86

**Salmonellae in Food—A Review**


---

**CLOSTRIDIUM BOTULINUM FOOD POISONING**

E. M. Foster, Janet S. Deffner, Thomas L. Bott, and Elizabeth McCoy

Department of Bacteriology, University of Wisconsin, Madison

**Summary**

The outbreaks of botulism in the United States during 1963 stimulated renewed interest in this food-borne disease, primarily because commercially prepared foods were involved. Three of the outbreaks were caused by *Clostridium botulinum* type E in fishery products. Two of these resulted from the consumption of smoked fish from the Great Lakes.

A survey has been started to see if *C. botulinum* type E is common on fish from the Great Lakes. Toxin neutralization tests have shown the organism to be present in cultures from nine of ten locations sampled in Lake Michigan. The organism was found more frequently in the intestinal tract than on gills, livers or the external surfaces of the fish. Over 75% of the cultures prepared from the intestines of fish caught in one large bay of Lake Michigan proved to contain type E toxin. The incidence of the organism in fish from the main body of the lake has been much lower than this.

The first recognized outbreak of botulism was observed over 200 years ago (10), although the causal organism was not isolated until 1895 (9). The disease is caused exclusively by the ingestion of food in which *Clostridium botulinum* has grown and produced its toxin.

---

1. The experimental work described in this report was supported in part by the Food and Drug Administration under contract No. FDA 63-77 (Neg.).

According to Lamanna (18), botulinum toxin is the most potent poison known to man. Less than 1 x 10^-10 gram will kill a mouse. Although the toxin is a protein and therefore a large molecule, it somehow passes into the lymphatic system from the upper part of the intestinal tract (18). By means that are not yet understood, the toxin acts on certain myoneural junctions, interfering with the release of acetylcholine and thus preventing the passage of nerve impulses. The muscles involved in respiration are particularly affected, and death results from asphyxiation.

Gastric symptoms frequently are the first sign of botulism, with nausea and vomiting often appearing in 12 to 18 hr. Patients may complain of a dry mouth during this time. Neurologic symptoms soon develop, with double vision, muscular weakness, and difficulty in talking and swallowing. Respiratory paralysis follows, with death in fatal cases usually coming in three to six days. Complete recovery in non-fatal cases may require several months (4, 5, 26).

Botulism is a disease of both man and animals. Reports of human botulism have come mainly from North America, Europe and Japan, although two outbreaks have been recorded in Argentina and two in Australia (19). The true incidence is unknown because of frequent failure to recognize the disease. In the United States there are usually no more than
10 or 12 verified outbreaks with 20 to 25 cases each year. For the past quarter century Germany has had 9 to 15 outbreaks with 30 to 40 cases annually (19).

Since World War II the incidence in France has been similar to that in Germany (19). Botulism was first recognized in Japan in 1951, but subsequently that country has averaged four outbreaks with about 25 cases per year. Between 1919 and 1954 Canada suffered a total of 14 known outbreaks, with an average of four cases each (19).

Although the incidence of botulism is low, the mortality rate is high. Almost two-thirds of the cases reported in the United States between 1899 and 1954 resulted in death (19). In Europe the mortality rate is much lower, averaging 19% for the more than 4,000 cases recorded by Meyer (19). The average mortality rate in Japan has been 26% (23). In some outbreaks the number of fatalities may be much higher or lower than the average figures.

The foods incriminated in outbreaks of botulism almost invariably are: (a) given an inadequate preliminary treatment such as heating, salting, smoking, drying or pickling; (b) allowed to stand at a temperature that will permit the growth of C. botulinum; and (c) eaten without cooking. In the United States, homogenized vegetables are incriminated in the vast majority of cases (20). Pork products are the major vehicles in Europe, with salted or pickled fish also frequently involved (19). A pickled relish called "izushi," which is made of raw fish, rice and diced vegetables, has caused over 90% of the outbreaks in Japan (23).

Botulism in animals has great economic importance (19). Feeding spoiled, discarded food to barnyard fowl has caused many outbreaks of "limberneck" with high losses in flocks of chickens, turkeys and geese. Mass intoxication of thousands of aquatic wild birds occurs with disturbing frequency on lakes and mud flats in the western United States. Fur ranchers have suffered enormous losses from feeding botulinogenic food to mink. Sheep ranchers in Australia and cattle ranchers in South Africa sometimes lose animals from feeding on forage or even carrion that contains botulinum toxin.

_Clostridium botulinum_ is a gram positive, anaerobic, sporeforming rod-shaped organism (3). Its spores are widely distributed in nature, having been found commonly in soil, mud, and the intestinal contents of animals. Thus the opportunity for contaminating food exists almost everywhere.

Botulinum toxin is stable in acid but is readily destroyed by alkali. Heating to 80°C for 30 min will inactivate it (26).

Six distinct types of _C. botulinum_ now are recognized. They are designated by the letters A, B, C, D, E and F, and are differentiated by the serological specificity of their toxins. Type C actually consists of two subtypes, C1 and C2, which differ in their effects on various animal species and in several other features (8, 19).

Types C and D only rarely have been implicated in outbreaks of human botulism (8), but they have caused huge losses in wild and domestic animals. Type F was first recognized in 1958 and has been involved in only one known outbreak, this being caused by home-prepared liver paste in Denmark (8). Thus, types A, B and E have been responsible for all but a few of the known outbreaks among humans (18, 27).

In addition to their serological differences, types A and B are distinguishable from type E by several other features.

1. **Heat resistance.** The spores of types A and B will survive boiling for several hours, whereas type E spores usually are killed by heating to 80°C for 30 min or less (4, 5, 28).

2. **Minimum growth temperature.** Types A and B grow slowly if at all at 50°F, whereas certain strains of type E have been observed to grow down to 38°F (21).

3. **Toxicity of cultures and activation of toxin by trypsin.** Cultures of type E show relatively much lower toxicities than types A and B when injected into mice (27). The potency of type E cultures can be increased 10 to 100 fold, however, by treating with trypsin. Trypsin does not ordinarily "activate" the toxins of types A and B, presumably because these organisms produce their own proteolytic enzymes, which may perform the same function as trypsin. However, Bonventre and Kempe (2) have shown that young cells of types A and B contain a toxic "precursor" that can be released by sonic disruption of the cells. The potency of this material, like that of type E toxin, can be increased by treatment with trypsin.

**Type E Botulism in Relation to Fishery Products**

Before last year the American public was hardly aware that type E botulism existed. Excluding a few small outbreaks in Alaska between 1950 and 1960, there had been only five known episodes of type E botulism in the United States. These involved a total of 15 cases with 6 deaths (22).

In March of 1963, however, three women in Detroit, Michigan, ate a lunch of tuna fish salad and two of them died (14). Type E botulism was diagnosed, and the causal organism was isolated from the empty can. Other cans from the same lot were recovered from grocers' shelves and some of them also contained the organism. Therefore, all cans bearing the incriminated code number were recalled from the market. The resulting publicity served effectively to
Clostridium Botulinum Food Poisoning

Table 1. Geographic Distribution of Verified Type E Botulism Outbreaks

<table>
<thead>
<tr>
<th>Place of occurrence</th>
<th>Outbreaks</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan: Hokkaido</td>
<td>29</td>
<td>222</td>
<td>42</td>
</tr>
<tr>
<td>Northern Honshu</td>
<td>20</td>
<td>82</td>
<td>37</td>
</tr>
<tr>
<td>U. S.: Alaska</td>
<td>7</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>Other states</td>
<td>8</td>
<td>36</td>
<td>15</td>
</tr>
<tr>
<td>Canada: British Columbia</td>
<td>8</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>Labrador</td>
<td>3</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Sweden</td>
<td>3</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Denmark</td>
<td>2</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>U.S.S.R.</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>TOTALS</td>
<td>82</td>
<td>404</td>
<td>122</td>
</tr>
</tbody>
</table>

Figures from Dolman and Iida (7) supplemented by more recent data for Japan (23) and the United States (22).

introduce C. botulinum type E to the general public. In this instance, the organism is believed to have entered the cans through defective seams after heating (14).

In late September and early October of 1963 two additional outbreaks of type E botulism occurred almost simultaneously. Both were traced to the consumption of smoked fish from the Great Lakes (22). A man and wife from Kalamazoo, Michigan, purchased a smoked whitefish while taking a motor trip through upper Michigan. Both contracted botulism and died. C. botulinum type E was recovered from the remains of the fish (15).

Immediately thereafter botulism was diagnosed in several patients at hospitals in Nashville and Knoxville, Tennessee. This outbreak eventually involved 17 patients with 5 deaths in the states of Tennessee, Alabama, and Kentucky (22). A single shipment of smoked whitefish chubs packed by a firm in Michigan was incriminated (1). All packages of the fish remaining in the stores were recalled, and the public was warned not to eat the product. C. botulinum type E was isolated from the remains of fish consumed by some of the victims (15). Samples of commercial smoked fish processed by three Michigan firms were incubated and proved to contain type E toxin (1).

On the basis of this experience, plus an earlier outbreak in Minneapolis, Minnesota, in 1960 (1), the Food and Drug Administration issued a warning on October 25, 1963, against the consumption and distribution of smoked fish from the Great Lakes area. Housewives were advised not to use smoked fish from the Great Lakes unless the product was known to have been either; (a) heated to at least 180 F for 30 min after packaging and thereafter kept under refrigeration, or (b) frozen immediately after packaging and maintained continuously in a frozen condition.

This action of the Food and Drug Administration promptly stimulated the issuance of processing regulations by several state and municipal regulatory agencies. The states of Michigan, Wisconsin, Minnesota and Illinois, and possibly others, have established requirements that smoked fish must be heated to an internal temperature of at least 180 F for 30 min during processing. The temperature requirements during distribution and the permissible methods of packaging vary considerably among the states. Freezing as an alternative to the heating requirement generally is allowed as originally suggested by the Food and Drug Administration.

Heretofore, there have been no processing regulations for smoked fish produced in the Great Lakes states. Each processor salted and smoked his product as he saw fit. There were no standards for salt, moisture, or heat treatment. The introduction of processing controls has revealed numerous technological problems, and until these are solved the Great Lakes smoked fish industry will have a difficult time.

The sudden awareness of type E botulism coming from the wide publicity given to the outbreaks in 1963 has led many people to wonder if the organism has been recently introduced into this country. Actually, the first outbreak of type E botulism ever recorded anywhere occurred in New York State in 1932. The vehicle was smoked salmon imported from Labrador (7). A second outbreak in New York State in 1934 was caused by canned sprats imported from Germany, and a third occurred in 1941 in California. The latter was traced to mushrooms from Yugoslavia and canned in San Francisco. Thus, the first outbreak of type E caused by a native U. S. food was the one in Minneapolis in 1960. This outbreak resulted from the consumption of smoked ciscoes from Lake Superior (I).

Type E botulism has occurred in many parts of the world. Table 1 lists the known outbreaks through 1963. These figures are taken from the review by Dolman and Iida (7), but are modified to include more recent outbreaks in Japan (23) and the 1963 smoked fish episodes in the United States.

C. botulinum type E has been found wherever outbreaks have occurred and the organism has been sought. Japanese workers (16, 24) have demonstrated its presence repeatedly in soil and mud samples on Hokkaido. Johannsen (11, 12) has isolated the organism from large numbers of soil, seashore, and sea bottom samples in and near Sweden. Pederson (25) has found it in soil and bottom mud in Denmark. And Dolman (7) has isolated the organism repeatedly from bottom samples off the coast of British Colum-
Several of these workers also have demonstrated *C. botulinum* type E in the intestinal contents of fish.

### Occurrence of *C. botulinum* Type E in Fish from Lake Michigan

The outbreaks of type E botulism that were traced to smoked fish from the Great Lakes strongly suggest that the organism occurs naturally in the northern United States. It is now generally accepted that type E is of terrestrial origin rather than marine, as was first supposed (6, 11, 16). There is no obvious reason, therefore, why the organism should not be native to the Great Lakes area.

To see if this is true, an intensive sampling program was begun on Lake Michigan in October, 1963. Most of the samples consisted of fish, water and mud. The results reported here will deal only with fish.

**Methods.**

Parts of fish (liver, gills, skin and contents of the intestinal tract) were inoculated into tubes of brain heart infusion broth (Difco) and incubated anaerobically at 30 C for three days. Each culture was centrifuged and 0.5 ml of the supernatant was injected intraperitoneally into a single mouse. Cultures that killed mice within 48 hr were subjected to routine mouse protection tests with antisera for types A, B and E toxin. For this purpose, each of four mice received 0.25 or 0.5 ml of culture supernatant. Three of the mice were protected with one international unit each of the respective antisera.

**Results and discussion.**

Results for almost 600 samples are given in Table 2. Over one-fourth of the cultures were toxic to mice on the initial test, at least 85% of them causing death in less than 24 hr. However, only a small fraction of the toxic cultures inoculated with gills, skin and liver gave typing patterns that suggested the presence of type E botulinum toxin. A few killed all of the mice and were classed as "nonspecific". About one-fourth to one-third were classed as "atypical", meaning that they killed one, two or three mice out of four but in no significant pattern. By far the greatest percentage of cultures from gills, skin and liver proved to be completely atoxic in the tests with antisera.

By contrast, almost one-third of the toxic cultures inoculated with intestinal contents gave typing patterns characteristic of *C. botulinum* type E toxin. But even in this group two-thirds of the cultures proved to be atypical or atoxic when tested with antisera.

The figures in Table 2 clearly indicate that intestinal contents of fish are more likely to give rise to cultures in which type E toxin can be demonstrated than are gills, skin or livers. Therefore, further tests

### Table 2. Occurrence of *C. botulinum* Type E Toxin in Cultures Inoculated With Parts of Fish from Lake Michigan

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Gills</th>
<th>Skin</th>
<th>Liver</th>
<th>Intestinal contents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of samples tested</td>
<td>102</td>
<td>103</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>No. of cultures toxic</td>
<td>25</td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>% of toxic cultures classed as:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Type E</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Type B</td>
<td>5</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Non-specific</td>
<td>0</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Atypical</td>
<td>29</td>
<td>32</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Lost toxicity</td>
<td>62</td>
<td>58</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>% of all cultures tested shown to contain type E toxin</td>
<td>1</td>
<td>1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

### Table 3. Representative Typing Patterns of Toxic Cultures Inoculated With the Intestinal Contents of Fish

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Initial test</th>
<th>Typing with antisera*</th>
<th>Classification of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1028-H</td>
<td>7</td>
<td>10 10 10 10</td>
<td>Type E</td>
</tr>
<tr>
<td>1544</td>
<td>5</td>
<td>4 4 4 4</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>2181</td>
<td>7</td>
<td>16</td>
<td>Atypical</td>
</tr>
<tr>
<td>1532</td>
<td>5</td>
<td>12 12 12 12</td>
<td>Atypical</td>
</tr>
<tr>
<td>1513-H</td>
<td>11</td>
<td>5 12 12 12</td>
<td>Atypical</td>
</tr>
<tr>
<td>1555</td>
<td>5</td>
<td>16 16</td>
<td>Atypical</td>
</tr>
<tr>
<td>1589</td>
<td>32</td>
<td></td>
<td>Atypical</td>
</tr>
</tbody>
</table>

*U = Unprotected mouse. A, B and E refer to mice protected with antisera for types A, B and E toxins.*
were made with intestinal contents as the inoculum. Examples of typing patterns are shown in Table 3. Experience has revealed that the majority of samples which give a pattern characteristic of type E toxin cause death of mice with typical symptoms of botulism in less than 10 hr. This is true in spite of wide variations in toxin levels, which have been found to range from 3 to more than 1000 mouse MLD per ml at the time of typing. Occasional cultures that contain type E toxin have been observed to take longer than 10 hr to kill an individual mouse (see sample 1504, Table 3). However, cultures that kill slowly rarely prove to contain type E toxin.

The typing pattern labelled “nonspecific” might result from several causes: the culture could contain a serological type of C. botulinum other than A, B or E; it might contain a mixture of serological types; or it could contain a toxic substance produced by an organism other than C. botulinum.

The “atypical” samples obviously contained very small amounts of toxin, since some of the mice survived without reference to the protective antisera. These results could represent extremely low levels of botulinum toxin, but judging from the relatively long death times they probably indicate toxins produced by other anaerobic organisms. Many species of clostridia produce substances that kill mice (3).

Loss of toxicity has been the most troublesome problem in the identification of lethal agents in mixed cultures. It has been observed with cultures that killed mice initially in as little as one or two hr, and has occurred in as little as one day. On the other hand, some cultures have remained toxic for weeks. Toxin destruction has been observed even at temperatures below 0 C.

Johannsen (13) and Kamizawa (16) also have observed loss of toxin from crude mixed cultures, the latter author attributing the destruction to enzymes produced by other organisms. This study has revealed nothing to contradict Kamizawa’s suggestion. Attempts to isolate type E from enrichment cultures (15) often show a predominance of other anaerobic sporeformers, many of which resemble Clostridium bifermantans (17). Much more needs to be learned about the nature of the organisms in the mixtures and their effect upon C. botulinum type E and its toxin.

Unfortunately, the transient nature of type E toxin was not fully realized at the beginning of this study, and some of the toxic cultures were held for several weeks before typing with antisera. This fact probably accounts in part for the high percentage of atypical and atoxic cultures in Table 2. From the death times in the initial toxicity tests it is probable that some—but by no means all—of these cultures originally contained type E toxin.

In further testing, special efforts have been made to record the death times accurately during the first 10 hr following injection and to look for symptoms of botulism in the mice. Because of the diversity of toxic agents that may be present, it is important, as Johannsen has suggested (13), to observe the symptoms preceding death. Merely finding a dead mouse in a cage on the morning following injection is little indication that the animal died of botulism. To minimize loss of toxicity it is also important to run the protection tests as quickly as possible after toxin is first demonstrated.

On the basis of toxin neutralization tests, fish from nine of ten locations sampled in Lake Michigan have yielded cultures that contained type E botulinum toxin. Little reliability can be placed on the percentages of positive cultures from some of the locations, although the overall incidence of C. botulinum type E likely is higher than the figures in Table 2 would indicate. Up to now little effort has been made to isolate the organism from the mixed cultures, but the alcohol method of Johnston, Harmon and Kautter (15) has been used successfully with a relatively small number of samples.

Evidence has been obtained to indicate that fish from certain locations may harbor C. botulinum type E in their intestines more frequently or possibly in greater numbers than do fish from other locations. Table 4 shows a comparison of the incidence of type E in fish from one place in Lake Michigan and of the same variety of fish taken at the same time from a large bay nearby. Fish from the lake gave the usual diversity of results with evidence of low toxin levels in many cultures, as judged by the percentages that were atypical or atoxic. By contrast, over three-fourths of the cultures prepared from fish in

### Table 4. Occurrence of C. botulinum Type E Toxin in Cultures Inoculated With the Intestinal Contents of Fish from a Bay of Lake Michigan and from the Main Body of the Lake

<table>
<thead>
<tr>
<th></th>
<th>Fish from late</th>
<th>Fish from bay</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples tested</td>
<td>109</td>
<td>94</td>
</tr>
<tr>
<td>No. of cultures toxic on initial test</td>
<td>56 (51%)</td>
<td>81 (86%)</td>
</tr>
<tr>
<td>% of toxic cultures classed as:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type E</td>
<td>11</td>
<td>90</td>
</tr>
<tr>
<td>Type A</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Nonspecific</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Atypical</td>
<td>29</td>
<td>1</td>
</tr>
<tr>
<td>Lost toxicity</td>
<td>55</td>
<td>7</td>
</tr>
<tr>
<td>% of all cultures tested shown to contain type E toxin</td>
<td>5</td>
<td>78</td>
</tr>
</tbody>
</table>
the bay clearly contained type E botulinum toxin. There were two differences in this experiment, either or both of which might have influenced the outcome of the tests. First, the fish from inside the bay were filled with food, whereas those from the lake contained very little material in the intestinal tract. Second, the temperature of the water in the bay was about 47 F, whereas that in the lake was 41 F.

The results of this study, while illustrating the inadequacy of present methods of demonstrating Clostridium botulinum type E in samples from nature, leave little doubt that the organism is a common contaminant of fish in Lake Michigan. Thus, Johannsen’s suggestion (12) that C. botulinum type E may be rare or nonexistent in the United States clearly was premature.

Further work is in progress to see if the organism is common in the other Great Lakes. Ecological studies are planned to learn how fish become contaminated and how the organism persists in the natural aquatic environment. Meanwhile, efforts are being made to improve the methods of demonstrating C. botulinum type E in samples from nature. These efforts will include attempts to isolate the organism in pure culture.

References