuromuscular junction is available.

C. botulinum can be isolated from stool, food, and other specimens by using anaerobic procedures and special enrichment techniques that have been well described (12, 20, 21, 28, 29, 30, 44, 48). All of the organisms capable of producing botulinum neurotoxins of types A through F possess the ability to produce lipase, which can be detected as an iridescent film surrounding the colony growth on egg yolk agar media (12, 20, 29). This characteristic has been a marker for isolating the various strains of *C. botulinum* until the recognition of the latest toxin-producing type G. *C. botulinum* type G organisms are not only lipase negative but also asaccharolytic. Neurotoxigenic *C. butyricum* and neurotoxigenic *C. baratii* are lipase negative. The usual screening method should include toxicity tests on enrichment cultures. This method should reveal the presence of toxigenic organisms by the mouse bioassay regardless of their lipase reaction. The procedure then requires the isolation of a lipase-negative organism that produces the toxin (27, 42, 67). The identity of toxin-producing organisms can be established by conventional cultural and biochemical procedures, as well as by gas chromatography (51). The toxin type of each pure culture isolate again is determined by the mouse toxin neutralization test.

PATHOGENICITY

Nature of the Neurotoxins

Publications and reviews on the molecular properties and pharmacological action of botulinum neurotoxins are available (29, 61, 62, 68). The active botulinum neurotoxins are proteins consisting of two polypeptide chains of approximately 100,000 and 50,000 Da joined by a disulfide bridge. The details of their structure have been reviewed recently by DasGupta (17). All toxin types are similar. They are synthesized as a single peptide with relatively low biological activity, requiring cleavage by a proteolytic enzyme to form the highly active dichain molecule. In culture, the neurotoxins exist as complexes with nontoxin proteins. The toxin in the complex is rather stable, especially under acidic conditions (pH 3.5 to 6.5), but the complex dissociates under slightly alkaline conditions, and the biological activity is readily inactivated in this state. The neurotoxin can be separated from the nontoxic components and purified by ion-exchange chromatography.

Pathogenesis

All four forms of botulism cause illness through a common pathway regardless of the manner in which the toxin gains access to the body. Botulinum toxins are absorbed from the intestinal tract or the infected wound site and are carried via the lymph and from the intestinal tract by the bloodstream to the neuromuscular endings. Toxin types differ in their affinity for nerve tissue, with type A having the greatest affinity. Bot-

ulinum neurotoxin acts by blocking release of acetylcholine at the neuromuscular junction in a three-step process: (i) the toxin molecule binds to receptors on the nerve ending; (ii) the toxin molecule, or a portion of it, is internalized; and (iii) within the nerve cell, the toxin interferes with the release of acetylcholine, which is needed to excite the muscle (62).

The mechanism of blocking of the release of acetylcholine has recently been elucidated. The active portion of the neurotoxin that has gained entrance to the nerve ending has peptidase activity that is specific for proteins that form the vesicle structures that contain the neurotransmitter and are involved in exocytosis. The action of the toxin prevents exocytosis of the neurotransmitter (50). Recovery of the nerve function in muscle fibers requires regeneration of terminal motor neurons and formation of new motor end plates.

Mouse Models

Using a mouse model system of intestinal colonization, Sugiyama and coworkers (10, 49, 69) have demonstrated that the intestinal microflora of adult animals ordinarily prevents colonization of the intestines by *C. botulinum*. When 10^6 type A spores were administered, they failed to colonize the intestine of normal adult mice, whereas after treatment for 2.5 days with a combination of oral antibiotics, erythromycin and kanamycin, half the mice could be colonized by just 2×10^4 spores. When the antibiotic-treated mice were placed in cages with normal mice, they lost their susceptibility to intestinal colonization after 3 days (10). In addition, adult germ-free mice could be colonized intestinally by just 10 *C. botulinum* type A spores. When the germ-free adult mice were placed in a room with conventional mice, the formerly germ-free animals became resistant to colonization by 10^5 spores after 3 days (49).

By contrast, normal infant mice were susceptible to intestinal colonization by *C. botulinum* spores (69). Like human infants, the normal infant mice were susceptible to colonization only for a limited period (7 to 13 days of age). The susceptibility of the infant mice peaked between days 8 and 11 in a pattern similar to the peaking of susceptibility between 2 and 4 months of age in human botulism (4, 69). The infective dose of spores for infant mice was much smaller than that of their antibiotic-treated adult counterparts; the 50% infective dose for normal infants was only 700 spores. In one experiment, 10 spores colonized an infant mouse (69).

The minimum infective dose of *C. botulinum* spores for human infants is not known, but on the basis of exposure to spore-containing honey, it has been estimated to be as low as 10 to 100 spores (7).

INCIDENCE

Infant botulism has been reported from countries on all the inhabited continents except Africa (19, 52–55, 74). The highest incidence has been reported in the United States, probably because of physician awareness. The yearly number of infant botulism cases now exceeds the number of food-borne and wound botulism cases combined. From 1976 through 1993, only five states did not report any cases. These were Maine, Minnesota, New Hampshire, Rhode Island, and South Carolina. In 1993, there were 60 cases of type A and type B infant botulism reported (48% type A and 52% type B). Of these, 29 cases were reported from California (13), which typically reports about half the cases in the United States. In 1994, there were 30 cases of infant botulism in California, which were equally distributed between *C. botulinum* type A and type B.

ENVIRONMENTAL ASPECTS

Habitat of *C. botulinum*

C. botulinum is commonly found in soil samples and aquatic sediments throughout the world. Smith and Sugiyama (64) and Hauschild (32) covered this subject well in their discussions on the natural occurrence of *C. botulinum*. Several soil surveys for *C. botulinum* were conducted in the United States. In the most recent survey (63), type A, B, C, D, and E strains were found. *C. botulinum* type A was found predominantly west of the Mississippi River, and type B was found predominantly east of it. Type F strains have been isolated from marine sediments from the Pacific Coast and from crabs in Chesapeake Bay (64). There is a strong association between the distribution of spore types and the causative toxin type of infant botulism. In the United States, type A infant botulism cases predominate from the Mississippi River westward, while type B cases predominate in the east (2). For example, the high incidence of infant botulism caused by type B strains in southeastern Pennsylvania indicates the regional occurrence of type B organisms as the type responsible for 43 of 44 cases reported in one study (40). Dodds (19) has reviewed the association of cases of infant botulism and the most prevalent toxin type in the soils of other countries.

Sources of *C. botulinum* for Infants

Two of the most recognized potential sources of *C. botulinum* spores are honey and dust (3). For most cases of infant botulism, the source of *C. botulinum* has not been found. Since the infection occurs in the intestinal tract, numerous food products that either were consumed by patients or would be consumed by infants were tested by various investigators. Infant foods such as sugar, cereal, dried-milk formula, vitamin supplements, and canned fruits and vegetables were not found to be significant sources of *C. botulinum* (34, 38, 44). The finding of *C. botulinum* spores in light and dark corn syrup samples indicated the possibility that this food was a source of the organism (38), but a subsequent study concluded that corn syrup on the market was not a food source of *C. botulinum* spores for infants (39). The ingestion of corn syrup is no longer considered a risk factor for infant botulism (3).

Honey is the only food item in common that is implicated as a source of *C. botulinum* organisms for ill infants (15, 22, 34, 53, 72, 73). Spores of *C. botulinum* are present naturally in some samples of honey (36, 46, 70).

In every instance, presently 31 cases, in which *C. botulinum* was isolated from a honey-fed infant who then developed infant botulism, the toxin type (A or B) of the organism in the honey was the same as the toxin type (A or B) responsible for the infant's illness (7). It should be noted that only a small minority of infant botulism patients have a history of honey consumption. Also, it should be emphasized that no investigator to date has reported the presence of preformed botulinum toxin in any honey sample. Other family members who had consumed the same honey that was fed to ill infants have remained well. During the early epidemiological and laboratory studies in California, approximately 30% of the cases were associated with the ingestion of honey containing *C. botulinum* spores (7, 15, 46). However, in recent years, less than 5% of California cases have been fed honey before the onset of illness, an indication of that state's successful educational program.

Studies have also implicated the natural environment as a source of *C. botulinum* spores. The organism causing infant botulism is usually the type found in the soil of the area where

the illness occurs (37, 41, 66). *C. botulinum* has been isolated from environmental samples such as yard soil and vacuum cleaner dust in comparable frequencies from both case-associated and control homes in California, but in every instance in which it was isolated from a case home, the environmental isolate had the same toxin type as the isolate from the patient (7). In Australia, the illnesses were associated with the organisms found in yard soils and drinking water (52). In Japan, *C. botulinum* type A organisms were detected not only in the honey fed to the infant before onset of illness but also in soil samples taken at the entry to the home and vacuum cleaner dust (72). Little is known about the ecology of neurotoxigenic strains of *C. butyricum* and *C. baratii*. After the recognition of *C. butyricum* infant botulism cases in Italy, Creti and coworkers (16) attempted to find the organism in the environment but were unable to do so. These rare toxigenic organisms are not likely to be found independent of cases of botulism which signal their presence.

RISK FACTORS ASSOCIATED WITH INFANT BOTULISM

Spika and coworkers (66) studied the risk factors for infant botulism in the United States other than California. The disease was found more often in infants who were white, had older mothers, and had mothers with more years of formal education. An important finding to these investigators was that illness in infants less than 2 months old was epidemiologically different from that in infants 2 months old or older. One risk factor for infants less than 2 months old was living in a rural area or on a farm; breast feeding was not a risk factor. There were three risk factors for infants 2 months of age and older: less than one bowel movement daily for at least 2 months, breast-feeding, and ingestion of corn syrup. One hypothesis that was considered to explain the differences between the two groups was that breast-feeding may prolong susceptibility to colonization by *C. botulinum*. Infants who were 2 months old or younger did not have a developed anaerobic intestinal microflora. One other factor that was considered was the disturbance of soils by agricultural and construction activities preceding the infant's illness. Possibly, disturbance of soils by earthquakes should also be included. Interestingly, there appeared to be a cluster of three infant botulism cases in Southern California following the January 1994 earthquake in the Northridge area, north of Los Angeles. Several parents of infant botulism cases remarked to the press about the clouds of dust that were encountered prior to the illness of their infants.

In Colorado, three infants developed type A botulism within a 6-month period in 1981. The families lived near each other in mobile homes. A common link was that two of the infants had used the same crib. The first infant had used the crib until 1 month after his illness. The crib then was used by a second nonaffected infant and then given to a third infant, who later developed infant botulism. However, the common finding in all three cases was the presence of a *C. botulinum* type A organism in environmental samples, including the crib, soils, and household dust (37).

In Pennsylvania, a case-control study (41) of risk factors associated with infant botulism revealed that the infants were white, were primarily breast-fed, and had type B botulism. More than half of the fathers had daily contact with soil.

In California studies, Arnon (1) reported that cases have occurred in all major racial and ethnic groups. Therefore, it appears that acquisition of illness does not depend upon racial differences in susceptibility. Identified risk factors for illness include the ingestion of honey and a slow intestinal transit time (less than one stool per day). Breast-feeding appears to slow the onset of infant botulism and to diminish the risk of respiratory arrest in infants in whom the disease develops. Formula-fed infants as well as breast-fed infants were susceptible to infant botulism. The mean age of onset of infant botulism for formula-fed babies was significantly less than that for breast-fed infants. Onset of infant botulism occurs at a significantly younger age in formula-fed infants (7.6 weeks) than in breast-fed infants (13.7 weeks), perhaps reflecting the earlier availability in formula-fed infants of suitable ecologic niches and the formula-fed infants' lack of the immune factors contained in human milk (1).

The role of breast-feeding as a risk factor is controversial. It is not clear whether it in fact increases the risk of infection or whether it offers some protection by slowing the progress of the illness. Bernshaw (9) reviewed the most popular hypothesis of the causes of SIDS, including severe infant botulism, and tried to explain through the scientific literature how breast-fed infants appear to be protected. In this review, she observed that the scientific literature lacks uniformity in the definitions of breast-feeding, whether partial and inclusive. This lack of uniformity may well be the cause of the differing conclusions in the risk assessment studies.

Diet may be one of the most important factors that influence the composition of the normal microflora. In comparison with the adult flora, the infant flora has fewer genera and species. The dominant members vary, depending in part on whether the infant is fed only breast milk, only formula milk, or a mixture of the two. Also, the composition of the intestinal flora is changed when solid foods such as cereals become part of the infant's diet. The normal human infant microflora contains several bacterial species, mainly *Bifidobacterium* and *Bacteroides* spp., that in vitro can inhibit the multiplication of *C. botulinum* (75). *C. botulinum* is not part of the normal flora of an infant's intestinal tract. Therefore, people are always exposed to spores with their occurrence in the local environment and the foods in their diet. Since infant botulism is not transmitted person to person, the risk of colonization is restricted to susceptible infants less than 1 year old and the rare adult whose special circumstances provide intestinal conditions that are amenable to the germination and outgrowth of spores.

CONCLUDING COMMENTS

In 1976, infant botulism was recognized as a distinct clinical and epidemiological entity. The purpose of this article is to present what has been learned about the disease in the 20 years since its recognition. Infant botulism occurs in infants between 1 week and 12 months of age and results from the absorption of botulinum toxin produced by toxigenic clostridia that colonize the intestinal tract. The clinical spectrum ranges from infants who are asymptomatic carriers through those who develop various degrees of paralysis or even sudden death. Meticulous supportive and respiratory care in an intensive-care setting is indicated in case management, whereas the use of antibiotics and equine botulinum antitoxin is not. Spores may be present in honey and dust, environmental factors associated with infection. Rarely, another form of botulism similar to infant botulism can occur in adults with anatomical or microbiological alterations to the gastrointestinal tract. During the investigations of infant botulism cases, it was found that clostridial species other than *C. botulinum* can produce the botulinum toxin that causes infant botulism. In the near future, some of the things that may lead to the successful control and treatment of infant botulism are (i) effective educational programs to increase physician awareness of the disease world-

wide, (ii) the use of modern technology for development of rapid, sensitive laboratory methods to replace the mouse bioassay for toxin detection and for the identification of organisms other than *C. botulinum* that produce botulinum toxin, and (iii) the ready availability of a nonequine antitoxin, such as human botulism immune globulin.

Continuing applications of modern microbiology techniques (18, 23, 24) for the detection of toxin (such as enzyme-linked immunosorbent assay) and molecular techniques (such as PCR) for the detection of nucleotide sequences will facilitate future studies on the incidence, risk factors, modes of transmission, and pathogenesis of the botulinum toxin-producing pathogens causing all forms of botulism.

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