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Kliniko-epidemiologicheskaya kharakteristika vspyshki botulizma, svyazannoi s upotrebleniem v pishchu konservirovannoi kambaly

(Clinical-epidemiological characteristic of an outbreak of botulism, connected with the use of canned flounder for food)

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(In Russian)

In December, 1959, in the Sovetskaya Gaven' three cases of botulism were registered, connected with the use of canned flounder for food. Fishing for this party of canned food was conducted in June of this same year in the north-eastern part of the Okhotsk Sea (near the shores of Kamchatka). According to literary data and reliable information, the Far Eastern fishes in the Soviet Union never served as a source of botulism cases. It is probable, therefore, that in the "Instruction about the order of sanitary-technical control of canned food" (1951) the requirement about compulsory "term-ostatirovannie" (keeping under constant temperature?) of 5% of cans in each autoclave sterilization does not cover the fish-canning factories of the Far East. Nevertheless, cases of botulism were repeatedly observed in the Khabarovsk Krai. (Begin p. 93). One of them was described, in 1959, by Shapiro and Konstantinova. There are no indications of the flounder infectiousness with Cl. botulinum in literature (Matveev), although flounder is a bottom-dwelling type of fish and the possibility of its infection (by the pathogen of botulism) is very probable.

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Cl. botulinum are widely spread and, probably, exist also in the region of the eastern shore of the Soviet Union. This is indirectly confirmed by data on the presence of cases of botulism in Alaska and Canada, caused by utilizing for food different kinds of fish from the Pacific Ocean (Gatveev). Therefore it is very important to establish the nature of the outbreak examined by us and determine the type of pathogen of botulism that is met in the given locality. Below we cite brief passages from the official report of the epidemiological examination, history of diseases and proceedings of legal-medical autopsy, as well as present the results of detailed bacteriological investigation, having in view, that botulism in men with fatal outcome is met very rarely in USSR, and in the region, where this outbreak was investigated, it was never described under such circumstances (using canned flounder for food).

Family G, where the cases were registered, consisted of 7 persons - two adults and 5 children. On December 21, 1959, 4 cans of fish were opened for supper; they were bought from a private person at the market. In the ration of the family, for the last 10 days preceding the poisoning, there were no products which could have been infected with Cl. botulinum. During supper flounder was eaten from two cans, as well as humpbacked salmon and Eleginus navaga. Two elder children ate only navaga in tomato sauce, the parents - the humpbacked salmon and flounder from one can; flounder in tomato sauce from the second can was eaten by the three younger children. The cited canned food was consumed in its entirety. Next day two younger boys fell ill, while in 3 days - the girl, who ate with them from the same can. Nobody else from the family ate flounder from this can and they had

no symptoms of disease. One should mention that the sick girl ate only one - two spoons of the suspected canned food. The empty cans from this food were found in the yard of the victims. One of them, which judging from the label contained flounder, had clear symptoms of bulging. A specialist found in it a difect in the can seaming. Consequently, from the epidemiological point of view, the cause of disease was canned flounder.

The incubation period of disease in boys was equal to 15-16 hours, while in the girl three 24-hour days. The first symptoms in boys appeared on the next day: general weakness set in, headache, vertigo, stomach-ache, nausea and copious vomiting. Temperature was normal. The pupils were dilated, reduction of vision was noted and disorder in the oculomotor musculature (divergent strabismus). The patients complained about the difficulty in swallowing and shortage of air. The disease progressed very rapidly in the first boy (Sergius, 7 years old) and he died with the symptoms of bulbar disorders before he was taken to the hospital. His brother (Valeril, 5 years old) was brought to the hospital in a very serious condition with a diagnosis of muscle paralysis of the esophagus. He remained conscious. At times he could not talk and it was hard to establish a contact with the patient. Pharyngismuses were observed in swallowing and speech. Temperature was 37.1°. Cyanosis of mucosa, of the skin of the face and extremities. Slow, superficial respiration, 42 per minute. Rhythmical pulse, 120 beats per minute, arterial pressure 80/50 mm "rt. st." (mercury column). Heart tones were deadened. Coated tongue at the root. The stomach was distended, painful over the pubis and in the area of the sigmoid. Liver and spleen were not diagnosed. The pupils were dilated,

reaction to the light was almost absent, there was a left strabismus. Botulism was diagnosed. Gastric lavage was done for the patient, a purgative administered, also cardiac drugs, oxygen. The state of the patient deteriorated rapidly and sharply, and in 2 hours he died with symptoms of respiration paralysis.

All the members of the family were hospitalized and they were administered the medicinal antibotulin serum, type A, brought in from Khabarovsk since by this time the results of the neutralization reaction of the toxin, isolated from the organs of the dead, were already known. In the girl (Valentina, 8 years old) the course of the disease was mild. The pulse was 80-90 beats a minute. The stomach was distended, painful in the lower part; some stool retention was noted. Botulin toxin was found in the intestine lavage water of the patient on the 2nd day after hospitalization. In all, during the course of treatment, the patient received 200,000 antitoxic units of the antibotulin serum, type A. Bicmycin and other antibiotics were administered to her, also glucose and isotonic solution subcutaneously and internally. Gastric and intestinal lavage was conducted.

In the pathologico-anatomical autopsy of corpses of the dead boys polyemia and certain symptoms of brain enema were found. In the histological examination were revealed the spleen polyemia, in the liver a cloudy swelling of liver cells with an unclear discomplexification of "balok" (beams, girders?), in the lungs, on the background of acute emphysema, an accumulation of transudate in alveoluses, in the heart focal non-uniformity in the staining of muscle fibers. The glia cells were not changed in the brain, occurrence of dystrophy (obliterated limits of cells, karyolysis and karyokinesis, vacuolization up to necrosis) in ganglionic cells.

Blood, brain and the spinal cord, stomach, large and small intestines with their contents were taken from the corpses of dead boys, in 6-12 hours after death, (Begin p. 94) also liver, spleen, kidneys, urea, mesenteric lymph nodes, as well as the vomitory masses, collected in the yard of the house. The lavage waters from stomach and intestines were taken from all the members of the family. First of all the suspensions from organs of the dead were examined in the reaction of neutralization on white mice with antibotulin sera of types A and B.

The suspensions were prepared by means of grinding the organs in a mortar with sterile quartz sand in a physiological solution in a proportion of 1:10. After centrifuging, at 3,000 rotations, during the course of 15 minutes, the supernatant liquid in the volume of 0.5 ml was administered, intraperitoneally, to 4 groups of white mice (4 in each), weighing 10-12 g, while the sediment was utilized for bacteriological research.

Mice of the first group received the native material, the second - the material that was preliminarily heated at 100° during the course of 30 minutes; to mice of the third and fourth groups, the tested suspension after a preliminary 20 minute contact, at 18°, with the antibotulin sera of types A or B. Sera of the Khar'kov Institute of Vaccines and Sera, purified by the "Diaferm-3" method were utilized as the antibotulin serum. In the type A serum 50,000 "AE" (antitoxic units) were contained in 4.2 ml, in the type serum - in 10 ml. We added 500 AE of the serum of each type to the 0.5 ml of the organ suspension in the experiment.

As a result, animals died which were infected with non-heated material and material with serum of type B. Mice, that received the heated organ suspension and the suspension, mixed with the serum of the A type, remained alive with the exception of 2 mice, that were infected with the suspension of the brain of the corpse of Valerii Ch. The first symptoms of disease in mice set in 2-30 hours after infection (table 1).

First an increased irritability was noted (the animals reacted sharply to the sound), dishevelling of hair, then torpidity appeared, uncertainty and unsteadiness of the gait. Pareses of extremities were noticed. A very characteristic symptom was the appearance of the phenomenon of the "wasp waist", which attested to the paralysis of the diaphragm; whereupon the respiration of animals became more frequent and superficial; expressed crepitation was observed. The death of animals set in, basically, in 2-6 hours after the appearance of the first symptoms of the disease.

Guinea pigs, which were infected intraperitoneally with 2 ml of the mixture of suspension of organs of the dead, became sick in 3-6 hours. The disease was characterized by adynamia, sharp weakening of the musculature (doughlike muscles) and spasms. Death of animals occurred in 12-18 hours. Frequent urinations and defecations were observed. Pathologico-histological changes, revealed in the organs of the dead animals were similar to changes in the organs of the dead men.

Table 1

Results of neutralization of toxin by antibotulin sera on white mice

Material	Disease and loss of animals in intraperitoneal infection with different suspensions			
	Native	mixed with type B serum (500 AE)	mixed with type A serum (500 AE)	heated
Small intestines of Sergius	2/4	6/9	----	---
" " of Valerii	10/14	22/24	----	---
Lymph nodes of Sergius	12/24	16/26	----	---
" " of Valerii	6/8	10/12	----	---
Liver of Sergius	30/36	13/24	----	---
" of Valerii	17/20	16/29	----	---
Blood of Sergius	4/7	3/5	----	---
" of Valerii	14/20	13/16	----	---
Brain of Sergius	12/14	18/28	----	---
" of Valerii	3/6	11/26	40/50	---
Spinal cord of Sergius	12/16	11/14	----	---
" " of Valerii	6/9	12/14	----	---
Vomitory masses of the dead boys	4/6	10/11	----	---

In order to find out the sensitivity of animals to the toxin depending on the method of its introduction, a titration was conducted of the supernatant liquid of the brain suspension of the dead child by way of subcutaneous, intraperitoneal and intravenous administration of the material to white mice in a dilution from 10^{-1} to 10^{-6}. The material was introduced in the volume (Begin p. 95) of 0.5 ml. The titer of the toxin, contained in the brain tissue, proved to be the highest in intravenous introduction - 4.3, in intraperitoneal it was equal to 2.8, and in subcutaneous - 2 LD_{50}. Incubation was sharply reduced (up to one hour) in the intravenous administration of the material. Apparently the intravenous method of administration of the tested material is the best for a rapid diagnosis of botulism.

Analysis of the obtained data (table 2) attests that the greatest amount of toxin was contained in the nervous tissue, in lymph nodes, small intestines, liver, blood and vomitory masses of the dead. No toxin was discovered in the rest of tissues and liquid of the corpses. The length of the incubation period and of the disease of mice also to a certain degree characterized the contents of toxin in tissues. Thus, in the infection if mice with the material from the organs of the corpse of Sergius Ch., who died first, the shortest time of incubation was observed in the administration to mice of blood and suspension of the small intestines. Into this experiment were introduced also the antibotulin sera of types C and E, which did not produce any preventive action. In the case of a later death (Valerii Ch.) the toxin acted the most rapidly in the administration of suspensions of brain and lymph nodes. The amount of toxin, which was in the brain tissues was so great that a dose of 500 AE of type A serum could not fully neutralize it, although it drew back considerably the time of falling ill and of death of animals. An additional experiment with the material from the brain tissue and a large amount of serum (1,000 AE) confirmed the result of the reaction of neutralization.

In the lavage waters from the intestines of the girl, sick with botulism, taken on the 2nd day after the appearance of the first symptoms of the disease, a large amount of the toxin was revealed also. It is hardly possible that such a dose of the toxin could have entered with the food taken. The toxin was not found in similar tests from other members of the family and in the contents of large intestines of the dead. It is left to suppose that reproduction of the pathogen took place in the intestines of the sick girl, which produced a large amount of toxin. This

coincides with the toxico-infection theory of pathogenesis of botulism. It is possible, that similar cases comprise a group of botulism patients in which one observes a prolonged incubation period.

Table 2

Results of titration of botulin toxin on white mice (0.5 ml of 10% suspension of organs and other materials in intravenous administration

Materials	Toxin titer (in LD_{50})
Brain of Valerii	3.29
Vomitory masses of the dead	3
Lavage waters from the intestines of the sick girl	2.6
Lymph nodes of Valerii	2.5
Small intestines of Sergius with their contents	1.71
Blood of Sergius	1.71
Liver of Valerii	1.25

Times of preservation of the toxin in the organ suspensions were also studied. It was established that by keeping it in the refrigerator (4°) the toxin did not lose its activity during the course of 6 months. The toxin titer was reduced sharply during the course of a 10-day stay in the diffused sunlight at 8°; at room temperature (in darkness) the toxin strength was reduced by two times in 1½ months.

It was not succeeded isolating the pathogen from the tested material.

We suppose that the results of investigation of this small outbreak of botulism are instructive and should attract the attention of workers of the Antiepidemic Service.

Conclusions

1. In December, 1959, an outbreak of botulism, connected with the use for food of the local canned flounder, was observed for the first time on the eastern shore of USSR (Soviet Gavan' city).

2. In the organs of two dead and in the lavage water from the intestines of the sick girl, botulin toxin of type A was detected.

3. In order to discover the botulin toxin, the examination of brain tissues, of vomitory masses and the lavage waters of intestines (in intravenous administration) in the experiments of neutralization on white mice is the most effective.

LITERATURE

Matveev, K. I., Botulism, M., 1959, p. 111.

Shapiro, S. K. and Konstantinova, I. A., Zhurnal Mikrobiologii, 1959 no. 5, p. 138.

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