ADDITIONAL TYPE E BOTULISM OUTBREAKS
IN HOKKAIDO, JAPAN

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In a previous paper (Nakamura, Iida, Saeki, Kanzawa and Karashimada, 1956) it was reported that nine outbreaks of proven type E human botulism had taken place in Hokkaido, Japan, since 1951. Eight additional episodes have been encountered subsequently, of which two were of particular interest from the epidemiological viewpoint. These two episodes form the chief subject of this communication.

In addition, observations on the activation of type E toxin are described. As Duff, Wright and Yarinsky (1956) reported, type E toxin is activated by trypsin, and the present authors applied this phenomenon to the demonstration of type E toxin in foods. The toxicity of incriminated foods is evidently increased by treatment with trypsin, a fact which seems to be of some importance in considering the pathogenesis of type E botulism.

GENERAL ASPECTS OF THE OUTBREAKS

Altogether, type E botulism has occurred 17 times in Hokkaido over the period 1951 to 1957. The salient data bearing on these outbreaks are shown in Fig. 1 and Table 1. The revised list includes these 17 episodes, involving 267 persons at risk, of whom 129 became ill—a morbidity rate of 48.3 per cent. Of these 129 cases, 39 died—a case fatality rate of 30.2 per cent.

Foodstuffs implicated in these outbreaks were exclusively “izushi” made of raw fish, except one which was caused by trout-eggs. The predilection of type E botulism for fish, emphasized by Dolman (1953), is definitely substantiated.

Perusal of the table shows that the outbreaks took place most frequently in autumn months. Of 17 outbreaks, 11 (65 per cent) occurred in September, October and November. As pointed out in the previous paper, “izushi” is usually prepared as a relish for winter nourishment. However, it is occasionally made in the warm season, and then has been responsible for type E botulism. It is certain that warm temperature favours the growth and toxin production of Clostridium botulinum type E, so advice has been repeatedly offered by the public health authorities not to prepare “izushi” in the warm season.
Various fish are used for preparing "izushi". Likewise, type E outbreaks have been caused by "izushi" made of various sorts of fish, as shown in Table 1. Sole and "hata-hata" (*Arctoscopus japonicus*) have been implicated most frequently, but herring, mackerel, salmon, "sanma" (*Cololabis saira*) and "yamabe" (a sort of dace) were also involved. As sole and "hata-hata" are...
caught in great quantity and are sold inexpensively, these fishes are widely used for preparing "izushi" in Hokkaido.

The frequent occurrence of outbreaks on the coast of the Sea of Okhotsk, which was pointed out in the previous paper, becomes less conspicuous in the revised figure. The outbreaks have taken place throughout the coastal districts of Hokkaido. It was demonstrated by the authors' investigation that the coastal sands of this island are considerably contaminated with spores of Clostridium botulinum type E, though the rate of type E isolation varies in different districts.

Human botulism outbreaks due to "izushi" have also occurred several times in Akita and Aomori Prefectures in Japan. All of these outbreaks proved to be of type E. The authors have as yet no information as to the occurrence of type A or type B botulism episodes in Japan.

Of the 17 outbreaks mentioned in Table 1, two which took place most recently are of particular interest from the epidemiological point of view. A descriptive outline of these two episodes now follows.

**Outbreak at Kitami-Esashi**

On October 31, 1957, an old woman tasted "izushi" which she had pickled about two months ago. She ate a small dish of it and found it delicious except for a slight acid odour. So next day she shared it with her family and with several boarders in the house. About 20 persons ate this "izushi", of whom none noted anything unusual except a 24 year-old housewife, who consumed it at supper on November 2.

On November 3, about noon, this young woman fell ill with nausea, vomiting and dizziness. In the afternoon, she developed ptosis, blurring of vision, difficulties in swallowing and speaking, hoarse voice, and extreme dryness of the mouth. The body temperature was subnormal. When she was sent to hospital that night, her mentality was a little disturbed and she developed retention of urine. She got rapidly weaker, and ceased breathing at 11 p.m. — some 28 hours after ingesting the "izushi".

The "izushi" incriminated in the outbreak was made of soles which were caught near Esashi on August 25. The fish had their heads chopped off, and were soaked in water for five days to remove blood. Then they were packed in a wooden tub together with cooked rice, malted rice ("Kōji"), and chopped cabbage. A small amount of vinegar, sugar and salt was added, and red pepper was mixed in as flavouring. Then the lid of the tub was put on and loaded with heavy stones. It was left to stand until October 31, when it was first tasted by the old woman who had pickled it.

The remnant of this "izushi" was sent from the Wakkanai Health Centre to the authors' laboratory. It had a sharp odour of lactic acid with signs of contaminated fermentation. As it was expected from the particularly low morbidity rate that the toxin might be present unequally in the container, sampling was made from the upper, middle and lower parts of the tub.

The fish samples thus obtained were each ground in a mortar with equal volumes of saline. The emulsions were centrifuged at 3,000 rpm for 30 minutes. Type E toxin was demonstrated in the supernatants of the materials from the
upper and middle parts of the tub, but not in the materials from the bottom. The toxicity of these supernatants was very low—about 10 mouse MLD per gram of fish.

The experiment on activation of the toxin by trypsin was carried out as follows: Five grams of fish meat was taken from the sample of the middle portion, ground in a mortar with 20 cc of saline, and centrifuged at 3,000 rpm for 30 minutes. The supernatant did not kill mice when injected with 0.5 cc intraperitoneously. To this supernatant was added an equal volume of 2 per cent trypsin suspension (suspended in a pH 6.2 phosphate buffer), and the mixture left to stand at 37°C for 1 hour. Then the toxicity of the mixture was tested. Type E toxin of about 200 mouse MLD per gram of fish was demonstrated after the treatment with trypsin. The potency of the toxin in the “izushi” was thus increased by about twenty times.

The isolation of a toxigenic type E strain was attempted repeatedly but unsuccessfully.

OUTBREAK AT MASHIKE

The last outbreak took place at Mashike, a little fishing port in Hokkaido, on November 18, 1957. It was also caused by a homemade “izushi” prepared by an old woman who was well experienced in pickling. About 60 persons ate it, of whom 35 fell ill. More than half of these patients were in a serious condition, and 9 of them died. This was the most extensive and the most tragic episode in the history of type E botulism.

On November 17, a festival was observed at a private house in Mashike. After the festival, a drinking party was held by about 40 men and women who had flocked there from various quarters. Festival dishes consisted of Japanese wine, sliced squid and lobster, hardboiled sole, vinegared dishes of carrot and garden radish, and “izushi” made of “hata-hata”. The party lasted till 10 p.m., after which some of the attendants took home the remnants of the “izushi” for their family. Thus the number of people who took this dangerous “izushi” amounted to sixty.

Several of these consumers fell ill during that night with nausea, vomiting and dizziness. Next morning, the number of patients increased, and many of them were examined by physicians of the Mashike Hospital. They were diagnosed as having botulism from their characteristic symptoms, such as ptosis, mydriasis, diplopia, and paralysis of the pharyngeal muscles.

An epidemiological investigation was immediately undertaken by the Rumoe Health Centre, which revealed the extensive scale of this outbreak. Of the 60 persons who ate the “izushi”, 35 fell ill—a morbidity rate of 58 per cent. There were 9 deaths—a case fatality rate of 26 per cent. The length of the incubation period in all the patients, and the duration of illness in the fatal cases, are shown in Tables 2 and 3, respectively.

From Table 2 it will be seen that most of the patients developed symptoms within 25 hours after consuming the “izushi”. This is in agreement with the view that the typical symptoms of botulism usually appear within 12-36 hours.

Table 3 indicates that all of the fatal cases died within 60 hours after the
development of symptoms. Two cases ceased breathing within as short a period as 10 hours. Such an abrupt and rapid ingrawescence was observed several times by the authors in other episodes of type E botulism.

Generally speaking, no relationship was observed between the quantity of the "izushi" consumed by a patient and the severity of his clinical symptoms. It appears that the clinical symptoms and prognosis of those who had shown intense vomiting and/or diarrhoea at the beginning of the illness were rather mild and benign. This apparently indicates the therapeutic importance of discharging the toxin as fast as possible.

Symptoms common to the majority of the patients were the following: (1) Digestive disturbances such as nausea, vomiting, distension of the abdomen, and constipation. Diarrhoea was observed in some cases. (2) Ocular symptoms such as mydriasis, diplopia, ptosis, blurring of vision, and tardiness or loss of light reflex. (3) Pharyngeal symptoms such as hoarse voice, dryness of the mouth, and difficulties in swallowing and speaking. (4) Other disturbances such as dizziness, retention of urine and semi-paralysis of the limbs. The rates of manifestation of such signs and symptoms are shown in Table 4.

The "izushi" implicated in this outbreak was pickled by an old woman living in the house where the unlucky festival was observed. The "hata-hata" used for preparing this "izushi" was caught off-shore at Mashike on September 25. Next day, the fish were gutted, washed, and soaked in water for about one week to remove blood. Then they were packed in a wooden container together with cooked rice, malted rice and diced carrot. A small amount of vinegar and Japanese wine was added. After pickling, the lid of the tub was put on and loaded with heavy stones to bring pressure on the contents. It was kept standing on an unfloored part of the house until the festival, November 17.

The remnant of this "izushi" was forwarded from the Rumoe Health Centre to the authors' laboratory. An odour of lactic acid was recognized besides the savoury odour of "izushi". Five grams of fish meat was removed from the remnant of the "izushi", ground in a mortar with 20 cc of saline, and centrifuged at 3,000 rpm for 30 minutes. The supernatant had a pH of 4.6, and was highly toxic for mice. Type E toxin of about 200 mouse MLD per gram of fish meat
Table 4. Clinical symptoms of the cases in the outbreak at Mashike

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Positive cases</th>
<th>Rate of manifestation (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestive disturbances</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>27</td>
<td>77.1</td>
</tr>
<tr>
<td>Abdominal distension</td>
<td>21</td>
<td>60.0</td>
</tr>
<tr>
<td>Constipation</td>
<td>18</td>
<td>51.5</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>13</td>
<td>37.1</td>
</tr>
<tr>
<td>Ocular symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mydriasis</td>
<td>22</td>
<td>62.9</td>
</tr>
<tr>
<td>Tardiness or loss of light reflex</td>
<td>17</td>
<td>48.6</td>
</tr>
<tr>
<td>Blurring of vision</td>
<td>12</td>
<td>34.3</td>
</tr>
<tr>
<td>Diplopia</td>
<td>10</td>
<td>28.6</td>
</tr>
<tr>
<td>Ptosis</td>
<td>10</td>
<td>28.6</td>
</tr>
<tr>
<td>Pharyngeal symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dryness of the mouth</td>
<td>31</td>
<td>88.6</td>
</tr>
<tr>
<td>Difficulty in swallowing</td>
<td>17</td>
<td>48.6</td>
</tr>
<tr>
<td>Difficulty in speaking</td>
<td>17</td>
<td>48.6</td>
</tr>
<tr>
<td>Other symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>21</td>
<td>60.0</td>
</tr>
<tr>
<td>Semi-paralysis of the limbs</td>
<td>10</td>
<td>28.6</td>
</tr>
<tr>
<td>Retention of urine</td>
<td>8</td>
<td>22.9</td>
</tr>
</tbody>
</table>

was demonstrated. After treatment with trypsin, the toxicity increased to more than 20,000 mouse MLD.

In this outbreak, it was observed that some people died after consuming relatively small amounts of the "izushi". The explanation might be that the toxicity of the consumed "izushi" was increased in the digestive canal, thus exerting the fatal toxic action on the patients. The possibility of this in vivo activation of type E toxin was successfully demonstrated by Dolman (1957a) in an outbreak due to salmon eggs in British Columbia.

From the sediment of the centrifuged material, a toxigenic strain of Clostridium botulinum type E was isolated.

**DISCUSSION**

The outbreak at Kitami-Esashi was characterized by its extremely low morbidity. Usually more than half of the consumers have developed symptoms in botulism due to "izushi" (See Table 1). However, of 20 persons at risk, only one person fell ill and died in this particular episode. This suggests that the toxin was not distributed throughout the tub but localized in an extremely limited part of it. In fact, the toxicity of the fish samples taken from the upper and middle part of the tub was very low, and no toxin was demonstrated in the samples from the bottom of the tub. On the other hand, the young woman (the single fatal case of this episode) died in about 28 hours after consuming only one or two
pieces of the fish. It is accordingly conceivable that the toxin had been contained in important quantity in just that portion of the fish which was taken by the victim. In compressed foods such as "izushi", a localized distribution of toxin is quite within the bounds of possibility.

By contrast, the outbreak at Mashike was distinguished by its extensive scale and outstanding severity. Clinically it was observed that, in some fatal cases, the apparently mild symptoms at the beginning of the illness turned abruptly serious, and this aggravation could not be inhibited by any treatment. The most important principle in therapy is to discharge any toxin which has remained unabsorbed in the digestive canal. Thorough lavage of the stomach or administration of purgatives have to be attempted for this purpose. As mentioned above, it was observed in this episode that the clinical signs and prognosis of those who had showed intense vomiting or diarrhoea at the beginning of the illness were rather mild and benign.

Finally, the observations on the activation of type E toxin by trypsin will be briefly discussed. As pointed out by Duff, Wright and Yarinsky (1956), type E toxin is activated by trypsin at about pH 6.0. This phenomenon was verified and extended by Dolman (1957 b). The present authors also verified the fact, and applied it to the demonstration of type E toxin in "izushi" in these two outbreaks. In one outbreak, the toxicity of the incriminated "izushi" increased from 10 mouse MLD per gram to 200 mouse MLD. In the other, the increase was more conspicuous—from 200 mouse MLD to more than 20,000 mouse MLD.

The discrepancy between the high case fatality rate of type E botulism and the low potency of the toxin of type E strains in routine culture media would be satisfactorily explained if this in vitro activation of the toxin could similarly occur in vivo. This was proved to be true by Dolman (1957 a) in an outbreak due to salmon eggs in British Columbia. Examination of the stomach contents of three victims revealed that enhancement of toxicity from nearly 20-fold to over 200-fold had occurred to the toxin after ingestion. As pointed out by him, this in vivo activation might be of crucial importance in the pathogenesis of type E botulism.

**SUMMARY**

1. Over the period 1951 to 1957, there have been 17 known outbreaks of proven type E botulism in Hokkaido, Japan. These have involved 129 persons, of whom 39 died.

2. "Izushi", a sort of relish made of raw fish, was implicated in all of these outbreaks, except one which was caused by trout-eggs.

3. Two outbreaks of particular interest from the epidemiological viewpoint are described. One took place at Kitami-Esashi on November 3, 1957. Of twenty persons who ate the "izushi" only one fell ill and died. Type E toxin was demonstrated, but it was unequally distributed in the remnant of the "izushi".

4. The other outbreak took place at Mashike on November 18, 1957. Sixty persons consumed deteriorated "izushi" at a drinking party after a festival. Thirty-five of them fell ill, of whom nine died. Type E toxin was demonstrated in the "izushi", and *Clostridium botulinum* type E was isolated from it.

5. The potency of type E toxin in these incriminated "izushi" was markedly increased by treatment with trypsin. The relationship between the high case...
fatality rate in some outbreaks of type E botulism and the activation of the toxin is discussed.

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