Epidemiological Report

**INCIDENCE OF *CLOSTRIDIUM BOTULINUM* IN COASTAL
AND INLAND AREAS OF WEST JAVA**

IKRAMUL HAQ* and FLORE SUHADI

*Pasar Jumat Atomic Energy Research Center, Kotak Pos 2,
Kebayoran Lama, Jakarta, Indonesia*

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Clostridium botulinum is widely distributed and its natural inhabitat is soil. Different types (A-F) have been reported in various environments including water, mud, soil, fish, etc. Fish generally carries spores of all types of *C. botulinum*, particularly type E, and is one of the main sources of human botulism (Hobbs, 1976; Tanasugarn, 1979; Huss, 1980).

The present investigation was carried out to determine the extent of contamination by *C. botulinum* of seawater and freshwater fish and fishing grounds around West Java in Indonesia.

A total of 350 samples was collected, which consisted of freshwater mud (50), seawater mud (46), freshwater (52), seawater (66), freshwater fish (52) and seawater fish (84). Fish samples comprised 70 species out of which 23 were of freshwater and 47 seawater origin. Mud and water samples were collected aseptically in pre-sterilized plastic bottles from the shores or banks of rivers and streams. Fish samples, obtained from fishermen at places where they dispose off their catch, were packed individually in pre-sterilized polyethylene bags. After collection, all the samples were transported under melting ice to the laboratory at Pasar Jumat Research Center, Jakarta, within 2-3 days. The samples were then stored in a deep freezer (-18 C) for further examination.

At a time 10 samples were taken out of the deep freezer and placed at room temperature (28-33 C) for thawing. After thawing, 10 ml of water, 10 g of mud, 10 g of fish (chopped muscle plus skin) and whole content of fish viscera were inoculated separately in 20-ml cooked meat medium of at pH 7.2 (Difco and Oxoid). All the tubes were incubated at 30 C in the air for 5 days to test for the production of botulinum toxin (Haq and Sakaguchi, 1980).

Mice used for botulinum toxicity tests were usually mixtures of strains of inbred white mice of both sexes. The cultures were centrifuged at 5,000 rpm for 15 min and the supernatants injected intraperitoneally in mice by a slightly

* Present address: Food Science Division, Nuclear Institute for Agriculture and Biology, P.O. Box 128, Faisalabad, Pakistan.

TABLE I

Contamination (%) with Clostridium botulinum of samples collected from West Java

Type of samples	Number of samples analyzed	Positive samples	% contamination
<i>Mud</i>	96	11	11.5
Freshwater	50	5	10.0
Seawater	46	6	13.0
<i>Water</i>	118	8	6.8
Freshwater	52	6	11.5
Seawater	66	2	3.0
<i>Fish</i>	136	21	15.4
Freshwater	52	7	13.5
Seawater	84	14	16.7
<i>Total</i>	350	40	11.4
Freshwater source	154	18	11.7
Seawater source	196	22	11.2

modified method of Carroll et al. (1966). In our studies, instead of 0.4 ml of the supernatant, a mixture of 0.2 ml of the supernatant and 0.3 ml of gelatin phosphate buffer (equal volumes of a 0.2% gelatin solution and 0.1 M phosphate buffer, pH 6.5) was injected. Heating a portion was done at 100 C for 10 min rather than at 80 C for 15 min. Prior to injection, the supernatants were stored overnight in the deep freezer to avoid non-botulinal deaths of mice (Bott et al., 1968). The unheated supernatant which caused deaths of mice with typical botulinum symptoms within 96 hr and no death after the heat treatment were further used for typing the toxin.

For typing the toxin, 0.2 ml of the toxic supernatant and 0.2 ml of gelatin phosphate buffer were mixed with 0.1 ml of each of the monovalent antitoxins (1 IU/ml of types A, B, C, E and F and 10 IU/ml of type D). Prior to injection, the mixtures were allowed to stand at room temperature for 15 min for neutralization. After injection, the mice were observed for 4 days for the appearance of botulism symptoms. The amount used for neutralization tests of such supernatants that requiring a higher titer of the antitoxin was 0.1 ml instead of 0.2 ml and gelatin phosphate buffer was increased to 0.3 ml. Antitoxins were obtained from the Center for Disease Control, Atlanta, GA, USA.

Table I indicates that out of 350 samples analyzed, 40 were positive for *C. botulinum*, giving an overall positive percentage of 11.4. The frequency of positive tests was the highest in seawater fish (16.7%), followed by freshwater fish (13.5%), seawater mud (13%), freshwater (11.5%), freshwater mud (10%) and seawater (3%). The higher contamination of *C. botulinum* in seawater mud than in seawater may be due to the fact that this organism is terrestrial in inhabitation and water bodies get contaminated through washed soil and later on these organisms settle down as sediment (Johanssen, 1963; Hobbs, 1976).

TABLE II

The incidence of botulinal toxin in mud and water samples

Sample No.	Type of samples	Place of sampling	Type of toxin detected
<i>Freshwater mud:</i>			
156	Mud (brown)	Garut	D
175	Mud (brown)	Ciranjang	C
229	Mud (brown)	Tanjunkait	E
295	Mud (brown)	Banjar	T
297	Mud (brown-black)	Rangkashitung	T
<i>Seawater mud:</i>			
1	Mud (black)	Jakarta bay front	D
187	Mud (brown)	Pelabuhanratu sea front	C
280	Mud (black)	Pagalaran sea front	B
311	Mud (shell mixed)	Seribu islands	B
313	Mud (shell mixed)	Seribu islands	C
315	Mud (shell mixed)	Seribu islands	C
<i>Freshwater:</i>			
72	Water (slightly muddy)	Plered	D
128	Water (clear)	Ciculang	C
174	Water (Muddy)	Ciranjang	C
225	Water (Muddy)	Mauk	C
231	Water (Muddy)	Balaraja	C
235	Water (Muddy)	Serang	C
<i>Seawater:</i>			
11	Water (dirty)	Cilincing sea front	F
275	Water (clear)	Labuhan sea front	C

T=Toxin disappeared.

The higher contamination in freshwater than in seawater may also be due to this reason, since the freshwater samples collected during the present investigations were muddy and well turbulated during their flow in rivers.

Typing *C. botulinum* showed that types C and D were dominant both in mud and water samples (Table II). Out of 19 positive samples, 13 were types C and D, 2 type B, 1 type E and 1 type F. In two samples, toxin disappeared during storage. Types C and D were also dominant in fish samples as indicated in Table III. From all the 21 positive samples, 13 were types C and D, 1 type A, 3 type B, 3 type E and 1 type F. This table also shows that the extent of contamination with types C and D was higher in freshwater fish than in seawater fish. It is interesting that in freshwater fish the majority of *C. botulinum* positive results were obtained with visceral contents, while in seawater fish with the muscle plus skin portions. The reason for high incidence of *C. botulinum* in the muscle and skin is not known and requires further investigation. However, detection of botulinum spores from the visceral content of fish has been reported (Craig, Hayes and Pilcher, 1968).

Dominance of types C and D has been reported from Northern Coastal

TABLE III

The incidence of botulinal toxin in fish samples

Sample No.	Fish sample		Place of sampling	Botulinal toxin type detected
	Local name	Scientific name		
Freshwater fish :				
15*	Kambe	<i>Cyprinus carpio</i>	Aratan	C
46**	Gurame	<i>Ophiocephalus</i> spp.	Sangkanurip	E
61**	Sepat	<i>Trichogaster trichopterus</i>	Sumedang	C
81**	Mas	<i>Cyprinus</i> spp.	Bandung	C
82**	Mas	<i>Cyprinus</i> spp.	Bandung	C
98**	Gurame	<i>Ophiocephalus</i> spp.	Kartahau	D
178*	Mas	<i>Cyprinus</i> spp.	Ciranjang	D
Seawater fish :				
4*	Teri	<i>Stolephorus commersonii</i> (lac)	Jakarta bay front	B
5*	Kerapu	<i>Lutjanus decessatus</i>	Jakarta bay front	C
6*	Sebelah	<i>Cynogrossus lingua</i> (H.B.)	Jakarta bay front	E
7*	Bawal	<i>Stromateus niger</i> (B.L.)	Jakarta bay front	C
163*	Tongkol	<i>Euthynnus alleteratus</i>	Sentolo sea front	B
192**	Cakul	<i>Puntinus brevis</i>	Pelabuhanratu sea front	F
216**	Kakap	<i>Lates calcarifer</i>	Tanjung sea front	D
239*	Baji baji	<i>Platycephalus scaber</i>	Karanghantu sea front	C
258**	Tongkol	<i>Euthynnus alleteratus</i>	Labuhan sea front	A
261*	Bentong	<i>Caranx crumenophthalus</i>	Labuhan sea front	T
285*	Lemah	<i>Lactarius lactarius</i>	Labuhan sea front	D
289***	Talang	<i>Chorinemus tala</i>	Baunangan sea front	C+D
303*	Biji rangha	<i>Upeneus sulphureus</i>	Seribu islands	B
304*	Tongkol	<i>Euthynnus alleteratus</i>	Seribu islands	E

* =Toxin was detected from the muscle plus the skin. ** =Toxin was detected from the viscera.

*** =Toxin was detected both from the muscle plus the skin and the viscera. Type C from the viscera and type D from the muscle plus the skin.

T=Toxin disappeared.

areas of Java, Bali, around Madura and East Sumatra (Mortojudo et al., 1973). Recently type D was found in the Gulf of Thailand and types C and D from Bangladesh (Tanasugarn, 1979; Huss, 1980). The exact reason for type distribution is not known; however, dominance of types C and D along the equator may be attributed to the higher temperature since 42°C is optimum for the growth of types C and D (Miyazaki and Sakaguchi, 1978).

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