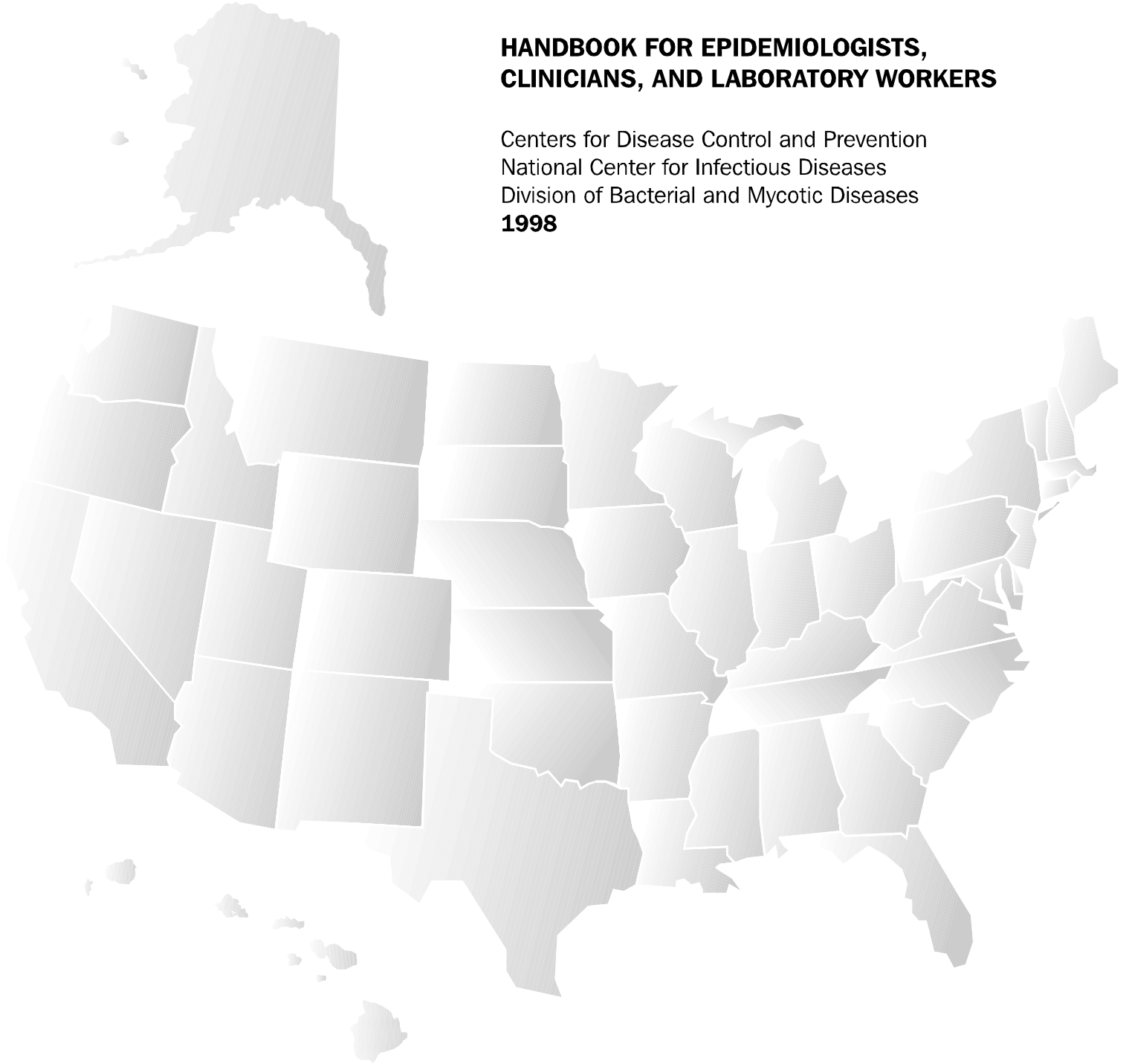


BOTULISM

IN THE UNITED STATES, 1899-1996

**HANDBOOK FOR EPIDEMIOLOGISTS,
CLINICIANS, AND LABORATORY WORKERS**

Centers for Disease Control and Prevention
National Center for Infectious Diseases
Division of Bacterial and Mycotic Diseases
1998



U.S. Department of Health and Human Services
Public Health Service
Centers for Disease Control and Prevention



Preface

This report, which updates handbooks issued in 1969, 1973, and 1979, reviews the epidemiology of botulism in the United States since 1899, the problems of clinical and laboratory diagnosis, and the current concepts of treatment. It was written in response to a need for a comprehensive and current working manual for epidemiologists, clinicians, and laboratory workers.

We acknowledge the contributions in the preparation of this review of past and present physicians, veterinarians, and staff of the Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases (DBMD), National Center for Infectious Diseases (NCID).

The excellent review of Drs. K.F. Meyer and B. Eddie, "Fifty Years of Botulism in the United States,"¹ is the source of all statistical information for 1899-1949. Data for 1950-1996 are derived from outbreaks reported to CDC.

Suggested citation

Centers for Disease Control and Prevention: Botulism in the United States, 1899-1996.
Handbook for Epidemiologists, Clinicians, and Laboratory Workers, Atlanta, GA.
Centers for Disease Control and Prevention, 1998.

¹ Meyer KF, Eddie B. Fifty years of botulism in the U.S. and Canada. George Williams Hooper Foundation, University of California, San Francisco, 1950.

Dedication

This handbook is dedicated to Dr. Charles Hatheway (1932-1998), who served as Chief of the National Botulism Surveillance and Reference Laboratory at CDC from 1975 to 1997. Dr. Hatheway devoted his professional life to the study of botulism; his depth of knowledge and scientific integrity were known worldwide. He was a true humanitarian and served as mentor and friend to countless epidemiologists, research scientists, students, and laboratory workers.

Table of contents

	Page
Preface	1
I. Emergency assistance	4
II. Introduction	5
III. Foodborne botulism	7
A. Incidence	7
B. Morbidity and mortality	7
C. Geographic distribution	7
D. Food sources and products causing outbreaks	8
E. Prevention and control	8
IV. Infant botulism	9
A. Epidemiology	9
B. Source of <i>C. botulinum</i>	10
C. Prevention and control	10
V. Wound botulism	10
VI. Child or adult botulism from intestinal colonization	11
VII. Clinical syndrome	11
VIII. Diagnosis	12
IX. Treatment	13
X. Public health response	14
XI. Laboratory confirmation	15
A. Overview	15
B. Safety precautions	15
C. Collection of specimens	16
D. Shipment of specimens	17
E. Examination of specimens for botulinum toxin	17
F. Isolation of <i>C. botulinum</i>	19
XII. References	22
XIII. Selected references for further reading	25
XIV. Tables and figures	26
XV. Appendix	42

I. Emergency assistance

Botulism is a public health emergency. Prompt diagnosis and early treatment of botulism are essential to minimize the otherwise great risk of death. Prompt epidemiologic investigation is critical to prevent further cases from occurring if a hazardous food is still available for consumption. State health departments and the Centers for Disease Control and Prevention (CDC) offer 24-hour diagnostic consultation, epidemic assistance, and diagnostic laboratory services. Trivalent botulinum antitoxin (for *Clostridium botulinum* types A, B, and E) and bivalent botulinum antitoxin (for types A and B) is available from CDC.

Health care providers who suspect they have a patient with botulism should contact their state health department epidemiology offices. The Foodborne and Diarrheal Diseases Branch of CDC (FDDB/DBMD/NCID/CDC) can provide emergency consultation and support to public health authorities.

Foodborne and Diarrheal Diseases Branch

Days	phone (404) 639-2206 (Monday-Friday, 8:30 AM-4:30 PM, eastern time)
Nights/weekends	phone (404) 639-2888

Diagnostic laboratory services are available at several state public health laboratories and CDC.

Names of manufacturers and trade names are provided for identification only, and inclusion does not imply endorsement by the U.S. Public Health Service or the U.S. Department of Health and Human Services.

II. Introduction

Botulism is a neuroparalytic illness resulting from the action of a potent toxin produced by the organism *Clostridium botulinum*. This microbe was first described in 1897 by E. van Ermengem after his investigation of a foodborne outbreak in Ellezelles, Belgium. Foodborne botulism is rare but it may kill rapidly, and contaminated products may expose many persons. Foodborne botulism therefore, represents a medical and a public health emergency that places a premium on rapid, effective communication between clinicians and public health officials.^(1,2)

“Botulism” comes from the Latin “botulus,” meaning sausage. When botulism was first recognized in Europe, many cases were caused by home-fermented sausages. This derivation, although historically important, has lost much of its significance, since plant rather than animal products are more common vehicles. Sausage now is rarely the cause of botulism in the United States.

Four distinct forms of botulism can occur, depending on the mode of acquisition of the toxin. Foodborne botulism results from the ingestion of food containing preformed toxin. Wound botulism is caused by organisms that multiply and produce toxin in a contaminated wound. Infant botulism is due to the endogenous production of toxin by germinating spores of *C. botulinum* in the intestine of the infant. Child or adult botulism from intestinal colonization is represented by those cases in which no food vehicle can be identified, there is no evidence of wound botulism, and there is the possibility of intestinal colonization in a person older than 1 year of age.

C. botulinum is a group of culturally distinct organisms that are alike only in that they are clostridia and produce antigenically distinct neurotoxins with a similar pharmacologic action. *C. botulinum* organisms are straight to slightly curved, gram-positive (in young cultures), motile, anaerobic rods, 0.5-2.0 μm in width, 1.6-22.0 μm in length, with oval, subterminal spores.⁽³⁾ The seven types of *C. botulinum* (A-G) are distinguished by the antigenic characteristics of the neurotoxins they produce. Types A, B, E, and in rare cases, F cause disease in humans. Types C and D cause disease in birds and mammals. Type G, identified in 1970, has not yet been confirmed as a cause of illness in humans or animals. Important epidemiologic features and some clinical characteristics distinguish the types of botulism that cause human illness. Rare cases of infant and adult botulism have been confirmed to be the result of intestinal colonization by non-*botulinum* *Clostridium* species that produced botulinum neurotoxin.

The structure and mechanism of action of each of the seven neurotoxins are similar. Each toxigenic Clostridia produces a polypeptide of 150 kDa which is activated by proteases following bacterial lysis. The active toxin consists of a heavy chain (H, 100 kDa) and a light chain (L, 50 kDa). The heavy chain consists of an amino-terminal 50 kDa domain (H_N) and a carboxy-terminal 50 kDa domain (H_C). Neuronal cell intoxication occurs through four steps: (1) binding of H_C to polysialoganglioside (and probably other protein) receptors on the neuronal membrane, (2) internalization of active toxin into endosomal-like compartments, (3) membrane translocation facilitated by H_N , and (4) enzymatic cleavage of target proteins by the L chain to prevent the release of the neurotransmitter acetylcholine from synaptic terminals of the motorneurons in muscle. The L chain of each neurotoxin type is a zinc-endopeptidase which has been shown to cleave a toxin-specific location of at least one of 3 proteins (VAMP, SNAP-25, or syntaxin). Although their absolute functions are unknown, these 3 proteins are members of a

group of proteins (SNARE proteins) which are essential to the docking and fusion of synaptic vesicles with the pre-synaptic membrane. The exact mechanism for neurotransmitter release is not known but most likely involves a fusion pore or a complete membrane fusion. Cleavage of one of the SNARE proteins by botulinum neurotoxin inhibits the release of acetylcholine from the synaptic terminal.⁽⁴⁾

The ability of *C. botulinum* to cause food poisoning in humans is directly related to the production of heat-resistant spores that survive preservation methods that kill nonsporulating organisms.⁽⁵⁾ The heat resistance of spores varies from type to type and even from strain to strain within each type; although some strains will not survive at 80°C, spores of many strains require temperatures above boiling to ensure destruction.^(6,7) The thermal resistance of spores also increases with higher pH and lower salt content of the medium in which the spores are suspended.⁽⁸⁾

In many cases, it is impractical or undesirable to treat a food product in a manner to eliminate all *C. botulinum* spores. As a result, most control methods focus on the inhibition of growth and toxin production. The main limiting factors for growth of *C. botulinum* in foods are: (1) temperature, (2) pH, (3) water activity, (4) redox potential, (5) food preservatives, and (6) competing microorganisms. All of these factors are interrelated and so changing one factor influences the effect of other factors. The interaction of factors may have a positive or negative effect on the inhibition of *C. botulinum*. In general, proteolytic strains grow optimally at 40°C; the lower limit is 10°C, upper limit is 45-50°C. Nonproteolytic strains, including type E can continue to grow even at 3.3°C. The minimum pH range for growth of proteolytic strains is 4.6-4.8; the limit is pH 5.0 for nonproteolytic strains. However, some food proteins, such as soy and beef, may have a protective effect on *C. botulinum* at or below pH 4.6. In addition, certain food preparations may contain low-acid “pockets” in which the pH may be high enough to support the production of toxin. Low water activity (a_w) inhibits the growth of *C. botulinum*. A minimum a_w of ~0.94 is needed to support growth and toxin production. Water activity can be limited by dehydration, but is in general controlled by the addition of NaCl. The minimum a_w of 0.94 corresponds to an approximate 10% NaCl solution. High redox potential (Eh) is usually due to the presence of O₂. The optimum Eh for growth of *C. botulinum* is low (~-350 mV) but toxin production has been observed at Eh of +250 mV. Because of this range, *C. botulinum* growth and toxin production can occur even in products considered to have a high oxygen level. In addition, vacuum-packaging used to lower Eh to preserve food increases anaerobic conditions and so may support the production of toxin. A number of food preservatives (nitrite, sorbic acid, parabens, phenolic antioxidants, polyphosphates and ascorbates) inhibit the growth of *C. botulinum* and limit toxin production. Lactic acid bacteria such as *Lactobacillus*, *Pediococcus*, and *Lactococcus* have been shown to produce acid and so inhibit *C. botulinum*.⁽⁵⁾

III. Foodborne botulism

A. Incidence

For the purpose of surveillance, we defined a foodborne outbreak as one or more cases of botulism in which a contaminated food source was implicated. In the period 1899-1949, 477 foodborne botulism outbreaks were recorded in the United States, and in the period from 1950 through 1996, an additional 444 outbreaks were reported to CDC for a total of 921. The average number of outbreaks per year has changed little, 9.7 per year for the earlier time period and 9.4 per year since 1950.

For the period 1899-1949, 1281 cases of botulism were reported, and in the time from 1950 through 1996, and additional 1087 cases were reported, bringing the total to 2368 cases. The average number of cases per outbreak has remained constant: 2.6 cases/outbreak in the first half of this century and 2.5 cases/outbreak so far in the second half.

Of the 444 foodborne botulism outbreaks since 1950, 37.6% were caused by type A botulinum toxin, 13.7% by type B, 15.1% by type E, 0.7% by type F, and 32.9% were unidentified with respect to toxin type (Table 1). The proportion of outbreaks for which the toxin type was not determined is decreasing. In 76.9% of the outbreaks for the period 1950-1959, the toxin type was not determined, compared with 59% of outbreaks for the period 1960-1969, 12.5% for 1970-1979, 2.5% for 1980-1989, and 3.6% for 1990-1996. From 1990 through 1996, outbreaks were most often caused by type A (44.6%), followed by type E (35.7%) and type B (12.5%).

B. Mortality

For the period 1899-1949, the case-fatality ratio was high at approximately 60%, but since about 1950, mortality has gradually decreased (Table 2). For the period 1950-1996, the case-fatality ratio was 15.5%. This decline in case-fatality ratio is due primarily to improvements in supportive and respiratory intensive care and perhaps to the prompt administration of antitoxin. The case-fatality ratio has generally declined over the years for all toxin types.

C. Geographic distribution

Foodborne botulism outbreaks have been reported from 46 states, Puerto Rico, and Washington D.C. from 1899 through 1996; only four states have never reported any foodborne botulism: Delaware, New Hampshire, South Carolina, and Vermont. Five western states (California, Washington, Colorado, Oregon, and Alaska) have accounted for more than half (53.8%) of all reported foodborne outbreaks since 1950 (Figure 1). Alaska alone accounts for 16.2% of outbreaks nationwide. This is because of the distinctive public health problem among the Alaska Native population, in which all botulism cases have been associated with improper preparation and storage of traditional Alaska Native foods.^(1,9)

There is a distinctive geographic distribution of botulinum toxin types in the United States. Of the 167 type A outbreaks recorded from 1950 through 1996, 144 (86.2%) occurred in states west of the Mississippi River. California, Washington, Oregon, and Colorado accounted for 58.7% of all reported type A outbreaks. Twenty states, most of them in the East, have not reported type A outbreaks (Figure 2).

Outbreaks of type B botulism have been reported by 26 states. Of the 61 type B outbreaks documented from 1950 through 1996, 37 (60.7%) were reported from eastern states; New York and Kentucky reported 13 of these Type B outbreaks (Figure 3).

Type E outbreaks have been reported from 10 states (Figure 4). The geographic distribution shows a predilection for Alaska, with 56 (83.6%) reported outbreaks from 1950 through 1996.

This regional distribution of outbreaks by toxin type is in keeping with the findings of a survey of soil samples which demonstrated a predominance of type A spores in soil specimens from the West and predominance of type B spores in soils of the northeast and central states.⁽¹⁰⁾ Type E spores have been found in marine life and sediment from the Pacific Northwest and the Great Lakes.^(11,12)

D. Food sources and products causing outbreaks

Until the early 1960s nearly all outbreaks of botulism in which toxin types were determined were caused by type A or B toxins and were usually associated with ingestion of home-canned vegetables, fruits, and meat products. Type E botulism was not recognized as a major problem in the United States until 1963, when 22 cases were reported.^(13,14) Sixty-one of the 67 outbreaks of type E botulism reported from 1950 through 1996 have been traced to marine products (fish or marine mammals); several cases have been attributed to beaver. The remainder are of an undetermined source. Only three outbreaks of Type F botulism have been reported in this country with one being traced to home-prepared venison jerky.⁽¹⁴⁻¹⁶⁾

From 1950 through 1996, 289 (65.1%) botulism outbreaks have been traced to home-processed foods and 31 (7%) to commercially processed foods, including foods served in restaurants. The type of food processing responsible for 124 (27.9%) outbreaks is unknown (Table 3).

Vegetables were the most important vehicle for the botulism toxin in the United States from 1950 through 1996. Fish and marine mammals were also responsible for a large number of the botulism outbreaks during this period. Of the 87 outbreaks caused by marine products (fish, or marine mammals), 61 were due to type E, 15 to type A, 8 to type B, and 3 were unknown. Beef, milk products, pork, poultry, and other vehicles caused fewer outbreaks.

E. Prevention and control

In the United States, foodborne botulism due to commercial foods has been largely controlled by safe canning and food manufacturing processes. Commercial canned foods are heated to a sufficient temperature and for a sufficient time to kill the spores. Unheated commercial foods in cans or jars can be made safe by acidification or other manipulations that inhibit the growth of the organism (e.g., addition of phosphoric acid to garlic in oil). Occasionally, commercial foods still cause botulism if they are prepared in a way that permits toxin production.

Many outbreaks of foodborne botulism in the United States result from eating improperly preserved home-canned foods.⁽¹⁸⁾ Persons doing home canning and other food preservation should be educated about the proper time, pressure, and temperature required to destroy spores, the need for adequately refrigerated storage of incompletely processed foods, and the effectiveness of boiling, with stirring, home-canned vegetables to destroy botulinum toxins.⁽¹⁹⁾ A pressure cooker

must be used to can vegetables at home safely because it can reach temperatures above boiling (>212°F [>100°C]), which is necessary to kill botulism spores.⁽¹⁹⁾ Although botulism spores are heat stable, botulinum toxin is heat labile. Botulinum toxin can be inactivated by heating to 176°F (80°C). Therefore, heating home-canned foods before consumption can reduce the risk of botulism intoxication.

C. botulinum may cause container lids to bulge and the contents to have “off-odors.” Commercial cans or home-canned products with bulging lids should not be opened, and foods with off-odors should not be eaten or “taste tested.”⁽¹⁹⁾ For more information about safe home-canning procedures, contact your local county extension home economist or see the website the Extension Service of the U.S. Department of Agriculture (USDA) at <http://ext.usu.edu/publica/foodpubs.htm>.⁽²⁰⁾

IV. Infant botulism

Since 1980, infant botulism has been the most common form of botulism reported in the United States. It is epidemiologically distinct from foodborne botulism, representing not ingestion of toxin preformed in contaminated foods but colonization (infection) of the intestine by spores of *C. botulinum*, with subsequent in vivo toxin production.⁽²¹⁾ Although infant botulism was first described in 1976,^(22,23) earlier cases have been identified retrospectively, and its detection only in recent years might reflect advances in diagnostic capabilities rather than the emergence of a new clinical syndrome.

The disease is characterized by the onset of constipation, which is followed shortly by neuromuscular paralysis that begins with the cranial nerves and progresses to peripheral and respiratory musculature. The spectrum in severity has ranged from mild lethargy and slowed feeding to severe hypotonia and respiratory insufficiency.

A. Epidemiology

Since the recognition of infant botulism in 1976, cases have been identified with increasing frequency. In the United States, 1442 cases were reported to CDC from 46 states between 1976 and 1996 (Figures 5 & 6). Type A accounted for 46.5% of these cases (Figure 7) and type B for 51.9% (Figure 8). Since reporting began to stabilize in 1980, the average annual incidence of reported infant botulism in the United States has been approximately 1.9/100,000 live births. Since 1976, 47.2% of all infant botulism cases have been reported from California. Between 1976 and 1994, the incidence of infant botulism was highest in Delaware, Hawaii, Utah, and California (9.0, 8.8, 6.3 and 5.7 per 100,000 live births, respectively). The reasons for the apparent geographic variation are unknown.

The characteristics of infant botulism cases have been clarified in recent years. Infants hospitalized with the disease tend to have had higher birth weights than other infants, and their mothers tend to be white, older, and better educated than mothers in the general population. Affected infants are also more commonly breast-fed,^(24,25) and breast-feeding is associated with an older age at onset in type B cases.⁽²⁵⁾ In general, affected infants are the product of normal gestation and delivery. No congenital abnormalities have been shown to be associated with infant

botulism, and the children were generally healthy until the onset of botulism. Approximately the same number of males as females have been affected. The mean age at onset has been 13 weeks, the range from 1 to 63 weeks. Clustering of cases of infant botulism has been noted in some suburban areas in the eastern United States and in some small towns and rural areas in the West.⁽²⁶⁻²⁹⁾

B. Source of *C. botulinum*

Infant botulism occurs when an infant ingests spores (less likely vegetative cells) of *C. botulinum* which in turn colonize the intestinal tract and produce toxin.⁽²¹⁾ In a prototypical case, type B organisms, but no toxin, were isolated from honey fed to an infant with infant botulism whose fecal specimens contained type B organisms and toxin. Family members who ate some of the same honey did not become ill. In several studies, more than 20% of affected infants had ingested honey before the onset of botulism.^(25,29,30) In many cases, *C. botulinum* spores of the same type were cultured from honey in the same households.

However, since most infants with infant botulism have had no exposure to honey, the risk factors and vehicles of transmission of *C. botulinum* for the majority of cases remain unclear.^(21,31) A survey of foods commonly fed to infants revealed *C. botulinum* in specimens of corn syrup as well as honey, but in no other category of foods tested.⁽³²⁾ In other studies, the same types of *C. botulinum* that caused disease were isolated from soil in an infant's yard and from vacuum cleaner dust. Investigators have also frequently noted environmental conditions that might expose infants directly to environmental sources of *C. botulinum* spores, such as a shared crib, dusty or windy locales, nearby building construction, or outdoor activities.^(26,28) These exposures have not, however, been fully evaluated by controlled studies. Infants hospitalized with botulism have also more typically been breast-fed than have control infants.^(25,31,33,34) Breast-feeding is known to affect the fecal flora differently than formula feeding; in mice, the fecal flora have been shown to be an important susceptibility factor in challenge experiments with *C. botulinum* type A spores.⁽³⁵⁾

C. Prevention and control

The risk factors for infant botulism are poorly described, but possible sources of spores include foods and dust. Honey should not be fed to infants because it has been identified as a food source.⁽¹⁹⁾

V. Wound botulism

Wound botulism is a rare disease resulting from the growth of *C. botulinum* spores in a contaminated wound with in vivo toxin production.⁽³⁶⁾ Neurologic findings are indistinguishable from those seen in foodborne botulism; however, gastrointestinal symptoms do not occur. Wounds might not be obvious or grossly infected. Between 1943, when the syndrome was first recognized, through 1985, 33 cases of wound botulism were reported in the United States. Of these, 25 were laboratory confirmed; 17 cases were type A, 7 type B, and 1 a mixture of type A and type B organisms.⁽³⁷⁾ The median age of patients was 21 years (range 6-44 years); 81% were male. The wounds were usually deep and contained avascular areas; many patients had compound fractures, and four had extensive crush injuries of the hand. The median incubation

period in cases of trauma was 7 days (range 4-21 days).⁽³⁸⁾ Since 1980, wound botulism cases have occurred in persons who used illicit drugs; these were associated either with needle puncture sites or with nasal or sinus lesions due to chronic cocaine sniffing.⁽³⁹⁾ From 1986 through 1996, 78 cases of wound botulism were reported in the United States; the majority were linked to injectable drug use, particularly with so-called "black tar heroin." Sixty-six cases were type A, 9 were type B, and the remaining 3 were of unknown toxin type. The median age of patients was 38 years (range 5-65 years); 60% were male.

VI. Child or adult botulism from intestinal colonization

Isolated cases of botulism in which extensive investigation failed to implicate a specific food as the cause of the disease have been recorded by CDC since 1978 as cases of "undetermined origin" rather than of foodborne botulism; through 1996, all of these cases have been in adults. Although there has been speculation since the 1920s, careful investigation has now demonstrated that some of these cases are caused by colonization of the gastrointestinal tract by *C. botulinum* or *C. baratii* with in vivo production of toxin, analogous to the pathogenesis of infant botulism.^(40,41) Support for the diagnosis of botulism from intestinal colonization is provided by the demonstration of the prolonged excretion of toxin and *C. botulinum* in the stool, and by the demonstration of spores of *C. botulinum* but not preformed toxin in suspected foods. In some cases of botulism strongly suspected of representing intestinal colonization, the patients had a history of gastrointestinal surgery or illnesses such as inflammatory bowel disease, which might have predisposed them to enteric colonization.⁽⁴²⁾ No other specific risk factors have been identified.

VII. Clinical syndrome

The clinical syndrome of botulism, whether foodborne, infant, wound, or intestinal colonization, is dominated by the neurologic symptoms and signs resulting from a toxin-induced blockade of the voluntary motor and autonomic cholinergic junctions and is quite similar for each cause and toxin type.^(21,34,38,43) Incubation periods for foodborne botulism are reported to be as short as 6 hours or as long as 10 days,⁽⁴⁴⁾ but generally the time between toxin ingestion and onset of symptoms ranges from 18 to 36 hours.⁽⁴⁵⁾ The ingestion of other bacteria or their toxins in the improperly preserved food or changes in bowel motility are likely to account for the abdominal pain, nausea and vomiting, and diarrhea that often precede or accompany the neurologic symptoms of foodborne botulism. Dryness of the mouth, inability to focus to a near point (prompting the patient to complain of "blurred vision"), and diplopia are usually the earliest neurologic complaints. If the disease is mild, no other symptoms may develop and the initial symptoms will gradually resolve. The person with mild botulism may not come to medical attention. In more severe cases, however, these initial symptoms may be followed by dysphonia, dysarthria, dysphagia, and peripheral-muscle weakness. If illness is severe, respiratory muscles are involved, leading to ventilatory failure and death unless supportive care is provided. Recovery follows the regeneration of new neuromuscular connections. A 2- to 8-week duration of ventilatory support is common, although patients have required ventilatory support for up to 7

months before the return of muscular function.⁽⁴³⁾ Death occurs in 5%-10% of cases of foodborne botulism; early deaths result from a failure to recognize the severity of disease or from secondary pulmonary or systemic infections, whereas deaths after 2 weeks are usually from the complications of long-term mechanical ventilatory management.⁽⁴³⁾

Perhaps because infants are not able to complain about the early effects of botulinum intoxication, the neurologic dysfunction associated with infant botulism often seems to develop suddenly. The major manifestations are poor feeding, diminished suckling and crying ability, neck and peripheral weakness (the infants are often admitted as "floppy babies"), and ventilatory failure.^(21,24,34) Constipation is also often seen in infants with botulism, and in some, has preceded the onset of neurologic abnormalities by many days. Loss of facial expression, extraocular muscle paralysis, dilated pupils, and depression of deep tendon reflexes have been reported more frequently with type B than with type A infant botulism.⁽³⁴⁾ Treatment with aminoglycoside antimicrobial agents may promote neuromuscular weakness in infant botulism⁽⁴⁶⁾ and has been associated with an increased likelihood of the requirement of mechanical ventilation.^(21,34) Fewer than 2% of reported cases of infant botulism result in death.

VIII. Diagnosis

Botulism is probably substantially underdiagnosed. The diagnosis is not difficult when it is strongly suspected, as in the setting of a large outbreak, but since cases of botulism most often occur singularly, the diagnosis may pose a more perplexing problem. Findings from many outbreaks have suggested that early cases are commonly misdiagnosed. They may be diagnosed only retrospectively after death, when the subsequent clustering of cases of botulism-like illness finally alerts public health personnel to an outbreak of botulism.⁽⁴⁷⁻⁴⁹⁾ Other cases are undoubtedly missed entirely. Entire outbreaks may even go undetected despite severe illness in patients; for example, one outbreak was recognized retrospectively only after a second cluster of cases was associated with the same vehicle.⁽⁴⁴⁾

Botulism should be suspected in any adult with a history of acute onset of gastrointestinal, autonomic (e.g., dry mouth, difficulty focusing), and cranial nerve (diplopia, dysarthria, dysphagia) dysfunction or in any infant with poor feeding, diminished suckling and crying ability, neck and peripheral muscle weakness, and/or ventilatory distress.⁽⁵⁰⁾ The demonstration of bilateral cranial nerve findings and the documentation of neurologic progression (descending peripheral muscle weakness, ventilatory compromise) increase the level of suspicion. The diagnosis is even more likely if an adult patient has recently eaten home-canned foods or if family members are similarly ill, or both. If the typical clinical syndrome is present and no food item can be pinpointed as a means of transmission, a contaminated wound should be sought. If the typical syndrome is seen and a wound is identified, the wound should be explored and specimens taken for culture and toxicity testing even if the wound appears clean.

The differential diagnosis includes myasthenia gravis, stroke, Guillain-Barré syndrome, bacterial and chemical food poisoning, tick paralysis, chemical intoxication (e.g., from carbon monoxide, barium carbonate, methyl chloride, methyl alcohol, organic phosphorus compound, or atropine), mushroom poisoning, medication reactions (e.g., from antibiotics such as neomycin,

streptomycin, kanamycin, or gentamicin), poliomyelitis, diphtheria, and psychiatric illness. In infant botulism, sepsis (especially meningitis), electrolyte-mineral imbalance, metabolic encephalopathy, Reye syndrome, Werdnig-Hoffman disease, congenital myopathy, and Leigh disease should also be considered.

Routine laboratory studies are not helpful in confirming the clinical suspicion of botulism. Serum electrolytes, renal and liver function tests, complete blood tests, urinalysis, and electrocardiograms will all be normal unless secondary complications occur. A normal cerebrospinal fluid (CSF) examination helps differentiate botulism from Guillain-Barré syndrome, although a slightly elevated CSF protein level is occasionally seen with botulism, and the protein level might be initially normal in Guillain-Barré syndrome.⁽⁴³⁾ A normal tensilon test helps to differentiate botulism from myasthenia gravis. Normal neuroradiologic studies, such as computed tomographic scans or magnetic resonance imaging, help to rule out stroke, another condition commonly confused with botulism.^(44,49)

Electromyography (EMG) may be helpful in distinguishing botulism from myasthenia gravis and Guillain-Barré syndrome, diseases that botulism often mimics closely. A characteristic EMG pattern observed in adult patients with botulism has been well described.⁽⁵¹⁻⁵³⁾ EMG is most useful, however, when conducted with repetitive stimulation at 50 Hz. EMGs should be performed on clinically involved muscles; positive results may be obtained from only one muscle even though many are weak. Because of variations in EMG results and their interpretation, EMG is most reliable if conducted by persons with experience in this procedure.

Toxicity testing of serum specimens, culture of tissues debrided from a wound, and toxicity testing, plus culture of stool specimens or epidemiologically incriminated foods or both are the best methods for confirming the diagnosis of botulism.⁽⁵⁴⁾ Cultures were positive for 51% of stool specimens and toxin testing was positive for 37% of sera and 23% of stool specimens collected from 309 persons with clinically diagnosed botulism reported to CDC from 1975 to 1988; at least one laboratory test was positive for 65% of patients.⁽⁴⁵⁾ Collecting stool and serum samples early during the course of illness increases the likelihood of obtaining positive results. However, in any given situation, these tests may not be helpful; large outbreaks have occurred in which none⁽⁵⁵⁾ or a very low percentage⁽⁵⁶⁾ of specimens yielded positive results. In addition, laboratory results may not be reported until many hours or days after the specimens are received. The administration of antitoxin is the only specific therapy available for botulism, and evidence suggests that it is effective only if given very early in the course of neurologic dysfunction.⁽⁵⁷⁾ Hence, the diagnosis of this illness cannot await the results of studies that may be long delayed and be confirmatory only in some cases. The diagnosis should be made on the basis of the case history and physical findings.

IX. Treatment

The mainstays of treatment of foodborne and wound botulism are as follows: 1) administration of botulinum antitoxin in an attempt to prevent neurologic progression of a moderate, slowly progressive illness, or to shorten the duration of ventilatory failure in those with a severe, rapidly progressive illness; 2) careful monitoring of respiratory vital capacity and aggressive respiratory care for those with ventilatory insufficiency (monitoring of respiratory vital

capacity should be performed as soon as diagnosis of botulism is made); and 3) meticulous and intensive care for the duration of the often prolonged paralytic illness.

Antitoxin therapy is more effective if undertaken early in the course of illness.⁽⁵⁷⁾ This is not surprising when one considers that equine antitoxin neutralizes only toxin molecules yet unbound to nerve endings.⁽⁵⁸⁾ More than 80% of persons reported with adult botulism in the United States are treated with antitoxin. Before administration of antitoxin, skin testing should be performed to test for sensitivity to serum or antitoxin (see package insert). Administration of one 10-ml vial of trivalent botulism antitoxin by the intravenous route results in serum levels of type A, B, and E antibodies capable of neutralizing serum toxin concentrations manyfold in excess of those reported for botulism patients. Therefore, after skin testing for sensitivity, contrary to the antitoxin package insert, administration of one vial of antitoxin intravenously is recommended and antitoxin need not be repeated since the circulating antitoxins have a half-life of 5 to 8 days.^{(58)*} However, treatment is not without risk, as approximately 9% of persons treated experience hypersensitivity reactions.⁽⁶⁰⁾ It is therefore extremely important that physicians recognize botulism as early in its course as possible, and yet not mistake other neurologic syndromes for botulism.

Equine antitoxin rarely has been used in infant botulism because of the risk of inducing lifelong hypersensitivity to equine antigens and lack of evidence of its benefit. Also, because of early concerns that anaphylactic reactions with the equine-derived product might be more severe in infants, few infants have been given the product.⁽²¹⁾ However, a human-derived botulism antitoxin, termed "botulism immune globulin," has been prepared, and a clinical trial of its efficacy when given early in the course of illness is in progress in California.^{(17)**} In infant botulism, antibiotics are used only to treat secondary infections because lysis of intraluminal *C. botulinum* may increase the amount of toxin available for absorption.⁽⁶⁰⁾

X. Public health response

Because cases of foodborne botulism result from ingestion of contaminated food that still may be available to cause illness in others, a single case of foodborne botulism represents a public health emergency that might herald a larger outbreak.⁽¹⁾ Therefore, it is critical for clinicians who suspect botulism to discuss the case immediately with local and state public health epidemiologists. State public health officials should then immediately contact CDC. If a commercial food product is a suspected vehicle for botulism, USDA or the U.S. Food and Drug Administration also should be notified. Investigation of a suspected case of botulism includes an immediate search for other possible cases and identification of suspected food exposures, as well as confirming the diagnosis. If a number of people are affected, a rapid and detailed epidemiologic investigation is launched to assure the source is identified and controlled. Diagnostic testing of both case specimens and foods should be performed as needed.

* Except for antitoxin administration instructions, other information included in antitoxin package insert is accurate.

** For more information about botulism immune globulin, call the Infant Botulism Prevention Program, California State Department of Health Services, phone number (510) 540-2646.

XI. Laboratory confirmation

A. Overview

Botulism is confirmed in the laboratory by identifying botulinum neurotoxin in the serum, feces, vomitus, or gastric contents of the patient, and/or in remnants of a food consumed by the patient. The only currently acceptable method for detection and identification of botulinum neurotoxin is the mouse toxicity and neutralization bioassay. In vitro methods (e.g., ELISA) for botulinum toxin detection are under development but have not been validated. Isolation of *C. botulinum* from the patient's feces or gastric specimen also provides good confirmatory evidence, since this organism is rarely, if ever encountered in human specimens in the absence of botulism.⁽⁵⁴⁾ Isolation of *C. botulinum* from remnants of consumed food does not provide confirmatory evidence of botulism in the absence of other supporting laboratory data. The organisms are recovered from enrichment cultures or from directly inoculated agar media. The identity of the organism is established on the basis of 1) lipase reaction; 2) Gram stain, observation of spores; 3) determining anaerobic requirement for growth; 4) demonstration of toxicogenicity; 5) identification of toxin by specific neutralization.

B. Safety precautions

Botulinum toxins are extremely poisonous for humans. Minute quantities acquired by ingestion, inhalation, or by absorption through the eye or a break in the skin can cause profound intoxication and death.⁽⁶²⁾ Therefore, all materials suspected of containing botulinum toxin must be handled with caution, and only experienced personnel, preferably immunized with botulinum toxoid*, should perform laboratory tests. Laboratory coats, disposable surgical gloves, face shields or bench top plexiglass shields should be used when handling specimens. Safety pipettes are recommended for transferring liquids. A biological safety cabinet (BSC) should be used when preparing extracts of tissue and solid foods to prevent the release of aerosols into the laboratory. All specimen containers, culture tubes, flasks, etc., containing botulinum toxin should be placed in leakproof, unbreakable containers during storage or incubation to prevent accidental spills. If spills occur, the toxin can be neutralized by the use of a strong alkaline solution such as 0.1 M sodium hydroxide. *C. botulinum* is inactivated with a 1:10 dilution of household bleach. The appropriate disinfectant solution must be in contact with the toxin or the organism for 15 to 20 minutes to ensure effective inactivation. If the material is suspected to contain both toxin and organisms, the spill must be sequentially treated with bleach and sodium hydroxide.

In the event of a laboratory incident with possible human exposure, the first steps to be taken are to assess the likelihood that an exposure actually occurred, the amount and serologic type of toxin involved, and the immune status of the person involved. What was the source of the material? Was it ingested, injected, inhaled? What quantity? If it was material from a clinical specimen, the quantity would probably be negligible (the risk of contracting hepatitis or HIV may indeed be greater). If it was from a culture or a concentrated toxin product, the exposure could be serious in an unimmunized person. If the exposure occurred during the injection of mice, the

*The botulinum toxoid vaccine is made available as an investigational agent through CDC. This vaccine is an activated polyvalent toxoid for laboratory personnel who work regularly with botulinum toxins. For more information, contact the National Botulism Surveillance and Reference Laboratory at CDC, phone number (404) 639-3867.

progress of the mouse test may indicate whether the accident involved a toxic sample, and may indicate the toxin type if neutralization tests were being performed. If the culture or toxin was of a type other than A, B, or E, therapeutic antitoxin would be useless. If the person was in the booster stage of immunization and was up to date in the program, he or she would most likely have adequate protection against toxin types A, B, C, D, and E.

In any case, the exposed person should be observed over the next 2 to 4 days and should be made aware of the early symptoms of botulism: blurred or double vision, dry mouth, slurred speech, peripheral muscle weakness. If any symptoms appear, the person should be hospitalized immediately and the case handled as possible botulism. With adequate supportive care, even severe cases of botulism are handled successfully, with or without administration of antitoxin.

C. Collection of specimens

Suitable materials for examination for botulinum toxin and *C. botulinum* in foodborne outbreaks include serum, feces, vomitus, gastric contents and suspected foods; in wound infections, serum, feces, exudate, debrided tissue, or swab samples from wounds of patients; and in infants with botulism, feces and serum samples. In some instances, examination of environmental specimens for *C. botulinum* can aid in establishing the probable source of the organism (e.g., in infant botulism).

All specimens except those from wounds should be refrigerated, preferably not frozen, and examined as quickly as possible after collection. Wound specimens should be placed in anaerobic transport devices such as Port-A-Cul tubes or vials (BioQuest Div., Becton, Dickinson & Co., Cockeysville, Maryland)⁽⁶³⁾, and sent to the appropriate laboratory without refrigeration for attempted isolation of *C. botulinum*. Foods should be left in their original containers if possible or placed in sterile unbreakable containers and labeled carefully. Empty containers with remnants of suspected foods can sometimes be examined. The date specimens are collected from patients should be indicated on the labels. Serum samples must be taken before antitoxin treatment to demonstrate the presence of botulinum toxin. Post treatment serum specimens are sometimes obtained, although uncommonly, to verify disappearance of the toxin, to determine the amount of antitoxin in circulating blood of the patient after treatment, and to determine how long antitoxin persists.

Ideally, 10 to 15 ml of serum should be obtained for laboratory tests. This quantity permits specific identification of the botulinum toxin involved, injection of higher volumes of serum, and repeat tests if necessary. Since it is impractical to obtain this much serum from some patients, especially from infants, as little as 0.5 ml can be sufficient to confirm botulism, although in many cases, volumes less than 3 ml will provide inconclusive results.

Ideally, 25 to 50 gm of feces should be collected, preferably before antitoxin treatment. However, confirmatory evidence of botulism has been obtained from much smaller quantities, and *C. botulinum* has been isolated from stools following antitoxin treatment. Botulism has been confirmed in infants with only "pea-sized" stools. If an enema must be given because of constipation, a minimal amount of fluid (preferably sterile nonbacteriostatic water) should be used to obtain the specimen so the toxin will not be unnecessarily diluted. If the patient has been taking any medication that might interfere with toxin assays or culturing of the stool, the laboratory should be notified. For example, it has been demonstrated that anticholinesterase drugs given orally to patients for myasthenia gravis can interfere with mouse botulinum toxin

assays of stool extracts.⁽⁶⁴⁾

D. Shipment of specimens

Specimens sent to a distant laboratory should be placed in sterile leakproof containers, then in insulated shipping containers with refrigerant, labeled **MEDICAL EMERGENCY, BIOLOGICAL HAZARD, REFRIGERATE ON ARRIVAL** and should be shipped by the most rapid means available. Cardboard containers are not suitable for stool specimens. Most of the major airlines have a special package handling service for expedited shipments. The receiving laboratory should be notified in advance by telephone as to when and how specimens will be shipped, and when they will arrive. If an unavoidable delay of several days is anticipated, the specimens (serum or stool) should be kept frozen and then packed in an insulated container with dry ice and proper cushioning material for shipment. It is worth noting that freezing does not affect the ability to detect botulinum toxin in specimens, but it does affect the ability to detect *C. botulinum*. Before shipment to CDC, please contact the National Botulism Surveillance and Reference Laboratory at (404) 639-3867. CDC form 50.34 (formerly 3.203) should be completed and submitted with the specimens if completion of the form does not delay shipment (see Appendix). All specimens must include an individual's name and telephone number to contact for the preliminary report. Preferably, the names of both the attending physician and the state health department contact should be given.

E. Examination of specimens for botulinum toxin

1. Mouse bioassay protocol

- a. Mice: 20 to 30 gm, white ICR strain mice give satisfactory results.
- b. Antitoxins: Monovalent (types A, B, C, D, E, and F) antitoxins distributed by CDC contain approximately 10 international units per ml when reconstituted. One unit of the corresponding type of antitoxin neutralizes 10,000 mouse IP LD₅₀ of toxin types A, B, C, D, and F, or 1000 IP LD₅₀ of toxin type E. Rehydrate as indicated with 50% glycerol.
- c. Mouse injection: Inject mice intraperitoneally with serum or extracts, alone or mixed with antitoxin, using a syringe with a 25-gauge, 5/8 inch needle.
- d. Observation of mice: Observe the mice at intervals (e.g., 4, 8, 12, 18, and 24 hours) and then daily for a period of 4 days for signs of illness or death. Although botulinum intoxication usually kills mice within 6 to 24 hours, delayed deaths are occasionally observed. If toxin is present in sufficient quantity, all mice injected with test materials will die except those receiving samples mixed with antitoxin specific for the botulinum toxin involved, and those injected with heated (held in boiling water for 10 minutes) extracts because botulinum toxins are heat-labile.

NOTE: Signs of botulism in mice begin with ruffling of the fur followed in sequence by labored abdominal breathing, weakness of the limbs, and total paralysis. Death is caused by respiratory failure. The time between the first sign of distress and death varies with the amount of toxin the mouse receives. Death without clinical signs is not adequate evidence that botulinum toxin was present in

the material injected.

2. Testing serum for toxin

- a. Place 1 ml of serum into each of 6 tubes and add 0.25 ml of the appropriate antitoxin to the last 5 tubes as indicated in Table 5.
- b. Mix antitoxin with serum by swirling the tubes. Try to prevent foaming, which can inactivate toxin.
- c. Incubate serum-antitoxin mixtures at room temperature for 30 to 60 minutes.
- d. Inject 2 mice intraperitoneally with 0.4 ml of untreated serum and 2 mice each with 0.5 ml of the serum-antitoxin mixtures.
- e. Mark each set of mice with dye in a distinctive pattern for identification.
- f. Observe mice for signs suggestive of botulism and death. If botulinum toxin is present in sufficient concentration, all mice will die within 96 hours, except those receiving the serum mixtures containing the antitoxin corresponding to the toxin type.
- g. A toxin test that is negative using 0.4 ml of patient's serum may show botulinum toxin when 0.8 ml is injected per mouse. Volumes larger than 0.8 ml are not recommended since normal serum will sometimes kill a mouse when 1 ml or more is injected. When deaths occur in mice injected with 0.8-ml quantities of serum, neutralization is carried out by adding 1/8 volume of antitoxin (e.g., 2 ml serum plus 0.25 ml antitoxin), incubating 30 to 60 minutes at room temperature, and injecting 0.9 ml into mice.
- h. If sufficient serum is not available for a complete toxin neutralization test, priority should be to demonstrate toxin by observing typical signs of botulism in mice after the intraperitoneal injection of untreated serum (0.4 or 0.8 ml). If toxin is demonstrated, the toxin type should be determined by the use of type-specific antitoxin if possible. The exact antitoxin (anti-A,B,E, etc) to employ in the performance of the neutralization test will depend upon the quantity of patient's serum available.

3. Testing stool/food specimens for toxin

- a. Prepare extract.
 - (i) Weigh specimen into a sterile container; if grinding is necessary transfer to a sterile mortar.
 - (ii) Add 1 ml of cold (4°C) gelatin diluent (0.2% gelatin, 0.4% Na₂PO₄; pH 6.4) for each gram of feces.
 - (iii) Mix until a uniform suspension is obtained.
 - (iv) Hold for 30 minutes in refrigerator (4°C); an extended holding period of several hours to several days is also acceptable since the toxin is stable under these conditions.
 - (v) Centrifuge at 12,000 x g in a refrigerated centrifuge (4°C) for 20 minutes, recover supernatant, and repeat centrifugation to clarify if necessary. Clarified supernatant is the extract used for testing.
- b. Test the extract in the same manner as serum. In addition, test a sample for heat lability and a sample for trypsin activation.
 - (i) For the heated sample, place 1 ml of extract in a tube, plug with cotton,

- and heat for 10 minutes in a boiling water bath.
- (ii) For the trypsinized sample, place 1 ml of extract in a tube, add 0.25 ml of 0.5% trypsin (Difco 1:250) solution, and incubate at room temperature for 30 to 60 minutes (Difco; Detroit, Michigan).
 - c. The schedule for testing extracts is outlined in Table 6.
 - d. If toxin is demonstrated only in the trypsinized sample, repeat the neutralization tests using trypsinized extract. Add 1.5 ml of 0.5% trypsin to 6 ml of extract and incubate 30 to 60 minutes at room temperature. Distribute 1.25 ml of trypsinized extract to each of 5 tubes, add 0.25 ml of appropriate antitoxin (monovalent types A,B,E, or polyvalent [A,B,C,D,E,F]) per tube, mix, and incubate again 30 to 60 minutes at room temperature. Inject 2 mice with each trypsinized extract-antitoxin mixture (0.6 ml per mouse). Table 7 shows details of tests on trypsinized extracts. Trypsinization is only rarely necessary for detecting botulinum toxin.
 - e. Toxic substances other than botulinum toxin may be present in the stool specimen. Diluting the sample in two-fold increments and titrating the extract, then repeating the neutralization test on the extract near the end point dilution may eliminate the interference and permit proper identification of botulinum toxin.
4. Testing food specimens for toxin
- a. Record all identifying information.
 - b. If canned foods are to be tested, scrub the top of the can with soap and water, rinse, remove excess water, wipe surface with 70% alcohol. Open can in a biological safety cabinet (BSC). If BSC is not available, place the can in a large plastic bag before opening it to prevent the formation of an aerosol in the laboratory. Open with a sterile can opener.
 - c. Record condition of food (gassy, dark, putrid, etc.) and remove a small sample for pH determination. Record pH.
 - d. Prepare extract as for stool specimen. In addition, 1 to 2 g sterile sand may be added to the material in the mortar to aid in grinding. Gelatin diluent is added gradually during grinding, and if the material is quite dry, the ratio (vol/wt) of diluent may be increased. Grinding of food specimens should be done inside a BSC.
 - e. Test extract in the same manner as described for stool extracts (Sections E3b-E3d, Table 6).

F. Isolation of *C. botulinum*

1. Specimen preparation

- a. Suspended stool and food specimens as described in Sections E3a and E4d are used for culturing. Tissue specimens should be cultured for *C. botulinum*. Since tissue may contain unknown organisms, including HIV or hepatitis B, these specimens must be handled within a BSC. Large tissue specimens may be ground as described for foods. Small tissue specimens should be divided into two or more portions so that at least two tubes of culture medium may be inoculated.
- b. Prepare smears of food or tissue suspensions and Gram stain. Observe and record

the staining reaction, morphologic features, and approximate number of the bacteria present. Record the size of the rods, if present, and the location of spores in sporulated cells.

2. Preparation of enrichment cultures

- a. Heat 2 tubes of chopped meat-glucose-starch (CMGS) medium in a boiling water bath for 10 minutes to remove oxygen. Cool to room temperature.
- b. Inoculate by adding 0.5-1.0 ml of the suspension into each of the tubes of medium near the bottom of the tubes with a capillary pipette. Try to avoid introducing air bubbles into the medium.
- c. Incubate one tube at 80°C for 10 minutes, then place it in cold water.
- d. After inoculation and heat treatment, incubate both tubes anaerobically at 30°C for 4 days.
- e. After incubation, remove a portion of culture, centrifuge, and recover supernatant fluid for toxin testing.
- f. Screen for toxicity by testing untreated, heated, and trypsinized culture fluids in mice, as shown for tubes 1,2, and 3 in Table 6.
- g. If untreated and trypsinized culture fluids are toxic, perform neutralization tests as shown with tubes 4,5,6,7, and 8 in Table 6.
- h. If only the trypsinized sample is toxic, trypsinize 6 ml of culture fluid and repeat neutralization tests as described in Section E3d and Table 7.
- i. Demonstration of botulinum toxin in enrichment cultures from a nontoxic specimen is presumptive evidence for the presence of *C. botulinum* in the specimen; however, it is desirable to isolate the organisms for definitive identification when possible.

3. Single colony isolation

- a. At the time of toxicity testing or 1 or 2 days earlier, subculture from both the unheated and the heated CMGS cultures onto Modified McClung-Toabe egg yolk agar plates and streak to obtain isolated colonies.⁽⁶⁵⁾ Incubate the plates anaerobically for 48 hours at 35°C.
- b. Examine plates with a hand lens and a stereoscopic microscope, pick typical lipase positive colonies, and inoculate into CMGS medium. Pick several colonies because some isolates may be nontoxigenic. Incubate CMGS cultures 4 days at 30°C.
- c. Observing lipase positive colonies only in the growth on the plate from the nonheated enrichment culture might mean that a strain of *C. botulinum* with heat sensitive spores is present. However, if large numbers of other bacteria are present, it may be impossible to isolate *C. botulinum* from the plate. If so, try alcohol treatment for spore selection.^(66,67)
 - (i) Transfer 1 ml of the unheated enrichment culture to a tube and mix with 1 ml of absolute alcohol. Hold 1 hour at room temperature and mix every 15 minutes.
 - (ii) Inoculate 0.5 to 1.0 ml of the alcohol mixture into CMGS medium and incubate anaerobically 48 hours.
 - (iii) Repeat isolation attempt with egg yolk agar.

- d. Perform toxicity and toxin neutralization tests on pure cultures.
- e. Confirm identity of isolates by biochemical characteristics as described by Dowell et al.⁽⁶⁵⁾

NOTE: Sometimes it is difficult to isolate *C. botulinum* type E from mixed cultures because of bacteriocin produced by nontoxigenic organisms similar to *C. botulinum*.⁽⁶⁸⁾ This can be overcome by using a medium containing trypsin, which inactivates the bacteriocin. Trypticase-peptone-glucose-yeast extract-trypsin medium may be used in addition to CMGS when such difficulties are encountered, or when one knows beforehand the strain being sought is type E. Dissolve ingredients, adjust pH to 7.0; dispense in tubes, 15 ml/tube. Autoclave 8 minutes at 121 °C. Prepare a 1.5% solution of aqueous trypsin (Difco 1:1250) and filter sterilize. Just before using the medium, drive off oxygen by heating in a boiling water bath 10 minutes, cool, and add 1 ml trypsin solution.

XII. References

1. Shapiro RL, Hatheway C, Swerdlow DL. Botulism in the United States: A clinical and epidemiologic review. *Ann Intern Med.* 1998; 129: 221-228.
2. Shapiro RL, Hatheway C, Becher S, Swerdlow DL. Botulism surveillance and emergency response. A public health strategy for a global challenge. *JAMA.* 1997;278: 433-435.
3. Cato EP, George WL, Finegold SM. Genus *Clostridium*. In: Sneath PHA, Mair NS, Sharpe ME, et al. eds. *Bergey's Manual of Systematic Bacteriology*, Vol. 2. Baltimore: Williams & Wilkins; 1986;1141-1200.
4. Schiavo G, Montecucco C. The structure and mode of botulinum and tetanus toxins. In: The Clostridia. Molecular Biology and pathogenesis. Eds. Rood J, McClane BA, Songer JG, Titball RW. San Diego, California: Academic Press; 1997; 295-322.
5. Kim J, Foegeding PM. Principles of control. In: Hauschild AHW, Dodds KL, eds. *Clostridium botulinum: Ecology and control in foods*. New York: Marcel Dekker Inc.; 1992;121-176.
6. Ito KA, Seslar DJ, Ercern WA, et al. The thermal and chlorine resistance of *Clostridium botulinum* types A, B, and E spores. In: Ingram M, Roberts TA, eds. *Botulism 1966*. London: Chapman & Hall; 1967; 108-122.
7. Roberts TA, Ingram M. The resistance of spores of *Clostridium botulinum* type E to heat and radiation. *J Appl Bacteriol* 1965;28:125-137.
8. Zezones H, Hutchings IJ. Thermal resistance of *Clostridium botulinum* (62A) spores as affected by fundamental food constituents. *Food Technol* 1965;19:1003-1005.
9. State of Alaska Department of Health and Social Services. *Botulism in Alaska: A guide for physicians and health care providers*. Anchorage: Alaska Printing; 1998.
10. Meyer KF, Dubovsky BJ. The distribution of the spores of *B. botulinus* in the United States. *J Infect Dis* 1922;31:559-594.
11. Ward BQ, Carroll BJ, Garrett ES, et al. Survey of the U.S. Gulf Coast for the presence of *Clostridium botulinum*. *Appl Microbiol* 1967;15:629-639.
12. Bott TL, Deffner JS, McCoy E, et al. *Clostridium botulinum* type E in fish from the Great Lakes. *J Bacteriol* 1966;91:919-924.
13. Rogers DE. Botulism, Vintage 1963. *Ann Intern Med* 1964;61:581-588.
14. Rogers DE, Koenig MG, Spickard A. Clinical and laboratory manifestations of type E botulism in man, in: Botulism. Lewis KH, Cassel K, editors. Proceedings of a symposium, (PHS Pub No. 999-FP-1), 1964;133-145.
15. Midura TF, Nygaard GS, Wood RM, et al. *Clostridium botulinum* type F: isolation from venison jerky. *Appl Microbiol* 1972;24:165-167.
16. Center for Disease Control. Botulism-California. *Morb Mort Wkly Rep* 1966;15:349.
17. Center for Disease Control. Botulism type F - California. *Morb Mort Wkly Rep* 1966;15:359.
18. Centers for Disease Control and Prevention. Foodborne botulism-Oklahoma, 1994. *Morb Mort Wkly Rep* 1995;44:200-202.
19. Benenson AS, ed. Botulism/infant botulism. In: Control of communicable diseases manual. 16th Edition. Washington, D.C.: American Public Health Association; 1995;66-69.

20. Extension Service, U.S. Department of Agriculture. Complete guide to home-canning. Washington, D.C.: U.S. Department of Agriculture, Extension Service, September 1994. (Agriculture information bulletin no.539).
21. Arnon SS. Infant botulism. In: Feigen R, Cherry J, eds. *Textbook of Pediatric Infectious Diseases* Philadelphia: W. B. Saunders; 1992; 1095-1102.
22. Midura TF, Arnon SS. Infant botulism: Identification of *Clostridium botulinum* and its toxin in faeces. *Lancet* 1976;2:934-936.
23. Pickett J, Berg B, Chaplin E, et al. Syndrome of botulism in infancy. *N Engl J Med* 1976;295:770-772.
24. Long SS, Gajewski JL, Brown LW, et al. Clinical, laboratory, and environmental features of infant botulism in southeastern Pennsylvania. *Pediatrics* 1985;75:935-941.
25. Morris JG, Snyder JD, Wilson R, et al. Infant botulism in the United States: An epidemiologic study of the cases occurring outside California. *Am J Public Health* 1983;73:1385-1388.
26. Istre GR, Compton R, Novotny T, et al. Infant botulism: three cases in a small town. *Am J Dis Child* 1986;140:1013-1014.
27. Long SS. Epidemiologic study of infant botulism in Pennsylvania. *Pediatrics* 1985;75:928-934.
28. Thompson JA, Glasgow LA, Warpinski JR. Infant botulism: Clinical spectrum and epidemiology. *Pediatrics* 1980;66:936-942.
29. Arnon SS, Midura TF, Damus K. Honey and other environmental risk factors for infant botulism. *J Pediatr* 1979;94:331-336.
30. Chin J, Arnon SS, Midura TF. Food and environmental aspects of infant botulism in California. *Rev Infect Dis* 1979;1:693-696.
31. Spika JS, Shaffer N, Hargrett-Bean N. Risk factors for infant botulism in the United States. *Am J Dis Child* 1989;143:828-832.
32. Kautter DA, Lilly T, Solomon HM, et al. *Clostridium botulinum* spores in infant foods: A survey. *J Food Protection* 1982;45:1028-1029.
33. Arnon SS, Damus K, Thompson B, et al. Protective role of human milk against sudden death from infant botulism. *J Pediatr* 1982;10:568-573.
34. Wilson R, Morris JG, Snyder JD, et al. Clinical characteristics of infant botulism in the United States: a study of the non-California cases. *Pediatr Infect Dis* 1982;1:148-150.
35. Wells CL, Sugiyama H, Bland SE. Resistance of mice with limited intestinal flora to enteric colonization by *Clostridium botulinum*. *J Infect Dis* 1982;146:791-796.
36. Weber JT, Goodpasture HC, Alexander H, et al. Wound botulism in a patient with a tooth abscess: case report and review. *Clin Infect Dis* 1993;16:635-639.
37. Hatheway CL. Botulism. In: Balows A, Hausler Jr. WJ, Ohashi M, et al., eds. *Laboratory Diagnosis of Infectious Diseases: Principles and Practice*, Vol. 1. Berlin: Springer-Verlag; 1988;111-133.
38. Merson MH, Dowell VR. Epidemiologic, clinical, and laboratory aspects of wound botulism. *N Engl J Med* 1973;289:1005-1010.
39. MacDonald KL, Rutherford GW, Friedman SM, et al. Botulism and botulism-like illness in chronic drug abusers. *Ann Intern Med* 1985;102:616-618.
40. McCroskey LM, Hatheway CL. Laboratory findings in four cases of adult botulism

- suggest colonization of the intestinal tract. *J Clin Microbiol* 1988;26:1052-1054.
41. Chia JK, Clark JB, Ryan CA, et al. Botulism in an adult associated with foodborne intestinal infection with *Clostridium botulinum*. *N Engl J Med* 1986;315:239-241.
 42. Griffin PM, Hatheway CL, Rosenbaum RB, Sokolow R. Endogenous antibody production to botulinum toxin in an adult with intestinal colonization botulism and underlying Chron's Disease. *J Infect Dis* 1997; 175:633-637.
 43. Hughes JM, Blumenthal JR, Merson MH, et al. Clinical features of types A and B food-borne botulism. *Ann Intern Med* 1981;95:442-445.
 44. St. Louis ME, Peck SH, Bowering D, et al. Botulism from chopped garlic: Delayed recognition of a major outbreak. *Ann Intern Med* 1988;108:363-368.
 45. Woodruff BA, Griffin PM, McCroskey LM, et al. Clinical and laboratory comparison of botulism from toxin types A, B, and E in the United States, 1975-1988. *J Infect Dis* 1992;166:1281-1286.
 46. L'Hommedieu C, Stough R, Brown L. Potentiation of neuromuscular weakness in infant botulism by aminoglycosides. *J Pediatr* 1979;95:1065-1070.
 47. Badhey H, Cleri DJ, D'Amato RF, et al. Two fatal cases of type E adult food-borne botulism with early symptoms and terminal neurologic signs. *J Clin Microbiol* 1986;23:616-618.
 48. Horwitz MA, Marr JS, Merson MH, et al. A continuing common-source outbreak of botulism in a family. *Lancet* 1975;1:1-6.
 49. Koenig GM, Spickard A, Cardella MA, et al. Clinical and laboratory observations on type B botulism in man. *Medicine* 1964;43:517-545.
 50. Terranova WA, Palumbo JN, Breman JG. Ocular findings in botulism type B. *JAMA* 1979;241:475-477.
 51. Cherington M. Botulism: ten-year experience. *Arch Neurol* 1974;30:432-437.
 52. Cherington M, Ginsberg S. Type B botulism: neurophysiologic studies. *Neurology* 1971;21:43-46.
 53. Kimura J. *Electrodiagnosis in Diseases of Nerve and Muscle*. Philadelphia: Davis; 1983.
 54. Dowell VR, McCroskey LM, Hatheway CL, et al. Coproexamination for botulinum toxin and *Clostridium botulinum*. *JAMA* 1977;238:1829-1832.
 55. Center for Disease Control. Follow-up: Botulism associated with commercial cherry peppers. *Morb Mortal Wkly Rep* 1976;25:148.
 56. Terranova WA, Breman JG, Locey RP, et al. Botulism type B: epidemiologic aspects of an extensive outbreak. *Am J Epidemiol* 1978;108:150-156.
 57. Tackett CO, Shandera WX, Mann JM, et al. Equine antitoxin use and other factors that predict outcome in type A foodborne botulism. *Am J Med* 1984;76:794-798.
 58. Sugiyama H. *Clostridium botulinum* neurotoxin. *Microbiol Rev* 1980;44:419-448.
 59. Hatheway CL, Snyder JD, Seals JD, et al. Antitoxin levels in botulism patients treated with trivalent equine botulism antitoxin to toxin types A, B, and E. *J Infect Dis* 1984;150:407-412.
 60. Black RE, Gunn RA. Hypersensitivity reactions associated with botulinum antitoxin. *Am J Med* 1980;69:567-570.
 61. American Academy of Pediatrics. Clostridium infections. In: Peter G, ed. 1997 Red Book: Report of the Committee on Infectious Diseases. 24th ed. Elk Grove Village, IL: American

- Academy of Pediatrics; 1997; 176.
62. Koenig MG, Drutz DJ, Mushlin AI, et al. Type B botulism in man. *Am J Med* 1967;42:208-219.
 63. Mena E, Thompson FS, Armfield AY, et al. Evaluation of Port-A-Cul system for protection of anaerobic bacteria. *J Clin Microbiol* 1978;8:28-35.
 64. Horwitz MA, Hatheway CL, Dowell VR Jr. Laboratory diagnosis of botulism complicated by pyridostigmine treatment of the patient. *Am J Clin Pathol* 1976;66:737-742.
 65. Dowell VR Jr, Hawkins TM. Laboratory methods in anaerobic bacteriology. In: CDC Laboratory Manual. US Dept of HEW, PHS, Center for Disease Control, Atlanta, Georgia. DHEW Publication No (CDC) 87-8272;1987.
 66. Johnston R, Harmon S, Kautter D. Method to facilitate the isolation of *Clostridium botulinum* type E. *J Bacteriol* 1962;88:1521-1522.
 67. Koransky JR, Allen SD, Dowell VR Jr. Use of ethanol for selective isolation of spore-forming microorganisms. *Appl Environ Microbiol* 1978;35:762-765.
 68. Lilly T Jr, Harmon SM, Kautter DA, et al. An improved medium for detection of *Clostridium botulinum* type E. *J Milk Food Technol* 1971;34:492-497.

XIII. Selected references for further reading

1. Angulo FJ & St. Louis ME. Botulism. In: Alfred S Evans, Philip Brachman, eds. *Bacterial Infections of Humans; Epidemiology and Control*. 3rd ed. New York: Plenum Medical Book Co.; 1998; 139-153.
2. Hatheway CL. Toxigenic Clostridia. *Clin Microbiol Rev* 1990;3:66-98.

XIV. Tables and Figures

Table 1. Outbreaks of foodborne botulism by toxin type, 1950-1996

Toxin type	<u>1950-1959</u>	<u>1960-1969</u>	<u>1970-1979</u>	<u>1980-1989</u>	<u>1990-1996</u>	<u>Total</u>
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
A	14 (13.5)	12 (15.4)	68 (53.5)	48 (60.8)	25 (44.6)	167 (37.6)
B	3 (2.9)	10 (12.8)	28 (22.0)	13 (16.5)	7 (12.5)	61 (13.7)
E	7 (6.7)	9 (11.5)	15 (11.8)	16 (20.3)	20 (35.7)	67 (15.1)
F	0	1 (1.3)	0	0	2 (3.6)	3 (0.7)
Unknown	80 (76.9)	46 (59.0)	16 (12.6)	2 (2.5)	2 (3.6)	146 (32.9)
Total	104	78	127	79	56	444

	1950-1959	1960-1969	1970-1979	1980-1989	1990-1996	Total
Toxin Type A						
Cases	39	30	164	119	60	412
Deaths	18	8	26	14	4	70
Case fatality ratio	46.2	26.7	15.9	11.8	6.7	17.0
Toxin Type B						
Cases	4	23	110	28	11	176
Deaths	2	4	6	1	0	13
Case fatality ratio	50.0	17.4	5.5	3.6	0.0	7.4
Toxin Type E						
Cases	21	35	28	62	47	193
Deaths	8	15	3	1	3	30
Case fatality ratio	38.1	42.9	10.7	1.6	6.4	15.5
Toxin Type F						
Cases	0	3	0	0	2	5
Deaths	0	0	0	0	0	0
Case fatality ratio	-	0.0	-	-	0.0	0.0
Total, toxin known						
Cases	64	91	302	209	120	786
Deaths	28	27	35	16	7	113
Case fatality ratio	43.8	29.7	11.6	7.7	5.8	14.4
Total, toxin unknown						
Cases	169	89	34	6	3	301
Deaths	30	15	11	0	0	56
Case fatality ratio	17.8	16.9	32.4	0.0	0.0	18.6
Percent cases due to unknown toxin type	72.5	49.4	10.1	2.8	2.4	27.7
Total						
Cases	233	180	336	215	123	1087

Table 3. Number (%) of foodborne botulism outbreaks, by place of food processing, 1950-1996

	<u>1950-1959</u>	<u>1960-1969</u>	<u>1970-1979</u>	<u>1980-1989</u>	<u>1990-1996</u>	<u>Total</u>
Process location	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Home processed	51 (49.0)	44 (56.4)	85 (66.9)	69 (87.3)	40 (71.4)	289 (65.1)
Commercially processed	2 (1.9)	10 (12.8)	9 (7.1)	6 (7.6)	4 (7.1)	31 (7.0)
Unknown	51 (49.0)	24 (30.8)	33 (26.0)	4 (5.1)	12 (21.4)	124 (27.9)
Total	104	78	127	79	56	444

Table 4. Number (%) of foodborne botulism outbreaks, by food category and toxin type, 1950-1996

Toxin type	<u>Vegetables</u>	<u>Fruits</u>	<u>Condiments</u>	<u>Dairy product</u>	<u>Beef</u>	<u>Pork</u>	<u>Poultry</u>	<u>Fish</u>	<u>Marine mammal</u>	<u>Other</u>	<u>Unknown</u>	Total
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
A	102 (61.1)	3 (1.8)	3 (1.8)	2 (1.2)	5 (3.0)	1 (0.6)	4 (2.4)	14 (8.4)	1 (0.6)	9 (5.4)	23 (13.8)	167
B	35 (57.4)	2 (3.3)	2 (3.3)	0	0	4 (6.6)	1 (1.6)	4 (6.6)	4 (6.6)	2 (3.3)	7 (11.5)	61
E	0	0	0	0	0	0	0	36 (53.7)	25 (37.3)	2 (3.0)	4 (6.0)	67
F	0	0	0	0	0	0	0	0	0	1 (33.3)	2 (66.7)	3
Unknown	43 (29.5)	3 (2.1)	1 (0.7)	1 (0.7)	1 (0.7)	2 (1.4)	1 (0.7)	3 (2.1)	0	3 (2.1)	88 (60.3)	146
Total	180 (40.5)	8 (1.8)	6 (1.4)	3 (0.7)	6 (1.4)	7 (1.6)	6 (1.4)	57 (12.8)	30 (6.8)	17 (3.8)	124 (27.9)	444

Table 5. Method of testing serum for botulinum toxin

Tube number	Volume of serum (ml)	Volume of antitoxin ^a (ml)	Type of antitoxin	Volume drawn into syringe	Volume injected into each mouse (ml)
1	1.0	0.0	None	0.8	0.4
2	1.0	0.25	A	1.0	0.5
3	1.0	0.25	B	1.0	0.5
4	1.0	0.25	E	1.0	0.5
5	1.0	0.25	F	1.0	0.5
6	1.0	0.25	ABCDEF ^b	1.0	0.5

^a Mix antitoxin with serum and incubate 30 to 60 minutes at room temperature.

^b Since type F toxin is rarely encountered, a trivalent antitoxin (types A, B, and E) reagent will be sufficient to confirm most cases of botulism; and tube number 5 (type F) can be prepared later if necessary.

Table 6. Method of testing extracts and culture fluids for botulinum toxin

Tube number	Volume of specimen (ml)	Treatment ^a	Type of antitoxin	Volume drawn into syringe (ml)	Volume injected into each mouse (ml)
1	1.0	None	None	0.8	0.4
2	1.0	Heat 100°C for 10 min	None	1.0	0.5
3	1.0	0.25 ml trypsin	None	1.0	0.5
4	1.0	0.25 ml antitoxin	A	1.0	0.5
5	1.0	0.25 ml antitoxin	B	1.0	0.5
6	1.0	0.25 ml antitoxin	E	1.0	0.5
7	1.0	0.25 ml antitoxin	F	1.0	0.5
8	1.0	0.25 ml antitoxin	ABCDEF ^b	1.0	0.5

^a Mix antitoxin or trypsin with specimen and incubate 30 to 60 minutes at room temperature.

^b Since type F toxin is rarely encountered, a trivalent antitoxin (types A, B, and E) reagent will be sufficient to confirm most cases of botulism; and, tube number 5 (type F) can be prepared later if necessary.

Table 7. Neutralization tests on trypsinized^a extracts and culture fluids

Tube number	Volume of trypsinized ^a test material (ml)	Volume of antitoxin (ml)	Type of antitoxin ^b	Volume drawn into syringe (ml)	Volume injected into each mouse (ml)
1	1.25	0.25	A	1.2	0.6
2	1.25	0.25	B	1.2	0.6
3	1.25	0.25	E	1.2	0.6
4	1.25	0.25	F	1.2	0.6
5	1.25	0.25	ABCDEF	1.2	0.6

^a Trypsinization: 6 ml test material + 1.5 ml 0.5% trypsin; incubate 30 minutes at 37°C.

^b Mix trypsinized test material and antitoxin and incubate 30 minutes at 37°C.

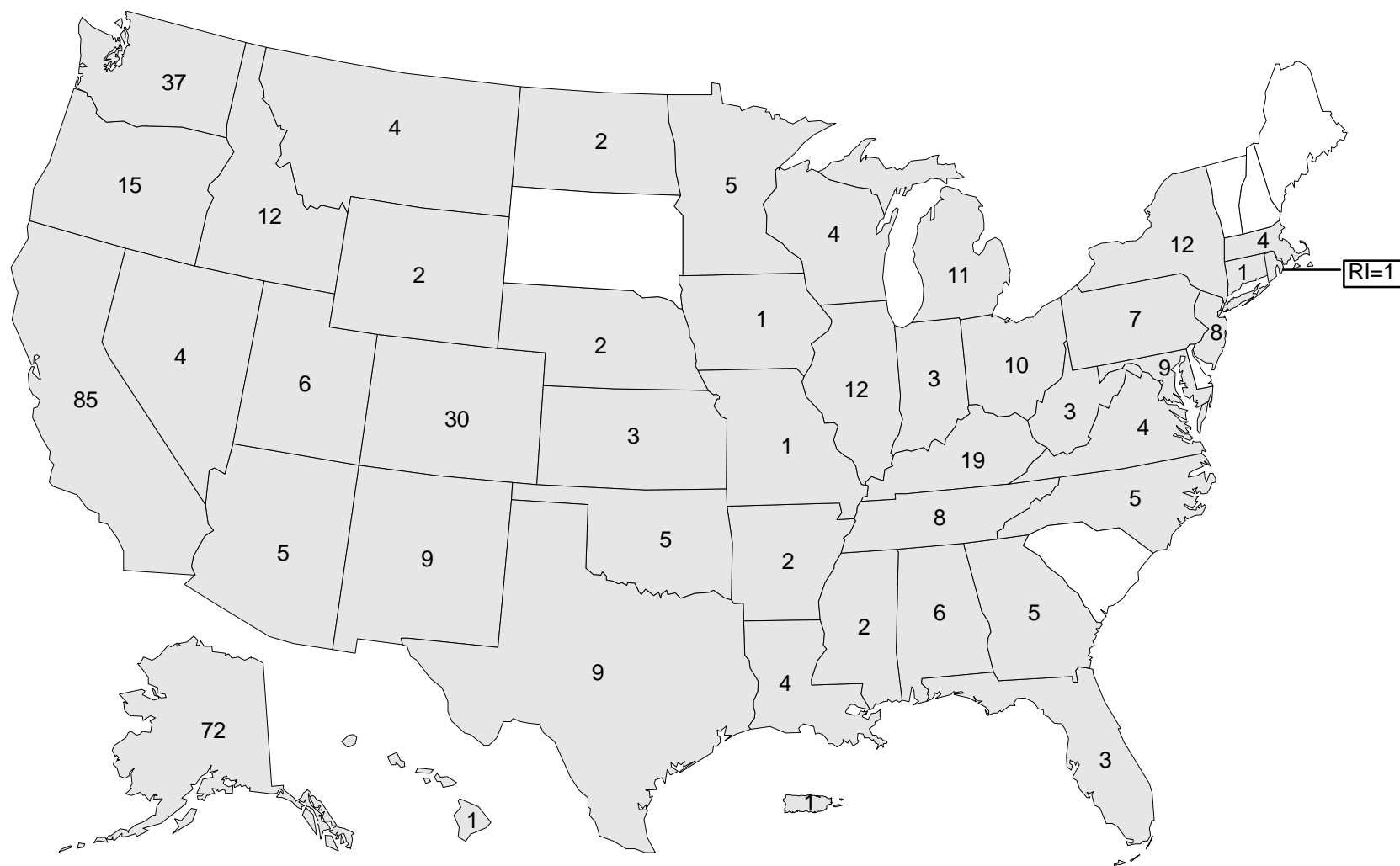


Figure 1. Outbreaks of foodborne botulism by state, 1950-1996*

* Multistate outbreaks are added to each involved state's total.

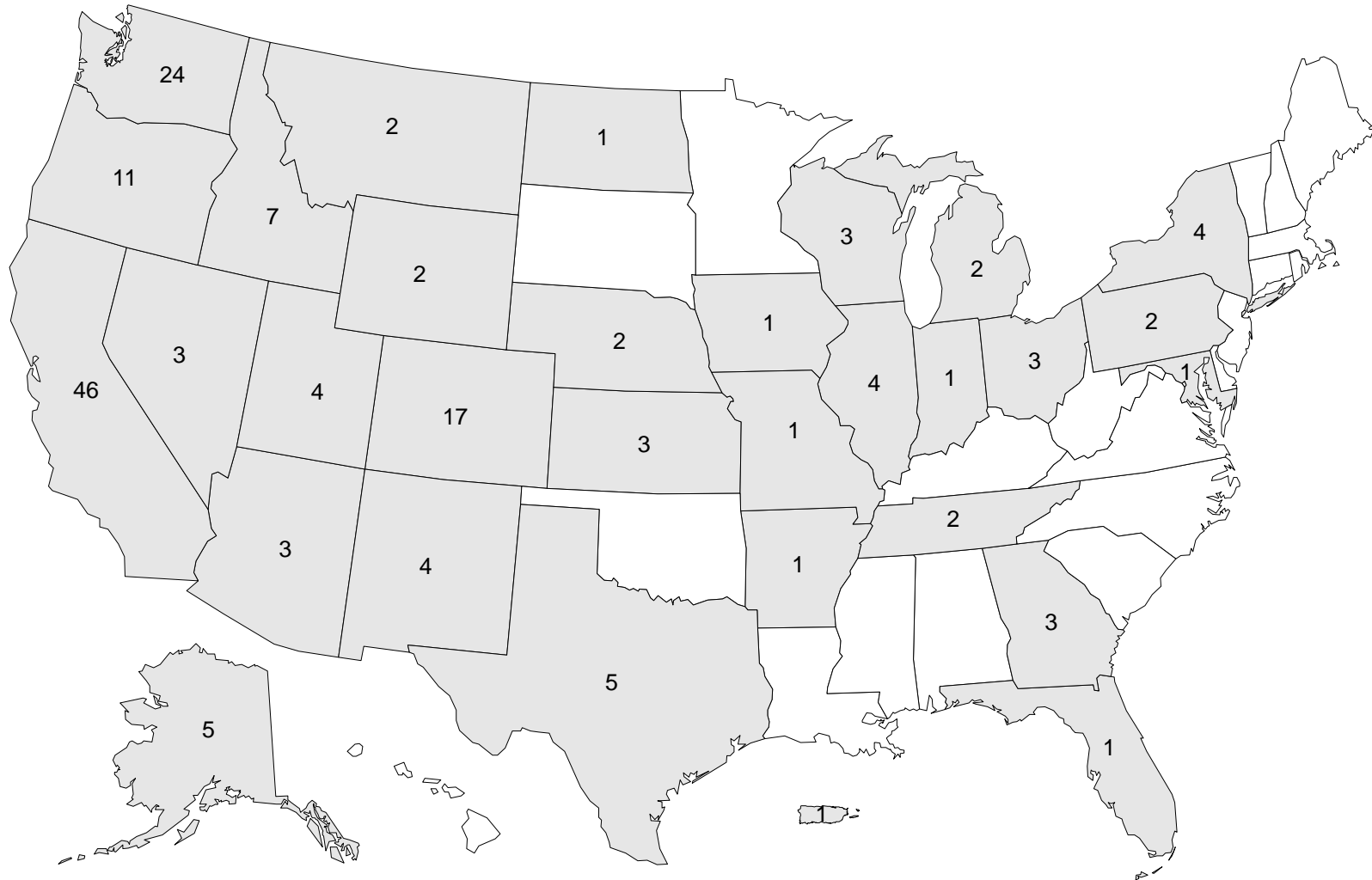


Figure 2. Outbreaks of foodborne botulism, toxin type A, by state, 1950-1996*

* Multistate outbreaks are added to each involved state's total.

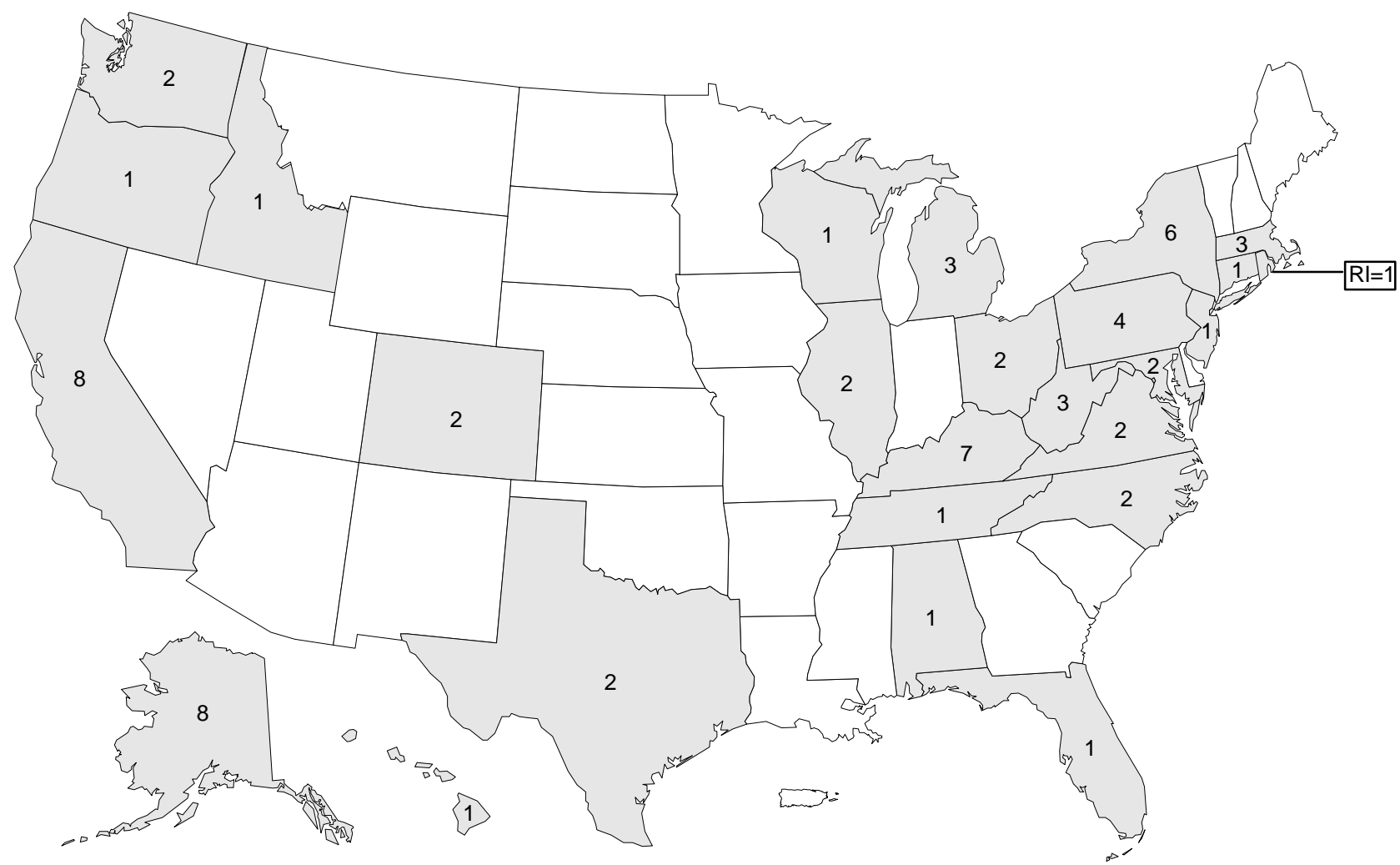


Figure 3. Outbreaks of foodborne botulism, toxin type B, by state, 1950-1996*

* Multistate outbreaks are added to each involved state's total.

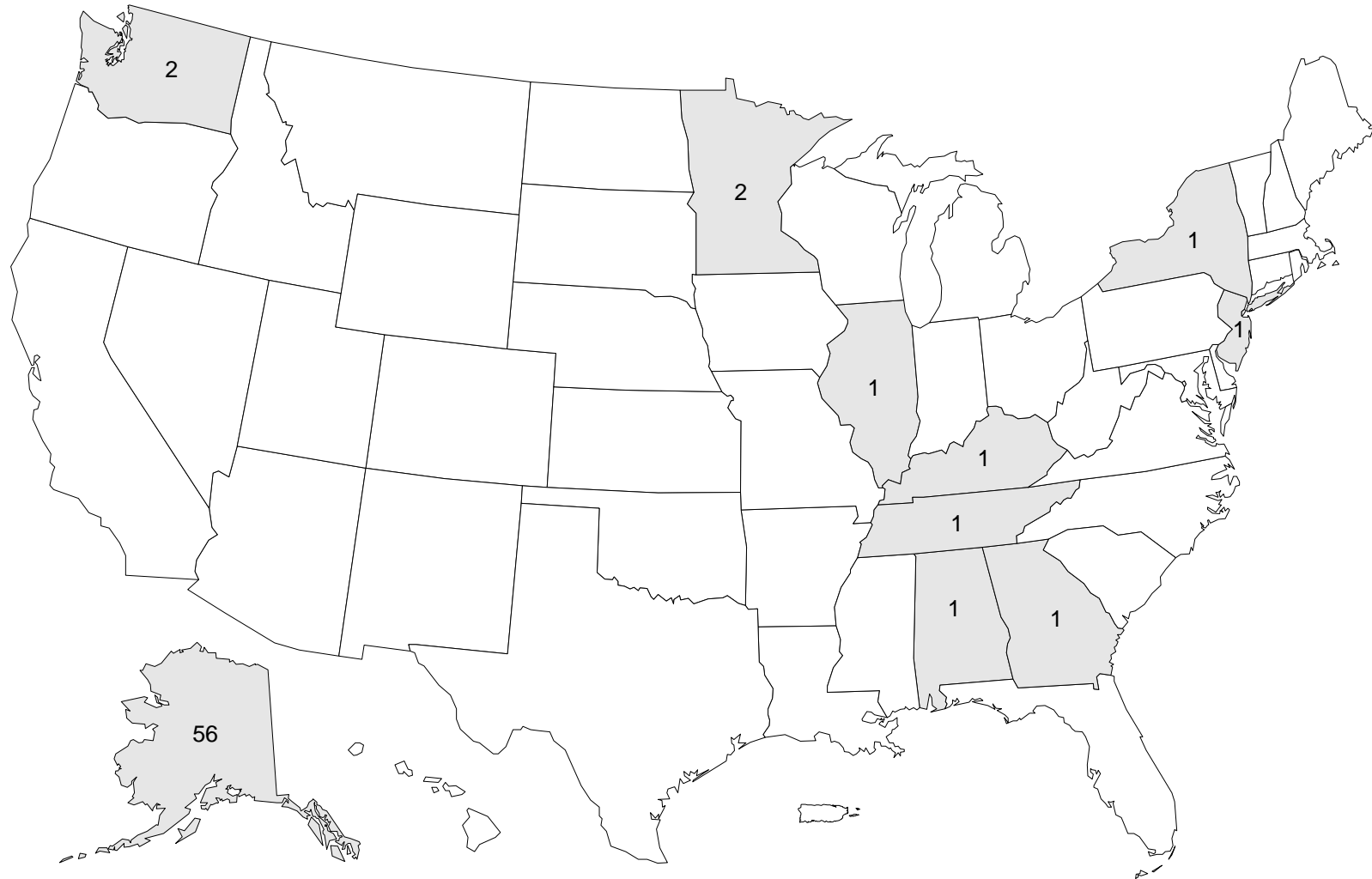


Figure 4. Outbreaks of foodborne botulism, toxin type E, by state, 1950-1996*

* Multistate outbreaks are added to each involved state's total.

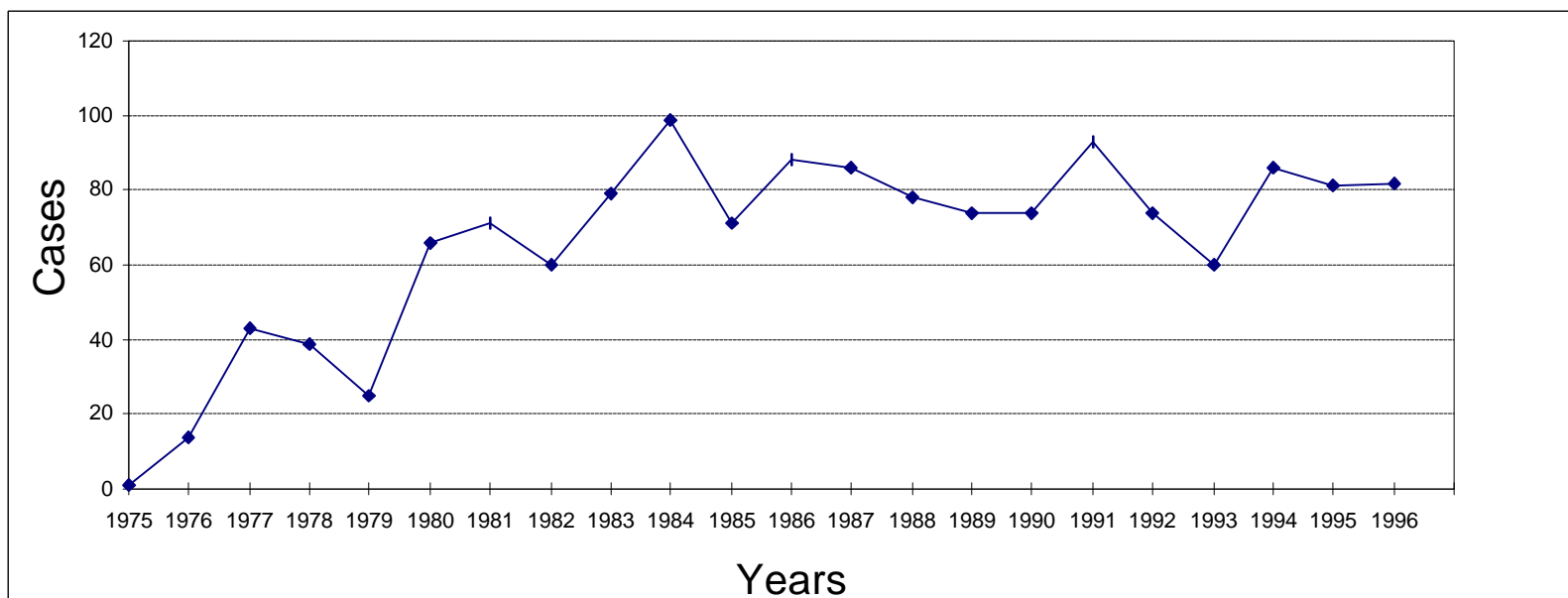


Figure 5. Cases of infant botulism, 1975-1996

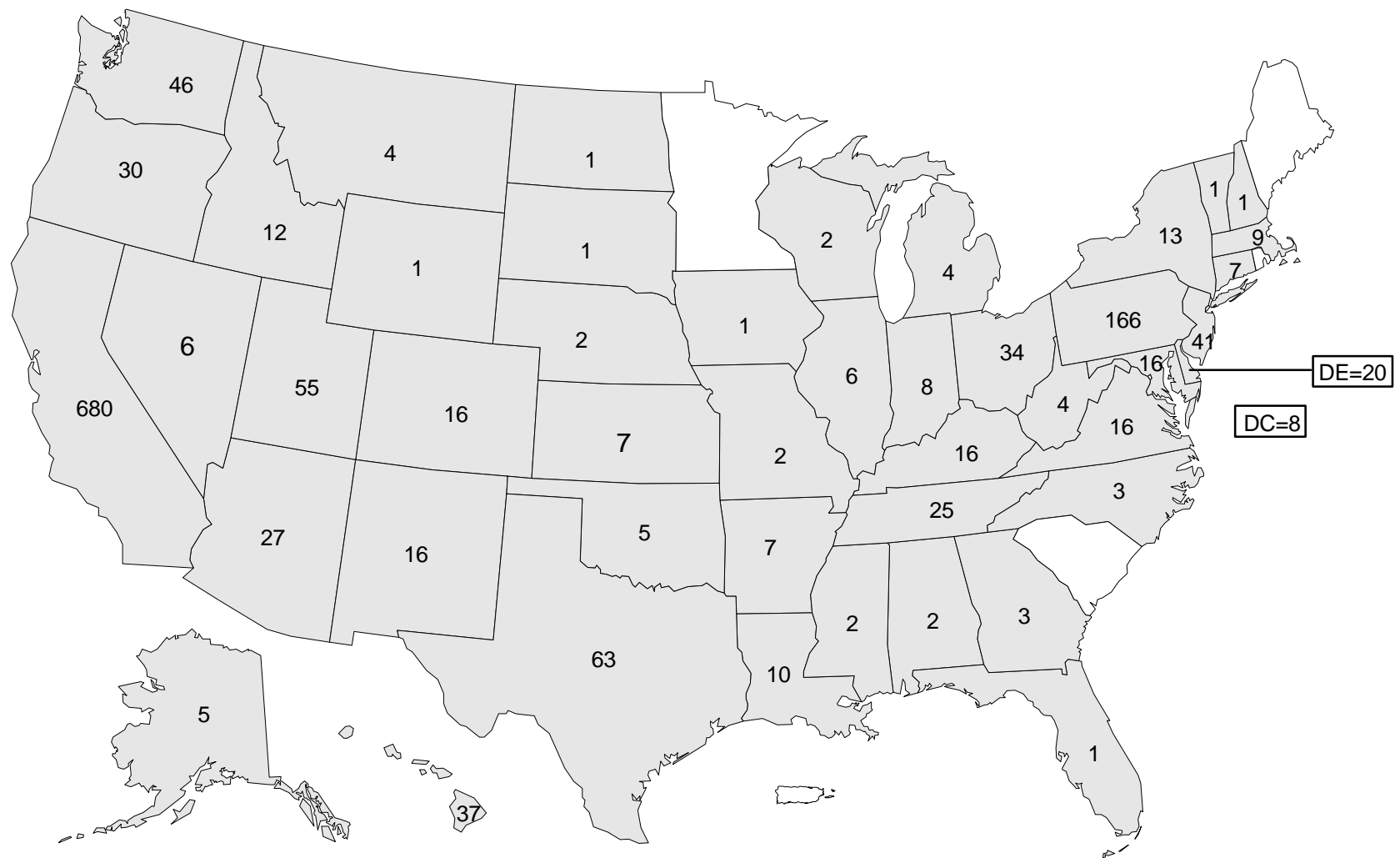


Figure 6. Outbreaks of infant botulism, by state, 1976-1996

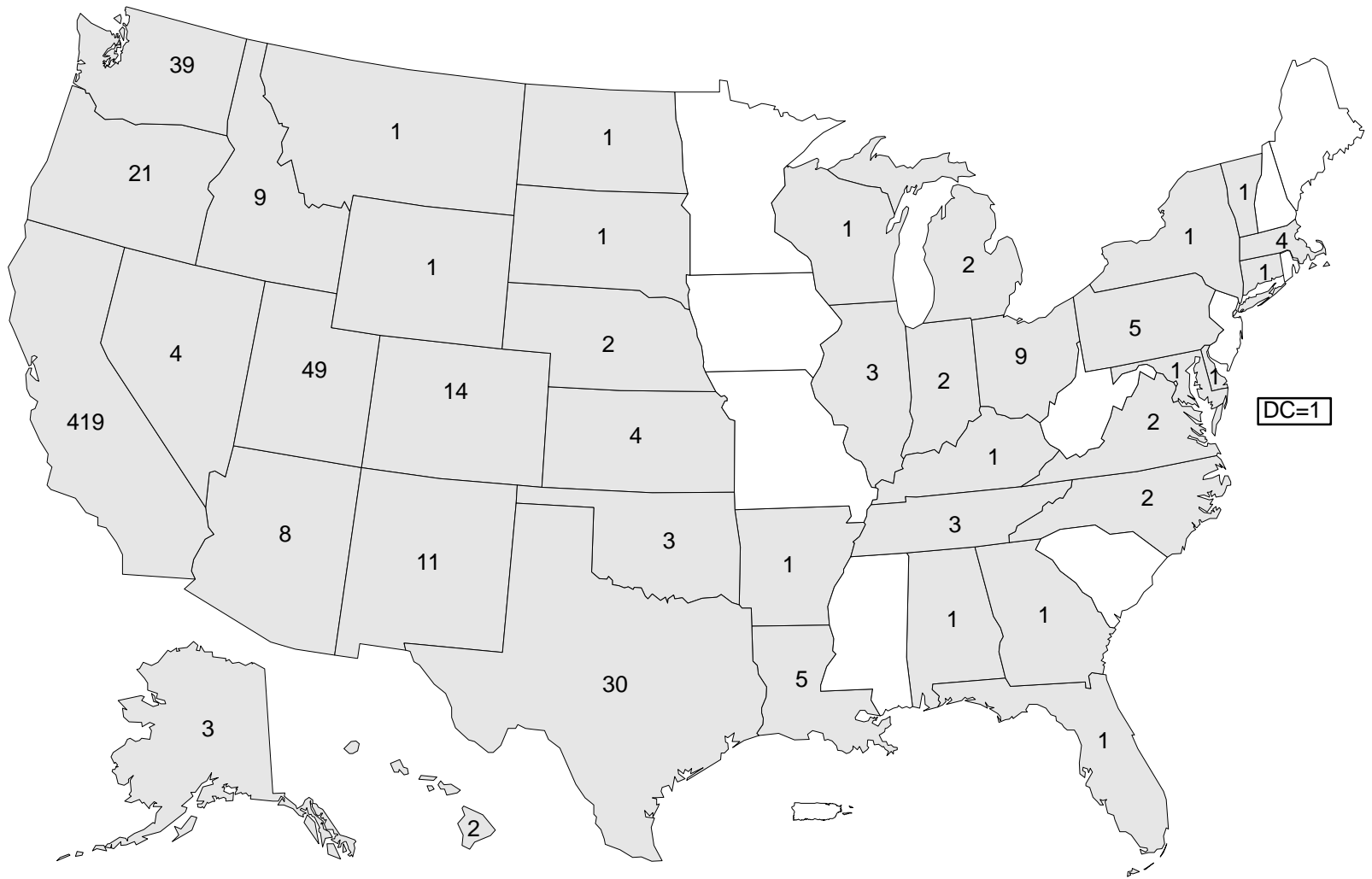


Figure 7. Outbreaks of infant botulism, type A, by state, 1976-1996

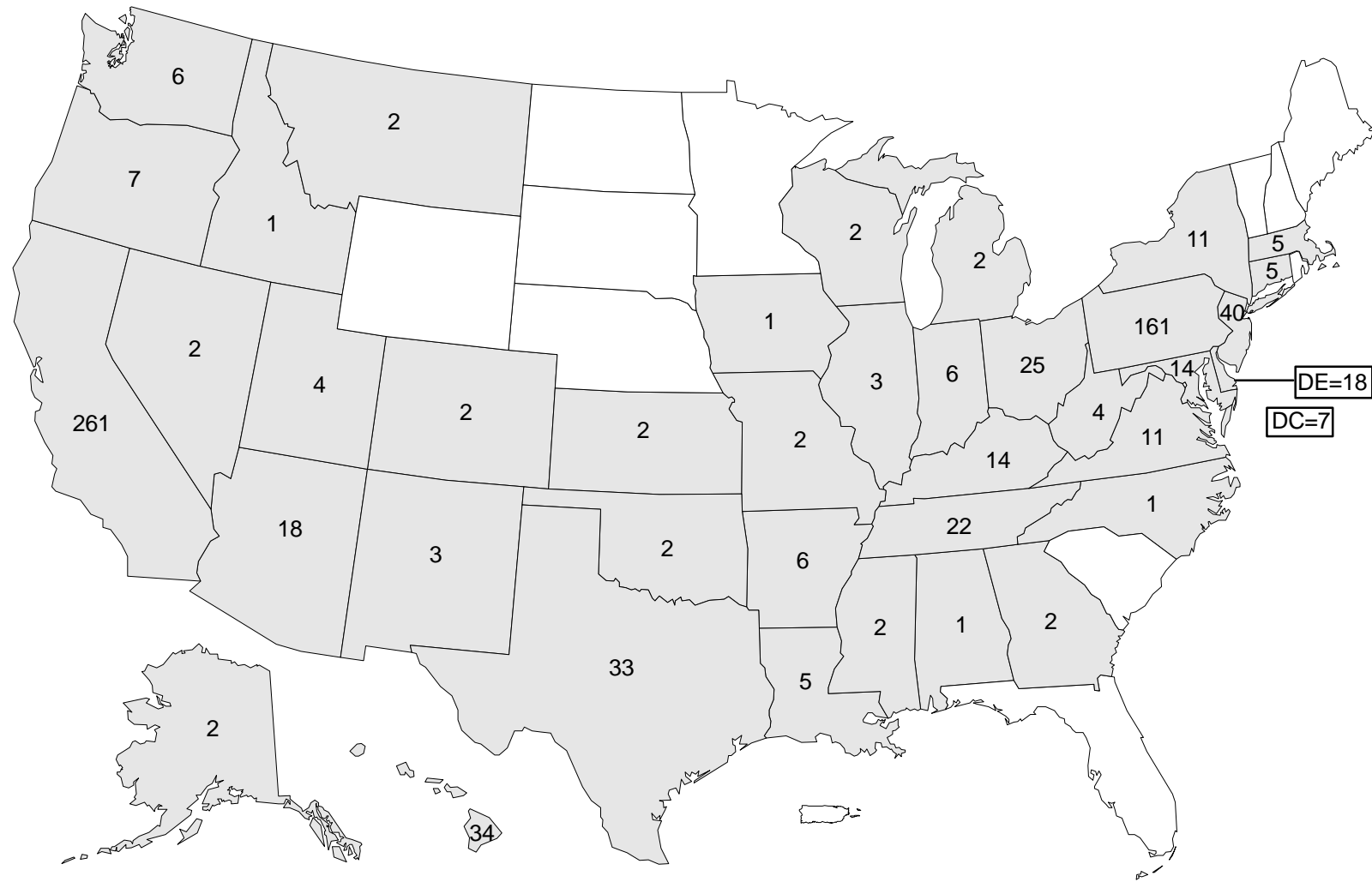


Figure 8. Outbreaks of infant botulism, type B, by state, 1976-1996

XV. Appendix