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NOTES

Distribution of *Clostridium botulinum* Around Fishing Areas of the Western Part of Indonesian Waters

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A survey was carried out to determine the presence of *Clostridium botulinum* in samples of sediment and seafoods from the fishing areas of the western part of Indonesian waters. Among the 3,433 samples, 82 (2.4%) were positive for *C. botulinum*. Type E was not found.

Fish preservation by irradiation is complicated by the presence of radiation-resistant pathogenic microorganisms (5, 11, 13). *Clostridium botulinum* is of particular concern because its spores are among the food-poisoning species and because it produces a potent toxin (1, 3). *C. botulinum* is widely distributed in nature and has been found on the five continents. The species is found in water, soil, vegetables, sediment, marine products, and some processed foods. The presence of certain types of *C. botulinum* in fishery products and marine environment has been reported by many investigators. Although no case of food poisoning by botulinum toxin has been reported in Indonesia, it does not mean that the Indonesian waters are free of this bacterium (10). This paper reports the distribution of *C. botulinum* in sediment and seafoods collected from the fishing areas of the western part of Indonesian waters.

Samples were collected from 109 sites around the fishing areas of the northern coast of Java, the eastern coast of Sumatra, and the west and east coastal areas of Kalimantan (Fig. 1). Sediment specimens were taken by grab-sampler at 1 to 2 km from the shore at water depths of 3 to 25 m. Seafood samples, such as fish, shellfish (clam, oyster, snails), shrimp, and crab, were collected directly from local commercial fishermen. Samples were separately packed in a plastic bag and put on ice for transport to the laboratory. Boiled fish samples (pindang) prepared in private homes were collected from 31 markets in Medan, Jakarta, Surabaya, and Madura. The 264 pindang samples were preparations of either species of fish. A total of 3,433 samples were collected from August to September from 1973 to 1976.

Test samples were sediments which were treated in 50% ethanol for 30 min. They consisted of gills and viscera (fish, crab), cephalothoraxes (shrimp), and whole animal (shellfish). About 5 g were cultured in screw-cap tubes (2.5 by 20 cm) containing 25 ml of cooked meat medium (Difco Laboratories). After incubation at ambient temperatures (about 27 to 32°C) for 5 to 7 days, the cultures were centrifuged at 3,000 × *g* at 5°C for 30 min. The supernatant fluids were kept overnight at −15°C to reduce the killing of mice by substances of nonbotulinal origin (14). One half of the extract was then treated with 0.2% trypsin (Difco Laboratories; 1:250 in 0.05 M K_2HPO_4 solution) at 37°C for 1 h. Detection for the presence of botulinum toxin was done by toxicity test on mice. After the supernatant was diluted 1:1 with gelatin-phosphate buffer (equal volumes of 0.4% gelatin solution and 0.1 M KH_2PO_4, pH 6.5), 0.4 ml was injected intraperitoneally into separate pairs of mice. If the mice given the heated (100°C, 10 min) supernatant fluid alone survived, a complete test series was carried out with types A-F specific botulinum antisera from the Centers for Disease Control, Atlanta, Ga. The dying mice were observed for botulism symptoms during 96 h.

The enrichment culture of 493 of the 3,433 samples was lethal for mice. Neutralization tests with type-specific antitoxins showed that 82 of the enrichment cultures contained *C. botulinum* toxin. The distribution of *C. botulinum* serotypes in the sampling areas is shown in Table 1.

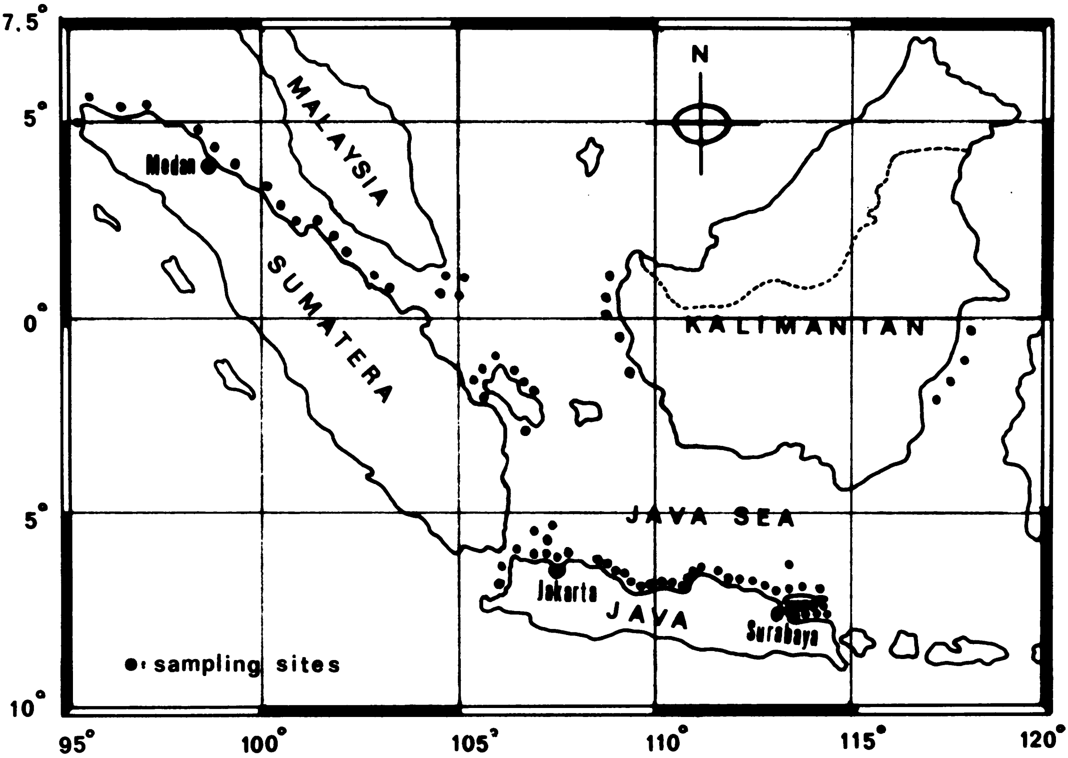

FIG. 1. *Sampling sites of sediment and fresh marine products around the northern coast of Java, the eastern coast of Sumatra, and the west and east coastal areas of Kalimantan.*

Of 82 (2.4%) positive samples, type A was present in 16, type B was present in 15, type C was present in 23, type D was present in 21, and type F was present in 7. Type E was not found, whereas this type is predominant in some other countries. Type E was reported to have been found between 36–49°N latitudes of the U.S. Pacific Coast (4) and around 30, 40, and 55°N latitudes of the U.S. Gulf Coast (16), Hokkaido (8), and Scandinavian waters (7, 12), respectively. The results obtained in this investigation correlate well with the opinion that type E is found in relatively cold areas. However, this cannot be taken as proof that tropical areas are free from type E. The Indonesian territory is situated around the equator between 5°N and 10°S latitudes. Recently, the presence of type E was reported around 12°N latitudes (14), whereas no type E was found around 34–43°S latitudes (2). The presence of *C. botulinum* in nature depends on the environmental conditions.

C. botulinum was found in 3.3% of samples collected from the northern coast of Java, 2.0% of samples from the eastern coast of Sumatra, and 1.2% of those from the west and east coastal areas of Kalimantan. Types C and D predominated on the northern coast of Java since these types were identified in 70% of the positive samples. It is interesting to note that out of 101 raw fish samples from Madura, an island in east Java territory, 14 (13.9%) were positive for type C or D, whereas 65% of positive samples from the eastern coast of Sumatra were types A and B.

The distribution of *C. botulinum* in samples of sediment and seafoods is shown in Table 2. Among the 592 sediment samples, 2,103 fish, 178 shellfish, 219 shrimp, and 77 crab samples, 11 (1.9%), 59 (2.8%), 2 (1.1%), 4 (1.8%), and 5 (6.5%) were positive, respectively, for *C. botulinum.* The average percent positive for all raw seafood samples was about 2.7%, and for seafoods other than fish (shell-fish, shrimp, and crab), it was 2.3%. Although *C. botulinum* was not restricted to specific types of sediment or seafood species, it was relatively common (10%) to *Mugil cephalus* (mullet). The 1 positive of 264 boiled fish (pindang) samples had type D. The positive sample, a preparation of *Clupea* sp., was obtained from a market in Kapaskrampung, Surabaya. The most important safety factor in prevention of botulism hazard in pindang fish is cooking these products before consumption.

The 2.4% contamination level of *C. botulinum*

TABLE 1. *Distribution of C. botulinum serotypes related to the sampling areas around the western part of Indonesian waters*

Sampling areas	No. of sites	No. of samples	No. of samples positive (%)	*C. bolutinum* serotypes					
				A	B	C	D	E	F
Java and Madura									
East Java & Madura	28	511	22 (4.3)	1	0	12	8	0	1
Central Java	8	304	11 (3.6)	2	2	4	2	0	1
West Java	32	510	11 (2.2)	1	2	2	4	0	2
Total	68	1,325	44 (3.3)	4	4	18	14	0	4
Sumatra									
South Sumatra	9	451	11 (2.4)	4	3	0	2	0	2
Riau	9	486	7 (1.4)	1	2	0	3	0	1
North Sumatra	9	304	8 (2.6)	5	2	1	0	0	0
Aceh	5	300	5 (1.7)	1	2	1	1	0	0
Total	32	1,541	31 (2.0)	11	9	2	6	0	3
Kalimantan									
West Kalimantan	5	307	3 (1.0)	1	1	1	0	0	0
East Kalimantan	4	260	4 (1.5)	0	1	2	1	0	0
Total	9	567	7 (1.2)	1	2	3	1	0	0
Total	109	3,433	82 (2.4)	16	15	23	21	0	7

TABLE 2. *Distribution of C. botulinum in samples of sediment and seafoods around fishing areas of the western part of Indonesian waters*

Sample	No. of samples	No. positive (%)	*C. botulinum* serotypes					
			A	B	C	D	E	F
Sediment	592	11 (1.9)	2	4	2	3	0	0
Raw seafoods								
Fish	2,103	59 (2.8)	12	10	16	16	0	5
Shellfish	178	2 (1.1)	1	0	1	0	0	0
Shrimp	219	4 (1.8)	0	1	1	0	0	2
Crab	77	5 (6.5)	1	0	3	1	0	0
Total	2,577	70 (2.7)	14	11	21	17	0	7
Boiled fish	264	1 (0.4)	0	0	0	1	0	0
Total	3,433	82 (2.4)	16	15	23	21	0	7

in sediment and seafoods around the fishing areas of the western part of Indonesian waters is lower than that reported for some other countries (4, 9, 16). The evidence indicates that the primary source of *C. botulinum* is soil or sediments. The contamination level in fish is variable, depending on environmental conditions, and *C. botulinum* is not able to establish itself and multiply in the intestines to fish (6).

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