

STANISŁAW ZALESKI
ELŻBIETA DACZKOWSKA
ANNA FIK
ALICJA JÓZWIAK

SURVEYS ON THE OCCURRENCE OF *CLOSTRIDIUM BOTULINUM* IN FRESH BALTIC HERRINGS

Institute of Marine Food Technology, Agricultural University, Szczecin

Key words: *Clostridium botulinum*, Baltic herrings.

Presence of *Clostridium botulinum* in fish is a result of the marine environment contamination with this bacteria. From among 152 herrings of Southern Baltic Sea origin in 30 cases intestinal canals of fish were contaminated with *Cl. botulinum*. Type E was identified in 28 cases while 2 samples were positive for *Cl. botulinum* type A. Percentage of positive results ranged from 4 to 50 depending of the batch of fish tested. An average percentage was equal to 19.7; for type E—18.4% and 1.3% for type A.

Observations have shown that the occurrence of *Clostridium botulinum* type E is limited to the northern hemisphere. Though its presence has been observed also in the terrestrial environment [1, 5, 8, 11], this type occurs mainly in the marine environment of the subarctic zone. This is why it has been defined as a marine type. Large concentrations of *Cl. botulinum*, type E mainly, were found along the northern-western sea coast of North America [14], along the western coasts of the Pacific [6], in the Baltic Sea [5, 7, 15]. Its presence has been also stated in water samples coming from the Great Lakes [2, 3], at the Canadian Sea coast [9, 10] and in the Mexican Bay [14].

Incidence of *Cl. botulinum* type E in fish is due to its presence in the marine environment. It has been isolated from such species of fish as plaice, lumpfish, cods, herrings and others [2, 5, 12]. The strongest contamination with this microorganism had been observed in fish caught in the Western Baltic Sea area [5].

This was the reason for studies into the frequency of *Cl. botulinum* occurrence in herrings caught in the Southern Baltic Sea fishing grounds.

MATERIAL AND METHODS

The tested material was constituted by the intestinal canals of herrings caught in the Southern Baltic Sea between November 1975 and May 1976. About 4 to 6 hours after they were taken from the ship, samples, collected aseptically from fresh, iced fish were placed in bottles containing 50 ml of TPGY enrichment medium (Trypticase 5%, peptone 0.5%, glucose 0.4%, yeast extract 2% and sodium tioglicolate 0.1%). After a 6 days incubation in 25°C, in anaerobical conditions each subsample was centrifuged for 10 min. at 16.000 g.

To detect the presence of the toxin supernatants (diluted 4:1 with phosphate buffer) were injected intraperitoneally to a pair of mice in the amount of 0.5 ml/mice. Samples that were lethal to the mice within 48 hours, were tested for the specific toxins of *Cl. botulinum*. Survival of mice protected by specific antitoxin and death, with typical symptoms, of the others, enabled to define the serotype of *Cl. botulinum*.

RESULT AND DISCUSSION

Results are given in Table. From among the total of 152 intestinal canals tested, 30 were positive for *Cl. botulinum*; in 28 cases the presence of type E was stated, while 2 were positive for type A toxin. The percentage of positive results ranged within 4 to 50% and varied according to batches of fish tested.

Table. Incidence of *Cl. botulinum* in herrings of Southern Baltic Sea fishery grounds origin

Date	Number of samples	Number of positive results	% of positive results	Type of toxin			
				A	B	E	F
14.11.75	24	1	4.17	—	—	1	—
4.12.75	36	10	27.8	—	—	10	—
6.01.76	30	3	10.0	—	—	3	—
28.02.76	18	9	50.0	2	—	7	—
6.05.76	20	4	20.0	—	—	4	—
7.05.76	24	3	12.5	—	—	3	—
Total	152	30	19.7	1.3	—	18.4	—

The percentage of fish contamination with *Cl. botulinum* type E is a reflection of environmental contamination with this microorganism. The number of positive results probably depends on the localization of the shoal and fishery similarly to the of *Cl. botulinum* in marine sediments which depends on the sampling place.

A very high percentage of positive results had been found in silt samples from the Western Baltic Sea area. Sediment samples of Sund, Kattegat and Skagerrak origin were positive for *Cl. botulinum* type E in 100%. The number of positive samples collected along the Swedish coastal line equalled 84%, while it decreased farther east from this area [5].

Similar results were obtained for herrings caught at the Sund fishery grounds, while herrings originating from the Western Baltic Sea were positive in 55% [5].

Silt samples collected in the Southern Baltic Sea area were positive for *Cl. botulinum* type E in 44% [7] and those of Polish coastal line origin were positive in about 32% [15]. Studies of *Cl. botulinum* incidence in herrings caught in this area showed its presence in about 20% of the fish. At the same time 18.4% of this number were identified as type E and 1.3% as type A.

Noteworthy is the isolation of *Cl. botulinum* type A. This type is commonly isolated in the USA and it is considered that its incidence in European waters is very rare [13]. Johannsen [5] did not show its presence either in the sediment samples or in fish from the Western Baltic Sea areas. Its presence in herrings may be connected with the appearance of this type in the mud samples collected along the Polish coast line, where *Cl. botulinum* type A had been identified in 1 case out of the 108 samples tested.

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Manuscript received: July, 1977.

Authors address: Kazimierza Królewicza 3, 70-550 Szczecin.

S. Zaleski, E. Daczowska, A. Fik, A. Józwiak

BADANIA NAD WYSTĘPOWANIEM *CLOSTRIDIUM BOTULINUM* W ŚWIEŻYCH ŚLEDZIACH BAŁTYCKICH

Instytut Technologii Żywności Pochodzenia Morskiego, AR Szczecin

Streszczenie

Wynikiem występowania *Clostridium botulinum* w środowisku morskim jest jego obecność w rybach tego środowiska. Spośród 152 śledzi pochodzących z łowisk Południowego Bałtyku u 30 szt. stwierdzono w przewodach pokarmowych obecność *Clostridium botulinum*, przy czym typ E zidentyfikowano w 28 przypadkach, podczas gdy w dwóch próbach stwierdzono obecność typu A.

Procent wyników dodatnich wahał się od 4 do 50 w zależności od badanej partii ryb. Średnio wynosił on 19,7%, z czego na typ E przypadało 18,4% a 1,3% na typ A.