Botulinum Toxin as a Biological Weapon
Medical and Public Health Management

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Objective The Working Group on Civilian Biodefense has developed consensus-based recommendations for measures to be taken by medical and public health professionals if botulinum toxin is used as a biological weapon against a civilian population.

Participants The working group included 23 representatives from academic, government, and private institutions with expertise in public health, emergency management, and clinical medicine.

Evidence The primary authors (S.S.A. and R.S.) searched OLDMEDLINE and MEDLINE (1960–March 1999) and their professional collections for literature concerning use of botulinum toxin as a bioweapon. The literature was reviewed, and opinions were sought from the working group and other experts on diagnosis and management of botulism. Additional MEDLINE searches were conducted through April 2000 during the review and revisions of the consensus statement.

Consensus Process The first draft of the working group's consensus statement was a synthesis of information obtained in the formal evidence-gathering process. The working group convened to review the first draft in May 1999. Working group members reviewed subsequent drafts and suggested additional revisions. The final statement incorporates all relevant evidence obtained in the literature search in conjunction with final consensus recommendations supported by all working group members.

Conclusions An aerosolized or foodborne botulinum toxin weapon would cause acute symmetric, descending flaccid paralysis with prominent bulbar palsies such as diplopia, dysarthria, dysphonia, and dysphagia that would typically present 12 to 72 hours after exposure. Effective response to a deliberate release of botulinum toxin will depend on timely clinical diagnosis, case reporting, and epidemiological investigation. Persons potentially exposed to botulinum toxin should be closely observed, and those with signs of botulism require prompt treatment with antitoxin and supportive care that may include assisted ventilation for weeks or months. Treatment with antitoxin should not be delayed for microbiological testing.

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THIS IS THE FOURTH ARTICLE IN A series entitled *Medical and Public Health Management Following the Use of a Biological Weapon: Consensus Statements of The Working Group on Civilian Biodefense*.[1-3] This article is the only one in the series to feature a biological toxin rather than a replicating agent. Botulinum toxin poses a major bioweapon threat because of its extreme potency and lethality; its ease of production, transport, and misuse; and the need for prolonged intensive care among affected persons.[4,5] An outbreak of botulism constitutes a medical emergency that requires prompt provision of botulinum antitoxin and, often, mechanical ventilation, and it constitutes a public health emergency that requires immediate intervention to prevent additional cases. Timely recognition of a botulism outbreak begins with an astute clinician who quickly notifies public health officials.

Botulinum toxin is the most poisonous substance known.[6,7] A single gram of crystalline toxin, evenly dispersed and inhaled, would kill more than 1 million people, although technical factors would make such dissemination difficult. The basis of the phenomenal potency of botulinum toxin is enzymatic; the toxin is a zinc proteinase that cleaves 1 or more of the fusion proteins by which neuronal vesicles release acetylcholine into the neuromuscular junction.[8]

It is regrettable that botulinum toxin still needs to be considered as a bioweapon at the historic moment when it has become the first biological toxin to become licensed for treatment of human disease. In the United States, botulinum toxin is currently licensed for treatment of cervical torticollis, strabismus, and blepharospasm associ-

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ated with dystonia. It is also used "off label" for a variety of more prevalent conditions that include migraine headache, chronic low back pain, stroke, traumatic brain injury, cerebral palsy, achalasia, and various dystonias.[9-13]

CONSENSUS METHODS

The working group included 23 representatives from academic, government, and private institutions with expertise in public health, emergency management, and clinical medicine. The 2 primary authors (S.S.A. and R.S.) conducted a literature search on use of botulinum toxin as a bioweapon. The OLDMEDLINE and MEDLINE databases were queried for all articles published between January 1960 and March 1999 that contained words referring to biological warfare (*bioterrorism*, *biowarfare*, *terrorism*, *war*, *warfare*, and *weapon*) in combination with terms related to *Clostridium botulinum* (*bacillus*, *botulin*, *botulinal*, *botulinum*, *botulinus*, *botulism*, *clostridia*, *clostridial*, and *Clostridium*). The articles identified in the databases were fully reviewed. In addition, published and unpublished articles, books, monographs, and special reports in the primary authors' collections were reviewed. Additional MEDLINE searches were conducted through April 2000 during the review and revisions of the consensus statement.

The first draft of the consensus statement was a synthesis of information obtained in the formal evidence-gathering process. Members of the working group provided written and oral comments about the first draft at their meeting in May 1999. Working group members then reviewed subsequent drafts and suggested additional revisions. The final statement incorporates all relevant evidence obtained in the literature search in conjunction with final consensus recommendations supported by all working group members.

The assessment and recommendations provided herein represent the best professional judgment of the working group based on currently available data and expertise. These conclusions and recommendations should be regularly reassessed as new information becomes available.

HISTORY OF CURRENT THREAT

Terrorists have already attempted to use botulinum toxin as a bioweapon. Aerosols were dispersed at multiple sites in downtown Tokyo, Japan, and at US military installations in Japan on at least 3 occasions between 1990 and 1995 by the Japanese cult Aum Shinrikyō. These attacks failed, apparently because of faulty microbiological technique, deficient aerosol-generating equipment, or internal sabotage. The perpetrators obtained their *C botulinum* from soil that they had collected in northern Japan.[14,15]

Development and use of botulinum toxin as a possible bioweapon began at least 60 years ago.[16,17] The head of the Japanese biological warfare group (Unit 731) admitted to feeding cultures of *C botulinum* to prisoners with lethal effect during that country's occupation of Manchuria, which began in the 1930s.[18] The US biological weapons program first produced botulinum toxin during World War II. Because of concerns that Germany had weaponized botulinum toxin, more than 1 million doses of botulinum toxoid vaccine were made for Allied troops preparing to invade Normandy on D-Day.[19,20] The US biological weapons program was ended in 1969-1970 by executive orders of Richard M. Nixon, then president. Research pertaining to biowarfare use of botulinum toxin took place in other countries as well.[21]

Although the 1972 Biological and Toxin Weapons Convention prohibited offensive research and production of biological weapons, signatories Iraq and the Soviet Union subsequently produced botulinum toxin for use as a weapon.[22,23] Botulinum toxin was 1 of several agents tested at the Soviet site Aralsk-7 on Vozrozhdeniye Island in the Aral Sea.[23,24] A former senior scientist of the Russian civilian bioweapons program reported that the Soviets had attempted splicing the botulinum toxin gene from *C botulinum* into other bacteria.[25] With the economic difficulties in Russia after the demise of the Soviet Union, some of the thousands of scientists formerly employed by its bioweapons program have been recruited by nations attempting to develop biological weapons.[25,26] Four of the countries listed by the US government as "state sponsors of terrorism" (Iran, Iraq, North Korea, and Syria)[27] have developed, or are believed to be developing, botulinum toxin as a weapon.[28,29]

After the 1991 Persian Gulf War, Iraq admitted to the United Nations inspection team to having produced 19 000 L of concentrated botulinum toxin, of which approximately 10 000 L were loaded into military weapons.[22,30] These 19 000 L of concentrated toxin are not fully accounted for and constitute approximately 3 times the amount needed to kill the entire current human population by inhalation. In 1990, Iraq deployed specially designed missiles with a 600-km range; 13 of these were filled with botulinum toxin, 10 with aflatoxin, and 2 with anthrax spores. Iraq also deployed special 400-lb (180-kg) bombs for immediate use; 100 bombs contained botulinum toxin, 50 contained anthrax spores, and 7 contained aflatoxin.[22,30] It is noteworthy that Iraq chose to weaponize more botulinum toxin than any other of its known biological agents.

Some contemporary analyses discount the potential of botulinum toxin as a bioweapon because of constraints in concentrating and stabilizing the toxin for aerosol dissemination. However, these analyses pertain to military uses of botulinum toxin to immobilize an opponent (William C. Patrick, unpublished data, 1998). In contrast, deliberate release of botulinum toxin in a civilian population would be able to cause substantial disruption and distress. For example, it is estimated that a point-source aerosol release of botulinum toxin could incapacitate or kill 10% of persons within 0.5 km downwind (William C. Patrick, unpublished data, 1998). In addition, terrorist use of botulinum toxin might be manifested as deliberate contamination of food. Misuse of toxin in this manner could produce either a large

botulism outbreak from a single meal or episodic, widely separated outbreaks.[31] In the United States, the Centers for Disease Control and Prevention (CDC) maintains a well-established surveillance system for human botulism based on clinician reporting that would promptly detect such events.[32]

MICROBIOLOGY AND VIRULENCE FACTORS

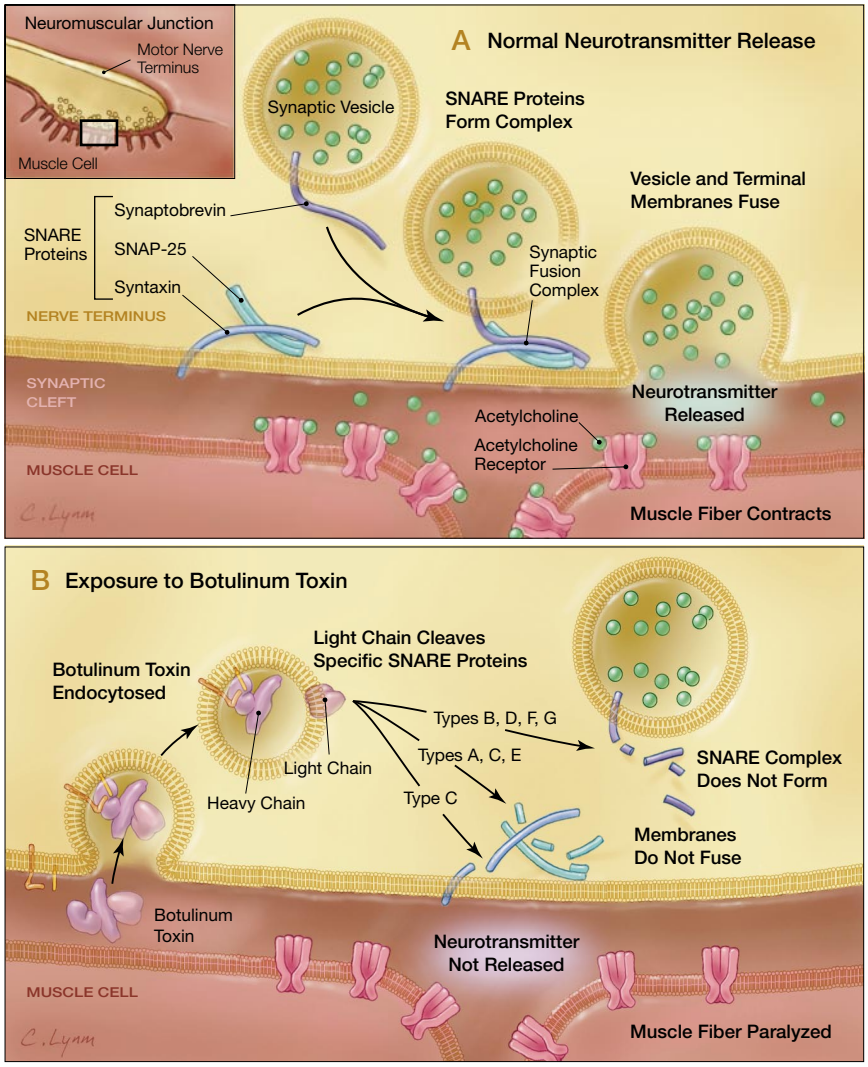
Clostridium botulinum is a spore-forming, obligate anaerobe whose natural habitat is soil, from which it can be isolated without undue difficulty. The species *C botulinum* consists of 4 genetically diverse groups that would not otherwise be designated as a single species except for their common characteristic of producing botulinum toxin.[33,34] Botulinum toxin exists in 7 distinct antigenic types that have been assigned the letters A through G. The toxin types are defined by their absence of cross-neutralization (eg, anti-A antitoxin does not neutralize toxin types B-G). The toxin types also serve as convenient epidemiological markers. In addition to *C botulinum*, unique strains of *Clostridium baratii* and *Clostridium butyricum* have the capacity to produce botulinum toxin.[35-37] Botulinum toxin is a simple dichain polypeptide that consists of a 100-kd "heavy" chain joined by a single disulfide bond to a 50-kd "light" chain; its 3-dimensional structure was recently resolved to 3.3 A.[38] The toxin's light chain is a Zn^{++}-containing endopeptidase that blocks acetylcholine-containing vesicles from fusing with the terminal membrane of the motor neuron, resulting in flaccid muscle paralysis (FIGURE 1).[8]

The lethal dose of botulinum toxin for humans is not known but can be estimated from primate studies. By extrapolation, the lethal amounts of crystalline type A toxin for a 70-kg human would be approximately 0.09-0.15 µg intravenously or intramuscularly, 0.70-0.90 µg inhalationally, and 70 µg orally.[10,39-41] Therapeutic botulinum toxin represents an impractical bioterrorist weapon because a vial of the type A preparation currently licensed in the United States contains only about 0.3% of the estimated human lethal inhalational dose and 0.005% of the estimated lethal oral dose.

PATHOGENESIS AND CLINICAL MANIFESTATIONS

Three forms of naturally occurring human botulism exist: foodborne, wound, and intestinal (infant and adult). Fewer than 200 cases of all forms of botulism are reported annually in the United States.[42] All forms of botulism result from absorption of botulinum toxin into the circulation from either a mucosal surface (gut, lung) or a wound. Botulinum toxin does not penetrate intact skin. Wound botulism and intestinal

Figure 1. Mechanism of Action of Botulinum Toxin

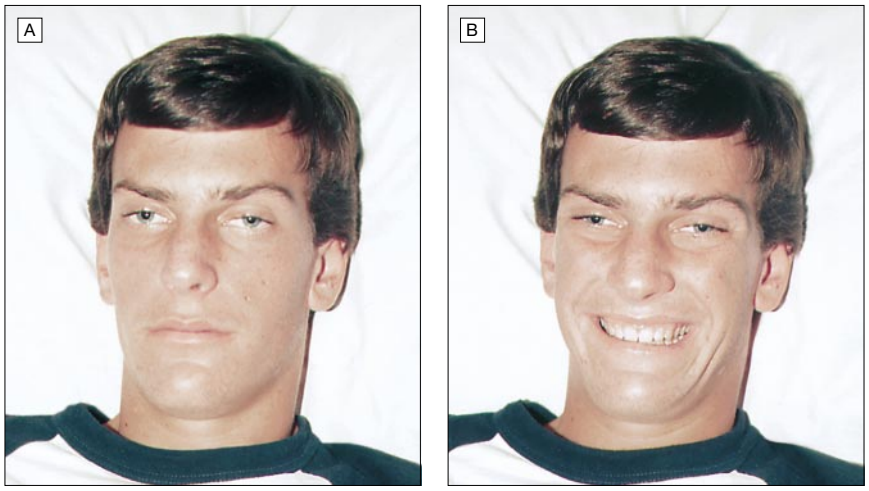


A, Release of acetylcholine at the neuromuscular junction is mediated by the assembly of a synaptic fusion complex that allows the membrane of the synaptic vesicle containing acetylcholine to fuse with the neuronal cell membrane. The synaptic fusion complex is a set of SNARE proteins, which include synaptobrevin, SNAP-25, and syntaxin. After membrane fusion, acetylcholine is released into the synaptic cleft and then bound by receptors on the muscle cell.

B, Botulinum toxin binds to the neuronal cell membrane at the nerve terminus and enters the neuron by endocytosis. The light chain of botulinum toxin cleaves specific sites on the SNARE proteins, preventing complete assembly of the synaptic fusion complex and thereby blocking acetylcholine release. Botulinum toxins types B, D, F, and G cleave synaptobrevin; types A, C, and E cleave SNAP-25; and type C cleaves syntaxin. Without acetylcholine release, the muscle is unable to contract.

SNARE indicates soluble NSF-attachment protein receptor; NSF, N-ethylmaleimide-sensitive fusion protein; and SNAP-25, synaptosomal-associated protein of 25 kd.

Figure 2. Seventeen-Year-Old Patient With Mild Botulism



A, Patient at rest. Note bilateral mild ptosis, dilated pupils, disconjugate gaze, and symmetric facial muscles. B, Patient was requested to perform his maximum smile. Note absent periorbital smile creases, ptosis, disconjugate gaze, dilated pupils, and minimally asymmetric smile. As an indication of the extreme potency of botulinum toxin, the patient had 40×10^{-12}g/mL of type A botulinum toxin in his serum (ie, 1.25 mouse units/mL) when these photographs were taken.

botulism are infectious diseases that result from production of botulinum toxin by *C botulinum* either in devitalized (ie, anaerobic) tissue[43] or in the intestinal lumen,[37] respectively. Neither would result from bioterrorist use of botulinum toxin.

A fourth, man-made form that results from aerosolized botulinum toxin is inhalational botulism. This mode of transmission has been demonstrated experimentally in primates,[39] has been attempted by bioterrorists,[14,15] and has been the intended outcome of at least 1 country's specially designed missiles and artillery shells.[22,30] Inhalational botulism has occurred accidentally in humans. A brief report from West Germany in 1962 described 3 veterinary personnel who were exposed to reaerosolized botulinum toxin while disposing of rabbits and guinea pigs whose fur was coated with aerosolized type A botulinum toxin. Type A botulinum toxin was detected in serum samples from all 3 affected individuals.[21]

Once botulinum toxin is absorbed, the bloodstream carries it to peripheral cholinergic synapses, principally, the neuromuscular junction, where it binds irreversibly. The toxin is then internalized and enzymatically blocks acetylcholine release (Figure 1). Accordingly, all forms of human botulism display virtually identical neurologic signs. However, the neurologic signs in naturally occurring foodborne botulism may be preceded by abdominal cramps, nausea, vomiting, or diarrhea.[44] These gastrointestinal symptoms are thought to be caused by other bacterial metabolites also present in the food[33] and may not occur if purified botulinum toxin is intentionally placed in foods or aerosols.

Botulism is an acute, afebrile, symmetric, descending flaccid paralysis that always begins in bulbar musculature. It is not possible to have botulism without having multiple cranial nerve palsies. Disease manifestations are similar regardless of botulinum toxin type. However, the extent and pace of paralysis may vary considerably among patients. Some patients may be mildly affected (FIGURE 2), while others may be so paralyzed that they appear comatose and require months of ventilatory support. The rapidity of onset and the severity of paralysis depend on the amount of toxin absorbed into the circulation. Recovery results from new motor axon twigs that sprout to reinnervate paralyzed muscle fibers, a process that, in adults, may take weeks or months to complete.[45,46]

Table 1. Symptoms and Signs of Foodborne Botulism, Types A and B*

	Cases, %
Symptoms	
Fatigue	77
Dizziness	51
Double vision	91
Blurred vision	65
Dysphagia	96
Dry mouth	93
Dysarthria	84
Sore throat	54
Dyspnea	60
Constipation	73
Nausea	64
Vomiting	59
Abdominal cramps	42
Diarrhea	19
Arm weakness	73
Leg weakness	69
Paresthesia	14
Signs	
Alert mental status	90
Ptosis	73
Gaze paralysis	65
Pupils dilated or fixed	44
Nystagmus	22
Facial palsy	63
Diminished gag reflex	65
Tongue weakness	58
Arm weakness	75
Leg weakness	69
Hyporeflexia or areflexia	40
Ataxia	17

*Data are from outbreaks of botulism reported in the United States in 1973-1974. The number of patients with available data varied from 35 to 55. Adapted from Hughes et al[44] with permission.

Patients with botulism typically present with difficulty seeing, speaking, and/or swallowing (TABLE 1 and TABLE 2). Prominent neurologic findings in all forms of botulism include ptosis, diplopia, blurred vision, often enlarged or sluggishly reactive pupils, dysarthria, dysphonia, and dysphagia.[5,44,47,48] The mouth may appear dry and the pharynx injected because of peripheral parasympathetic cholinergic blockade. Sensory changes are not observed except for infrequent circumoral and peripheral paresthesias from hyperventilation as a patient becomes frightened by onset of paralysis.

As paralysis extends beyond bulbar musculature, loss of head control, hy-

potonia, and generalized weakness become prominent. Dysphagia and loss of the protective gag reflex may require intubation and, usually, mechanical ventilation. Deep tendon reflexes may be present initially but diminish or disappear in the ensuing days, and constipation may occur. In untreated persons, death results from airway obstruction (pharyngeal and upper airway muscle paralysis) and inadequate tidal volume (diaphragmatic and accessory respiratory muscle paralysis).

Because botulism is an intoxication, patients remain afebrile unless they also have acquired a secondary infection (eg, aspiration pneumonia). The toxin does not penetrate brain parenchyma, so patients are not confused or obtunded. However, they often appear lethargic and have communication difficulties because of bulbar palsies (Figure 2). Botulism may be recognized by its classic triad: (1) symmetric, descending flaccid paralysis with prominent bulbar palsies in (2) an afebrile patient with (3) a clear sensorium. The prominent bulbar palsies can be summarized in part as "4 Ds": diplopia, dysarthria, dysphonia, and dysphagia.

Table 2. Symptoms and Signs of Inhalational Botulism in Order of Onset

Humans (n = 3)[21]	Monkeys (n = 9)[39]*
Third day after exposure	12-18 hours after exposure
Mucus in throat	Mild muscular weakness
Difficulty swallowing solid food	Intermittent ptosis
Dizziness	Disconjugate gaze
Fourth day after exposure	Followed by
Difficulty moving eyes	Severe weakness of postural neck muscles
Mild pupillary dilation and nystagmus	Occasional mouth breathing
Indistinct speech	Serous nasal discharge
Unsteady gait	Salivation, dysphagia
Extreme weakness	Mouth breathing
	Rales
	Anorexia
	Severe generalized weakness
	Lateral recumbency
	Second to fourth day after exposure
	Death in some animals

*After exposure to 4 to 7 monkey median lethal doses of botulinum toxin. The time to onset and pace of paralysis were dose-dependent. Adapted from Middlebrook and Franz[48] with permission.

EPIDEMIOLOGY

Early recognition of outbreaks of botulism, whether natural or intentional, depends on heightened clinical suspicion. Aerosol dissemination may not be difficult to recognize because a large number of cases will share a common temporal and geographical exposure and will lack a common dietary exposure. However, identification of the common exposure site initially may be difficult because of the mobility of persons exposed during the incubation period. Botulism and botulinum toxin are not contagious and cannot be transmitted from person to person. In contrast, a microbe intentionally modified to produce botulinum toxin might be contagious.

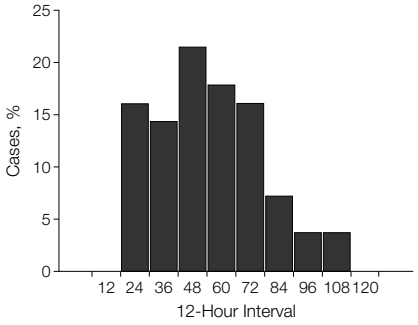
No instances of waterborne botulism have ever been reported.[42,49,50] Although the potency of botulinum toxin has led to speculation that it might be used to contaminate a municipal water supply, this scenario is unlikely for at least 2 reasons.[51] First, botulinum toxin is rapidly inactivated by standard potable water treatments (eg, chlorination, aeration).[52] Second, because of the slow turnover time of large-capacity reservoirs, a comparably large (and technically difficult to produce and deliver) inoculum of botulinum toxin would be needed.[53] In contrast with treated water, botulinum toxin may be stable for several days in untreated water or beverages.[52,54] Hence, such items should be investigated in a botulism outbreak if no other vehicle for toxin can be identified.

If food were deliberately used as a vehicle for the toxin, the outbreak would need to be distinguished from naturally occurring foodborne botulism. During the past 20 years, the epidemiology of foodborne botulism has expanded beyond its traditional association with home-preserved foods and now includes nonpreserved foods and public eating places,[47] features that could make terrorist use of botulinum toxin more difficult to detect. Characteristics of outbreaks of botulism include:

Incubation Period

The rapidity of onset and severity of botulism depend on the rate and amount of toxin absorption. Symptoms of foodborne botulism may begin as early as 2 hours or as long as 8 days after ingestion of toxin.[55,56] Typically, cases present 12 to 72 hours after the implicated meal. In 1 large foodborne outbreak, new cases presented during the ensuing 3 days at a fairly even rate before decreasing (FIGURE 3).[57] The time to onset of inhalational botulism cannot be stated with certainty because so few cases are known. Monkeys showed signs of botulism 12 to 80 hours after aerosol exposure to 4 to 7 multiples of the monkey median lethal dose.[39] The 3 known human cases of inhalational botulism had

Figure 3. Fifty-Nine Cases of Botulism, by Interval Between Eating at a Restaurant and Onset of First Neurologic Symptom—Michigan, 1977



Reproduced from Terranova et al[57] with permission of Oxford University Press.

Box 1. Features of an Outbreak That Would Suggest a Deliberate Release of Botulinum Toxin

Outbreak of a large number of cases of acute flaccid paralysis with prominent bulbar palsies

Outbreak with an unusual botulinum toxin type (ie, type C, D, F, or G, or type E toxin not acquired from an aquatic food)

Outbreak with a common geographic factor among cases (eg, airport, work location) but without a common dietary exposure (ie, features suggestive of an aerosol attack)

Multiple simultaneous outbreaks with no common source

Note: A careful travel and activity history, as well as dietary history, should be taken in any suspected botulism outbreak. Patients should also be asked if they know of other persons with similar symptoms.

onset of symptoms approximately 72 hours after exposure to an unknown but probably small amount of reaerosolized toxin.[21]

Age and Sex

Persons of all ages are potentially susceptible to botulism. There are no sex differences in susceptibility.

Agent and Vehicles

Botulinum toxin in solution is colorless, odorless, and, as far as is known, tasteless. The toxin is readily inactivated by heat (≥85°C for 5 minutes).[33,34,52] Thus, foodborne botulism is always transmitted by foods that are not heated, or not heated thoroughly, before eating. Almost every type of food has been associated with outbreaks of botulism, but the most commonly implicated foods in the United States are vegetables, particularly "low-acid" (ie, higher pH) vegetables such as beans, peppers, carrots, and corn.[42,50,58]

A novel epidemiological development is the occurrence of foodborne botulism after eating various nonpreserved foods in restaurants or delicatessens. Foil-wrapped baked potatoes are now known to be capable of causing restaurant-associated foodborne botulism[59] when held at room temperature after baking and then served plain,[60] as potato salad,[61,62] or as a Mediterranean-style dip.[59] Other outbreaks that originated in restaurants resulted from contaminated condiments such as sautéed onions,[63] garlic in oil,[64] and commercial cheese sauce.[65] Additional examples of notable commercial foods that have caused botulism outbreaks include inadequately eviscerated fish,[66] yogurt,[67] cream cheese,[68] and jarred peanuts.[69]

Incidence and Outbreak Size

Naturally occurring foodborne botulism is a rare disease. Approximately 9 outbreaks of foodborne botulism and a median of 24 cases occur annually in the United States.[42,47] The mean outbreak size has remained constant over the years at approximately 2.5 cases per outbreak. The largest outbreak of foodborne botulism in the United States in the last 100 years occurred in Michigan in 1977; 59 cases resulted from eating home-preserved jalapeño peppers at a restaurant.[57] However, only 45 of the 59 patients had clinically evident weakness and hypotonia.

Toxin Types

Of the 135 foodborne outbreaks in the 16 years from 1980 to 1996 in the United States, the toxin types represented were: type A, 54.1%; type B, 14.8%; type E, 26.7%; type F, 1.5%; and unknown, 3.0%.[42] Type F foodborne outbreaks are rare in the United States; a 1962 outbreak resulted from homemade venison jerky,[70] while other type F cases actually may have had intestinal botulism.[71] Toxin types C and D cause botulism in wildlife and domestic animals but have not caused human foodborne disease. However, humans are thought to be susceptible to these toxin types because they have caused botulism in primates when ingested.[72-74] Toxin type G is produced by a bacteria species discovered in South American soil in 1969 that has never caused recognized foodborne botulism.[75] Aerosol challenge studies in monkeys have established the susceptibility of primates to inhaled botulinum toxin types C, D, and G.[48]

Distribution

Although outbreaks of foodborne botulism have occurred in almost all states, more than half (53.8%) of the US outbreaks have occurred in just 5 western states (California, Washington, Oregon, Colorado, and Alaska). East of the Mississippi River, 60% of the foodborne outbreaks have resulted from type B toxin, while west of the Mississippi River, 85% have resulted from type A toxin. In the 46 years between 1950 and 1996, 20 states, mainly in the eastern United States, did not report any type A botulism outbreaks, while 24 states, mostly in the western United States, did not report any type B outbreaks.[42] In Canada and Alaska, most foodborne outbreaks resulted from type E toxin associated with native Inuit and Eskimo foods.[50,76]

Bioterrorism Considerations

Any outbreak of botulism should bring to mind the possibility of bioterrorism, but certain features would be particularly suggestive (BOX 1). The availability and speed of air transportation mandate that a careful travel and activity history, as well as a careful dietary history, be taken. Patients should also be asked whether they know of other persons with similar symptoms. Absence of a common dietary exposure among temporally clustered patients should suggest the possibility of inhalational botulism.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Clinical diagnosis of botulism is confirmed by specialized laboratory test-

ing that often requires days to complete. Routine laboratory test results are usually unremarkable. Therefore, clinical diagnosis is the foundation for early recognition of and response to a bioterrorist attack with botulinum toxin.

Any case of suspected botulism represents a potential public health emergency because of the possibility that a contaminated food remains available to others or that botulinum toxin has been deliberately released. In these settings, prompt intervention by civil authorities is needed to prevent additional cases. Consequently, clinicians caring for patients with suspected botulism should notify their local public health department and hospital epidemiologist immediately to coordinate shipment of therapeutic antitoxin, laboratory diagnostic testing, and epidemiological investigation (BOX 2). In most jurisdictions of the United States, botulism suspected on clinical grounds alone by law must be reported immediately by telephone to local public health authorities. The attending clinician needs to be both prompt and persistent in accomplishing this notification.

Box 2. Clinicians Caring for Patients With Suspected Botulism Should Immediately Contact Their:

(1) Hospital epidemiologist or infection control practitioner

and

(2) Local and state health departments

Consult your local telephone operator; the telephone directory under "government listings," or the Internet at: http://www.cdc.gov/other.htm#states or http://www.astho.org/state.html

If the local and state health departments are unavailable, contact the Centers for Disease Control and Prevention: (404) 639-2206; (404) 639-2888 [after hours].

Differential Diagnosis

Botulism is frequently misdiagnosed, most often as a polyradiculoneuropathy (Guillain-Barré or Miller-Fisher syndrome), myasthenia gravis, or a disease of the central nervous system (TABLE 3). In the United States, botulism is more likely than Guillain-Barré syndrome, intoxication, or poliomyelitis to cause a cluster of cases of acute flaccid paralysis. Botulism differs from other flaccid paralyses in its prominent cranial nerve palsies disproportionate to milder weakness and hypotonia below the neck, in its symmetry, and in its absence of sensory nerve damage.

A large, unintentional outbreak of foodborne botulism caused by a restaurant condiment in Canada provides a cautionary lesson about the potential difficulties in recognizing a covert, intentional contamination of food.[64] During a 6-week period in which the condiment was served, 28 persons in 2 countries became ill, but all were misdiagnosed (Table 3). The 28 were identified retrospectively only after correct diagnoses in a mother and her 2 daughters who had returned to their home more than 2000 miles away from the restaurant. Four (14%) of the cases had been misdiagnosed as having psychiatric disease, including "factitious" symptoms. It is possible that hysterical paralysis might occur as a conversion reaction in the anxiety that would

Table 3. Selected Mimics and Misdiagnoses of Botulism*

Conditions	Features That Distinguish Condition From Botulism
Common Misdiagnoses	
Guillain-Barré syndrome† and its variants, especially Miller-Fisher syndrome	History of antecedent infection; paresthesias; often ascending paralysis; early areflexia; eventual CSF protein increase; EMG findings
Myasthenia gravis†	Recurrent paralysis; EMG findings; sustained response to anticholinesterase therapy
Stroke†	Paralysis often asymmetric; abnormal CNS image
Intoxication with depressants (eg, acute ethanol intoxication), organophosphates, carbon monoxide, or nerve gas	History of exposure; excessive drug levels detected in body fluids
Lambert-Eaton syndrome	Increased strength with sustained contraction; evidence of lung carcinoma; EMG findings similar to botulism
Tick paralysis	Paresthesias; ascending paralysis; tick attached to skin
Other Misdiagnoses	
Poliomyelitis	Antecedent febrile illness; asymmetric paralysis; CSF pleocytosis
CNS infections, especially of the brainstem	Mental status changes; CSF and EEG abnormalities
CNS tumor	Paralysis often asymmetric; abnormal CNS image
Streptococcal pharyngitis (pharyngeal erythema can occur in botulism)	Absence of bulbar palsies; positive rapid antigen test result or throat culture
Psychiatric illness†	Normal EMG in conversion paralysis
Viral syndrome†	Absence of bulbar palsies and flaccid paralysis
Inflammatory myopathy†	Elevated creatine kinase levels
Diabetic complications†	Sensory neuropathy; few cranial nerve palsies
Hyperemesis gravidarum†	Absence of bulbar palsies and acute flaccid paralysis
Hypothyroidism†	Abnormal thyroid function test results
Laryngeal trauma†	Absence of flaccid paralysis; dysphonia without bulbar palsies
Overexertion†	Absence of bulbar palsies and acute flaccid paralysis

*CSF indicates cerebrospinal fluid; EMG, electromyogram; CNS, central nervous system; and EEG, electroencephalogram.
†Misdiagnoses made in a large outbreak of botulism.[64]

follow a deliberate release of botulinum toxin.

Diagnostic Testing

At present, laboratory diagnostic testing for botulism in the United States is available only at the CDC and approximately 20 state and municipal public health laboratories.[42] The laboratory should be consulted prospectively about specimen collection and processing. Samples used in diagnosis of botulism include serum (≥30 mL of blood in "tiger"-top or red-top tubes from adults, less from children), stool, gastric aspirate, and, if available, vomitus and suspect foods. Serum samples must be obtained before therapy with antitoxin, which nullifies the diagnostic mouse bioassay. An enema may be required to obtain an adequate fecal sample if the patient is constipated. Sterile water should be used for this procedure because saline enema solution can confound the mouse bioassay. Gastric aspirates and, perhaps, stool may be useful for detecting inhaled aerosolized botulinum toxin released in a bioterrorist attack.[77] A list of the patient's medications should accompany the diagnostic samples because anticholinesterases, such as pyridostigmine bromide, and other medicines that are toxic to mice can be dialyzed from samples before testing. All samples should be kept refrigerated after collection.

The standard laboratory diagnostic test for clinical specimens and foods is the mouse bioassay,[42] in which type-specific antitoxin protects mice against any botulinum toxin present in the sample. The mouse bioassay can detect as little as 0.03 ng of botulinum toxin[10] and usually yields results in 1 to 2 days (range, 6-96 hours). Fecal and gastric specimens also are cultured anaerobically, with results typically available in 7 to 10 days (range, 5-21 days). Toxin production by culture isolates is confirmed by the mouse bioassay.

An electromyogram with repetitive nerve stimulation at 20 to 50 Hz can sometimes distinguish between causes of acute flaccid paralysis.[78,79] The characteristic electromyographic findings of botulism include normal nerve conduction velocity, normal sensory nerve function, a pattern of brief, small-amplitude motor potentials, and, most distinctively, an incremental response (facilitation) to repetitive stimulation often seen only at 50 Hz. Immediate access to electrophysiological studies may be difficult to obtain in an outbreak of botulism.

Additional diagnostic procedures may be useful in rapidly excluding botulism as the cause of paralysis (Table 3). Cerebrospinal fluid (CSF) is unchanged in botulism but is abnormal in many central nervous system diseases. Although the CSF protein level eventually is elevated in Guillain-Barré syndrome, it may be normal early in illness. Imaging of the brain, spine, and chest may reveal hemorrhage, inflammation, or neoplasm. A test dose of edrophonium chloride briefly reverses paralytic symptoms in many patients with myasthenia gravis and, reportedly, in some with botulism.[64] A close inspection of the skin, especially the scalp, may reveal an attached tick that is causing paralysis.[80] Other tests that require days for results include stool culture for *Campylobacter jejuni* as a precipitant of Guillain-Barré syndrome and assays for the autoantibodies that cause myasthenia gravis, Lambert-Eaton syndrome, and Guillain-Barré syndrome.

Foods suspected of being contaminated should be refrigerated until retrieval by public health personnel. The US Food and Drug Administration and the US Department of Agriculture can assist other public health laboratories with testing of suspect foods by using methods similar to those applied to clinical samples.

THERAPY

The mortality and sequelae associated with botulism have diminished with contemporary therapy. In the United States, the percentage of persons who died of foodborne botulism decreased from 25% during 1950-1959 to 6% during 1990-1996, with a similar reduction for each botulinum toxin type.[42] Despite this increase in survival, the paralysis of botulism can persist for weeks to months with concurrent requirements for fluid and nutritional support, assisted ventilation, and treatment of complications.

Therapy for botulism consists of supportive care and passive immunization with equine antitoxin. Optimal use of botulinum antitoxin requires early suspicion of botulism. Timely administration of passive neutralizing antibody will minimize subsequent nerve damage and severity of disease but will not reverse existent paralysis.[81,82] Antitoxin should be given to patients with neurologic signs of botulism as soon as possible after clinical diagnosis.[47] Treatment should not be delayed for microbiological testing. Antitoxin may be withheld at the time of diagnosis if it is certain that the patient is improving from maximal paralysis.

In the United States, botulinum antitoxin is available from the CDC via state and local health departments (Box 2). The licensed trivalent antitoxin contains neutralizing antibodies against botulinum toxin types A, B, and E, the most common causes of human botulism. If another toxin type was intentionally disseminated, patients could potentially be treated with an investigational heptavalent (ABCDEFG) antitoxin held by the US Army.[83] However, the time required for correct toxin typing and subsequent administration of heptavalent antitoxin would decrease the utility of this product in an outbreak.

The dose and safety precautions for equine botulinum antitoxin have changed over time. Clinicians should review the package insert with public health authorities before using antitoxin. At present, the dose of licensed botulinum antitoxin is a single 10-mL vial per patient, diluted 1:10 in 0.9% saline solution, administered by slow intravenous infusion. One vial provides between 5500 and 8500 IU of each type-specific antitoxin. The amount of neutralizing antibody in both the licensed and the investigational equine antitoxins far exceeds the highest serum toxin levels found in foodborne botu-

lism patients, and additional doses are usually not required. If a patient has been exposed to an unnaturally large amount of botulinum toxin as a biological weapon, the adequacy of neutralization by antitoxin can be confirmed by retesting serum for toxin after treatment.

There are few published data on the safety of botulinum antitoxins. From 1967 to 1977, when the recommended dose was larger than today, approximately 9% of recipients of equine botulinum antitoxin in the United States displayed urticaria, serum sickness, or other reactions suggestive of hypersensitivity.[84] Anaphylaxis occurred within 10 minutes of receiving antitoxin in 2% of recipients. When the US Army's investigational heptavalent antitoxin was given to 50 individuals in a large Egyptian outbreak of type E foodborne botulism in 1991, 1 recipient (2%) displayed serum sickness, and 9 (18%) had mild reactions.[83] To screen for hypersensitivity, patients are given small challenge doses of equine antitoxin before receiving a full dose. Patients responding to challenge with a substantial wheal and flare may be desensitized over 3 to 4 hours before additional antitoxin is given. During the infusion of antitoxin, diphenhydramine and epinephrine should be on hand for rapid administration in case of adverse reaction. Although both equine antitoxins have been partially despeciated by enzymatic cleavage of the allogenic F_c region, each contains a small residual of intact antibody that may sensitize recipients to additional doses.

Botulism patients require supportive care that often includes feeding by enteral tube or parenteral nutrition, intensive care, mechanical ventilation, and treatment of secondary infections. Patients with suspected botulism should be closely monitored for impending respiratory failure. In nonventilated infants with botulism, a reverse Trendelenburg positioning with cervical vertebral support has been helpful, but applicability of this positioning to adults with botulism remains untested. This tilted, flat-body positioning with neck support may improve ventilation by reducing entry of oral secretions into the airway and by suspending more of the weight of the abdominal viscera from the diaphragm, thereby improving respiratory excursion (FIGURE 4). In contrast, placing a botulism patient in a supine or semirecumbent position (trunk flexed 45° at the waist) may impede respiratory excursion and airway clearance, especially if the patient is obese. The desired angle of the reverse Trendelenburg position is 20° to 25°.

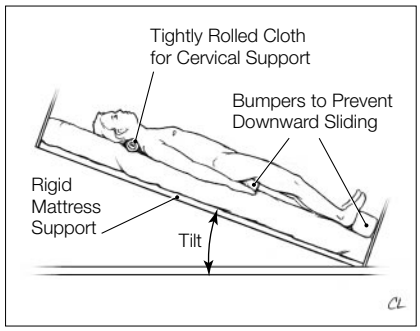
Botulism patients should be assessed for adequacy of gag and cough reflexes, control of oropharyngeal secretions, oxygen saturation, vital capacity, and inspiratory force. Airway obstruction or aspiration usually precedes hypoventilation in botulism. When respiratory function deteriorates, controlled, anticipatory intubation is indicated. The proportion of patients with botulism who require mechanical ventilation has varied from 20% in a foodborne outbreak[64] to more than 60% in infant botulism.[85] In a large outbreak of botulism, the need for mechanical ventilators, critical care beds, and skilled personnel might quickly exceed local capacity and persist for weeks or months. Development of a reserve stockpile of mechanical ventilators in the United States is under way[86] and will require a complement of staff trained in their use.

Antibiotics have no known direct effect on botulinum toxin. However, secondary infections acquired during botulism often require antibiotic therapy. Aminoglycoside antibiotics and clindamycin are contraindicated because of their ability to exacerbate neuromuscular blockade.[87,88] Standard treatments for detoxification, such as activated charcoal,[89] may be given before antitoxin becomes available, but there are no data regarding their effectiveness in human botulism.

SPECIAL POPULATIONS

Based on limited information, there is no indication that treatment of children, pregnant women, and immunocompromised persons with botulism should differ from standard therapy.

Figure 4. Preferred Positioning of Nonventilated Botulism Patients



Note flat, rigid mattress tilted at 20°, tightly rolled cloth to support cervical vertebrae, and bumpers to prevent downward sliding. Use of this position may postpone or avoid the need for mechanical ventilation in mildly affected patients because of improved respiratory mechanics and airway protection.

Despite the risks of immediate hypersensitivity and sensitization to equine proteins, both children[43,90] and pregnant women[91,92] have received equine antitoxin without apparent short-term adverse effects. The risks to fetuses of exposure to equine antitoxin are unknown. Treatment with human-derived neutralizing antibody would decrease the risk of allergic reactions posed by equine botulinum antitoxin, but use of the investigational product, Botulism Immune Globulin Intravenous (Human) (California Department of Health Services, Berkeley), is limited to suspected cases of infant botulism.[82,93]

PROPHYLAXIS

Botulism can be prevented by the presence of neutralizing antibody in the bloodstream. Passive immunity can be provided by equine botulinum antitoxin or by specific human hyperimmune globulin, while endogenous immunity can be induced by immunization with botulinum toxoid.

Use of antitoxin for postexposure prophylaxis is limited by its scarcity and its reactogenicity. Because of the risks of equine antitoxin therapy, it is less certain how best to care for persons who may have been exposed to botulinum toxin but who are not yet ill. In a small

study of primates exposed to aerosolized toxin in which supportive care was not provided, all 7 monkeys given antitoxin after exposure but before the appearance of neurologic signs survived, while 2 of 4 monkeys treated with antitoxin only after the appearance of neurologic signs died.[39] Moreover, all monkeys infused with neutralizing antibody before exposure to toxin displayed no signs of botulism. In a balance between avoiding the potential adverse effects of equine antitoxin and needing to rapidly neutralize toxin, it is current practice in foodborne botulism outbreaks to closely monitor persons who may have been exposed to botulinum toxin and to treat them promptly with antitoxin at the first signs of illness.[47] To facilitate distribution of scarce antitoxin following the intentional use of botulinum toxin, asymptomatic persons who are believed to have been exposed should remain under close medical observation and, if feasible, near critical care services.

In the United States, an investigational pentavalent (ABCDE) botulinum toxoid is distributed by the CDC for laboratory workers at high risk of exposure to botulinum toxin and by the military for protection of troops against attack.[94] A recombinant vaccine is also in development.[95] The pentavalent toxoid has been used for more than 30 years to immunize more than 3000 laboratory workers in many countries. Immunization of the population with botulinum toxoid could in theory eliminate the hazard posed by botulinum toxins A through E. However, mass immunization is neither feasible nor desirable for reasons that include scarcity of the toxoid, rarity of natural disease, and elimination of the potential therapeutic benefits of medicinal botulinum toxin. Accordingly, preexposure immunization currently is neither recommended for nor available to the general population. Botulinum toxoid induces immunity over several months and, so, is ineffective as postexposure prophylaxis.

DECONTAMINATION

Despite its extreme potency, botulinum toxin is easily destroyed. Heating to an internal temperature of 85°C for at least 5 minutes will detoxify contaminated food or drink.[52] All foods suspected of contamination should be promptly removed from potential consumers and submitted to public health authorities for testing.

Persistence of aerosolized botulinum toxin at a site of deliberate release is determined by atmospheric conditions and the particle size of the aerosol. Extremes of temperature and humidity will degrade the toxin, while fine aerosols will eventually dissipate into the atmosphere. Depending on the weather, aerosolized toxin has been estimated to decay at between less than 1% to 4% per minute.[96] At a decay rate of 1% per minute, substantial inactivation (≥13 logs) of toxin occurs by 2 days after aerosolization.

Recognition of a covert release of finely aerosolized botulinum toxin would probably occur too late to prevent additional exposures. When exposure is anticipated, some protection may be conferred by covering the mouth and nose with clothing such as an undershirt, shirt, scarf, or handkerchief.[97] In contrast with mucosal surfaces, intact skin is impermeable to botulinum toxin.

After exposure to botulinum toxin, clothing and skin should be washed with soap and water.[98] Contaminated objects or surfaces should be cleaned with 0.1% hypochlorite bleach solution if they cannot be avoided for the hours to days required for natural degradation.[33,52,98]

INFECTION CONTROL

Medical personnel caring for patients with suspected botulism should use standard precautions. Patients with suspected botulism do not need to be isolated, but those with flaccid paralysis from suspected meningitis require droplet precautions.

RESEARCH NEEDS

Additional research in diagnosis and treatment of botulism is required to minimize its threat as a weapon. Rapid diagnostic and toxin typing techniques currently under development would be useful for recognizing and responding to a bioterrorist attack. Although polymerase chain reaction assays can detect the botulinum toxin gene,[99] they are unable, as yet, to determine whether the toxin gene is expressed and whether the expressed protein is indeed toxic. Assays that exploit the enzymatic activity of botulinum toxin have the potential to supplant the mouse bioassay as the standard for diagnosis.[100] Detection of botulinum toxin in aerosols by enzyme-linked immunosorbent assay[101] is a component of the US military's Biological Integrated Detection System for rapid recognition of biological agents in the battlefield.[17]

The distribution of botulinum antitoxin to local hospitals from regional depots takes several hours. In contrast, standard detoxification techniques can be applied immediately. Studies are needed to assess whether activated charcoal and osmotic catharsis can prevent gastrointestinal tract absorption or reduce circulating levels of botulinum toxin. Enteral detoxification may be less useful in inhalational botulism than in foodborne disease.

The competing needs for immunity to weaponized botulinum toxin and for susceptibility to medicinal botulinum toxin could be reconciled by supplying human antibody that neutralizes toxin. With a half-life of approximately 1 month,[102] human antibody would provide immunity for long periods and avoid the reactogenicity of equine products. Existing in vitro technologies could produce the stockpiles of fully human antibody necessary both to deter terrorist attacks and to avoid the rationing of antitoxin that currently would be required in a large outbreak of botulism.[103-106] A single small injection of oligoclonal human antibodies could, in theory, provide protection against toxins A through G for many months. Until such a product becomes available, the possibilities for reducing the population's vulnerability to the intentional misuse of botulinum toxin remain limited.

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The views, opinions, assertions, and findings contained herein are those of the authors and should not be construed as official US Department of Defense or US Department of Army positions, policies, or decisions unless so designated by other documentation.

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current national point prevalence data are available. In addition, there are no quantitative data suggesting isotretinoin misuse, and the informed consent specifically indicates that the patient has been diagnosed with the FDA-approved indication. It is important to note that Roche Laboratories promotes the use of isotretinoin exclusively for patients with this approved indication.

Finally, it is important to state that the clinical criteria for the use of this drug in an individual patient must be left to the judgment of the physician, who is the only appropriate person to define the treatment plan for that patient.

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RESEARCH LETTER

Persistent Pain in Nursing Home Residents

To the Editor: More than 1.5 million people in the United States reside in nursing homes and an estimated 43% of adults 65 years and older will enter a nursing home prior to death.[1] Previous research using an early version of the Minimum Data Set (MDS), a nationally mandated nursing home resident assessment instrument, noted that daily pain was prevalent among nursing home residents diagnosed with cancer who had been discharged from a hospital, as well as among the residents of nursing homes in general.[2] Prior research was restricted by a limited MDS pain frequency measure of "none" or "daily," but since 1998, information on both frequency (none, daily, or less than daily) and severity of pain (mild, moderate, or excruciating at times) has been collected. We report the rates of persistent severe pain among US nursing home residents by analyzing a national repository of MDS data, which represents all nursing home residents in all 50 states.

Methods. We determined the rate of persistent severe pain among all 2.2 million residents of US nursing homes within 60 days of April 1, 1999. The term "persistent pain" indicates residents with pain at an assessment around that time who were also reported to be in daily moderate or excruciating pain at a second assessment, 60 to 180 days later. Using state as the unit of analysis, we adjusted observed rates of persistent severe pain for the nursing home discharge rate and the prevalence of severe pain among all 1999 admissions.

Results. Nationwide, 14.7% of residents in a nursing home for 2 assessments were in persistent pain and 41.2% of residents in pain at first assessment were in severe pain 60 to 180 days later. This rate varied from 37.7% (Mississippi) to 49.5% (Utah). Forty-one states had rates of persistent pain between 39.5% and 46.1%. Individual state reports are available online at http://www.chcr.brown.edu/dying/factsondying.htm.

Comment. We believe that these results underestimate the true pain burden experienced by nursing home residents because the data were reported by nursing home staff rather than by patients. States in which pain is not adequately assessed may report lower rates of persistent pain. Although facilities in states with higher rates of reported pain may be doing a better job of recognizing pain, nearly half of these residents were apparently not afforded adequate palliation. The high rate of persistent pain is consistent with previous research noting that pain is often not appropriately treated in nursing home residents.[2,3] Untreated pain results in impaired mobility, depression, and diminishes quality of life.[3-5] These population results indicate that pain control represents an often neglected need of this vulnerable population.

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CORRECTION

Incorrect Wording and Web Site Address: In the Consensus Statement entitled "Botulinum Toxin as a Biological Weapon: Medical and Public Health Management" published in the February 28, 2001, issue of THE JOURNAL (2001;285:1059-1070), 3 errors appeared. In the third introductory paragraph on page 1059, the word "biological" should be "microbial." In the paragraph labeled "Toxin Types" on page 1064, the word "bacteria" should be "bacterial." Finally, on page 1069, the Web site address for reference 27 should be http://www.state.gov/www/global/terrorism/1999report/1999index.html.