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Presence of *Clostridium botulinum* in the Gulf of Venezuela and the Gulf of Darién

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To broaden the scope of a survey for the presence of *Clostridium botulinum* in the estuarine areas of the Gulf of Mexico (B. Q. Ward and B. J. Carroll, Appl. Microbiol. **13**:502, 1965; Ward, Atomic Energy Commission Conf. 651024, p. 124, 1965), our collections have been supplemented by specimens from various laboratories and commercial sources. Personnel of the vessel *Oregon* (Exploratory Fishing and Gear Research, Bureau of Commercial Fisheries) have provided numerous whole shrimp (*Panaeus aztecus*) from a number of stations in the Gulf of Darién, and several specimens of Lane's snapper (*Lutjanus synagris*) from stations in the Gulf of Darién and the Gulf of Venezuela. A single sand trout (*Cynoscion* sp.) was also included among the Gulf of Venezuela fish. All specimens were taken from the net, placed whole in plastic bags, frozen immediately aboard the vessel, and returned to the Pascagoula Laboratory in the frozen state. Collections were made between 6 October 1965 and 18 October 1965, at a uniform depth of 45 ft (13.7 meters).

At the Laboratory, specimens were allowed to thaw. The "heads" (cephalothorax) of 20 shrimp from one collecting station were removed aseptically and homogenized in a sterile blender for 2 min with 450 ml of sterile Butterfield's buffer. Since "heads" are roughly 1 pint, the dilution factor was grossly estimated to be 1:2. Into each of 10 large tubes, labeled A to J, containing 20 ml of Cooked Meat Medium (Difco), 2 ml of a homogenate was introduced. Each fish was opened along the ventral surface with flamed scissors. Viscera was detached aseptically and removed to a sterile blender jar. Viscera of six fish from a single station were homogenized with 450 ml of the buffer, and a single tube of Cooked Meat Medium was inoculated with 6 ml of the homogenate. All tubes were incubated at 30 C for 4 days. After incubation, 5 ml of fluid was withdrawn from each tube and centrifuged.

Two mice were injected intraperitoneally immediately from each tube with 0.4 ml of the supernatant fluid or 0.4 ml of supernatant fluid heated at 80 C for 15 min. The remainder of the supernatant fluid (diluted 1:2 with gelatin-phosphate buffer) was retained in duplicate tubes in the frozen state. Supernatant fluids producing the initial requisite results in mice (death within 96 hr from unheated injection, and no death from heated) were then examined critically. Each of eight mice was injected intraperitoneally with unheated supernatant fluid (0.4 ml), heated supernatant fluid (0.4 ml), or 0.5 ml of one of six (A to F) antitoxin-supernatant fluid mixtures [0.4 ml of supernatant fluid with 0.1 ml of monospecific antitoxin (obtained from the Communicable Disease Center, Atlanta, Ga.) incubated at room temperature for 30 min]. Mice were then observed for 96 hr (Table 1). Trypsinization was not employed.

We may have failed to detect certain feebly toxic cultures, but in this instance we have sought only indications of presence with no intention of exhaustively pursuing the problem of incidence at this time. Supernatant fluids producing perfect type-specific patterns of death (of eight mice, only one antitoxin-protected and the heated control surviving) were retested from the second frozen tube held in reserve. In all instances, the second series verified the first. In several instances, all antitoxin-protected mice of the first series died, but the control (heated) survived. From the second frozen replicate of such tubes, three mice were inoculated: unheated supernatant fluid, heated supernatant fluid, or unheated fluid reacted with both A and C antitoxins simultaneously. When only the unheated supernatant fluid caused death, the tubes were considered mixed types, and are tentatively recorded as A + C. From results tabulated, it is seen that we have also encountered instances of heat-stable toxic materials and heat-labile toxins not countered by any of the six known antitoxins or the combination of A + C. Inasmuch as these latter deaths were delayed and atypical, we assume the presence of nonbotulinic toxin, although we cannot dismiss the possibilities of mixtures other than A + C or of new undescribed types beyond "F."

In conversation, L. DS. Smith mentioned

TABLE 1. *South American samples and test results*

Coordinates	Sample	Sample no.	Mouse test indications
N11°33′ W71°31′	*Cynoscion* sp.	23	Atoxic
N11°33′ W71°31′	*Cynoscion* sp.	24	Type A
N11°33′ W71°31′	*Cynoscion* sp.	25	Atoxic
N 8°46′ W77°21′	*Lutjanus synagris*	13–15, 17–22, 26–28, 30–33	Atoxic
N 8°46′ W77°21′	*L. synagris*	16	Heated controls toxic
N 8°46′ W77°21′	*L. synagris*	29	Type A
N 8°46′ W77°21′	*Penaeus aztecus*	1 A–J	Atoxic
N 8°46′ W77°21′	*P. aztecus*	3 A, 3 J	Types A + C
N 8°46′ W77°21′	*P. aztecus*	3 B	Type C
N 8°46′ W77°21′	*P. aztecus*	3 C	Type A
N 8°46′ W77°21′	*P. aztecus*	3 D–I	Atoxic
N 8°46′ W77°21′	*P. aztecus*	4 A–J	Atoxic
N 8°46′ W77°21′	*P. aztecus*	5 A, 5 C–D, 5 F–J	Atoxic
N 8°46′ W77°21′	*P. aztecus*	5 B	Type C
N 8°46′ W77°21′	*P. aztecus*	5 E	Types A + C
N 8°46′ W77°21′	*P. aztecus*	7 A–J	Toxic, no antitoxin protection
N 8°46′ W77°21′	*P. aztecus*	8 A–J	Atoxic
N 8°46′ W77°21′	*P. aztecus*	9 A–J	Toxic, no antitoxin protection
N 8°41′ W77°12′	*P. aztecus*	2 A–J	Atoxic
N 8°41′ W77°12′	*P. aztecus*	6 A–J	Atoxic
N 8°41′ W77°12′	*P. aztecus*	11 A–F, 11 I–J	Toxic, no antitoxin protection
N 8°41′ W77°12′	*P. aztecus*	11 G	Type A
N 8°41′ W77°12′	*P. aztecus*	11 H	Type B
N 8°41′ W77°12′	*P. aztecus*	12 A–B, 12 D–E, 12 G–J	Atoxic
N 8°41′ W77°12′	*P. aztecus*	12 C	Types A + C
N 8°41′ W77°12′	*P. aztecus*	12 F	Type E
N 8°59′ W76°31′	*P. aztecus*	10 A–E, 10 G–J	Atoxic
N 8°59′ W76°31′	*P. aztecus*	10 F	Type C

essentially negative results for all attempted detections of *C. botulinum* northward toward the Virgin Islands from the Amazon River mouths. Ward (*unpublished data*) found no indications of type A along the U.S. Gulf Coast. Inasmuch as types A and C predominated in the samples reported here, it would seem that examinations of distribution patterns on both the Mexican Gulf Coast and in the Caribbean area might be of interest.

ERRATUM

Identification of Fluorescent-Antibody Labeled Group A Streptococci by Fluorometry

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Volume 14, no. 3, page 386, col. 2, next to last line: Change "(sharp cut at 610 mμ)" to "(sharp cut at 510 mμ)."