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Botulism in a Tidal Estuary in New Jersey

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ABSTRACT

Clostridium botulinum, type C β was identified as the causative organism in an epornitic which occurred on a tidal estuary in New Jersey. Approximately 1,000 individuals of twelve species representing five families of birds were intoxicated. Muskrats and killifish were also affected. Seven species previously unreported as affected by botulinus intoxication were recorded. The role of the food web in the epizootiology of botulinus intoxication and the intraspecific relationship between the killifish and Least Terns in the continuance of the outbreak is discussed.

INTRODUCTION

In the first week of August 1959, during a period of hot, humid weather, an epizootic occurred among aquatic birds in the tidal marshes of the Hakensack River estuary in Bergen and Hudson Counties, New Jersey. Approximately one thousand representatives of twelve avian species representing five families were affected. These species were: Great Blue Heron (*Ardea herodias*), American Egret (*Casmerodine albus egretta*), Snowy Egret (*Leucophoyx thula thula*), Black-crowned Night Heron (*Mycticoras mycticoras hoactli*), Mallard Duck (*Anas platyrhynchos platyrhynchos*), Black Duck (*Anas rubripes*), Florida Gallinule (*Gallinula chloropus cachinnans*), American Coot (*Fulica americana americana*), Herring Gull (*Larus argentatus*), Ring-billed Gull (*Larus delawarensis*), Laughing Gull (*Larus atricilla*) and Least Tern (*Sterna albigrons antillarum*). Other vertebrates were the Muskrat (*Ondatra zibethica zibethica*) and the Common Killifish (*Fundulus heteroclitus*).

An area of approximately 30 acres was

the focus of this epornosis. This portion of the marsh was separated from the open river by a sanitary land fill, and an unincorporated bulkhead of stone, broken concrete paving and building rubble, and a pipe line dike. The area was subject to tidal flooding through the unincorporated bulkhead and through culverts under the dike. It consisted principally of an open water area, 2 to 3 feet in depth at high tide, interspersed with several small low islands, whose dominant vegetation was reed grass, *Phragmites communis*, and smooth cord grass, *Spartina alterniflora*. At low tide, the area was a mud flat with numerous shallow pools, 1 to 6 inches in depth.

Botulism was suspected when numerous Least Terns were observed which exhibited various degrees of muscular weakness and flaccid paralysis pathognomonic of botulism intoxication. Some terns could only fly a few yards and could be caught by hand in flight or easily captured on the ground. Terns still in possession of full powers of flight were observed actively feeding on dying

killifish. The presence of numerous dead terns, egrets and other aquatic birds, several dead muskrats and hundreds of dead killifish throughout the marsh, also suggested an outbreak of botulism.

MATERIALS AND METHODS

Five moribund terns were taken to the laboratory, killed and autopsied. Their livers were removed aseptically, and small pieces were cultured in deep-tubes containing trypticase-soy extract broth (Difco) and incubated 48 hours at 37°C. Killifish which had lost equilibrium and the hydrostatic function of their swim bladders were collected and their organs cultured in a similar manner. No attempt was made to culture tissues of other animal species involved due to the advanced stage of decomposition of their carcasses.

The supernates of these cultures were centrifuged and filtered through a U. F. fritted glass bacterial filter. Five 20 gm. white mice were given intravenous injections of 0.1 ml of this filtrate to determine its toxicity. To eliminate the possibility that a contaminant might kill the mice, cultures were heated in a boiling water bath for 10 minutes to destroy all vegetative forms and the material was reinoculated into deep tubes of chopped meat trypticase-soy extract broth. Subcultures were incubated at 37°C for 72 hours, their supernates treated as above and injected into five additional mice.

Aliquots of this supernate were mixed with equal amounts of various types of *C. botulinum* antitoxin: Type A obtained from Fort Detrick, Maryland; Types C α and C β and Type D of rabbit origin prepared by us; and Type B of horse origin obtained from the Division of Laboratories and Research, New York State Department of Health, Albany, New York. Five groups of five 20 gm. white mice were injected intravenously with 0.2 ml of these supernate - antitoxin mixtures. A control group of five mice received 0.1 ml of supernate.

The technique of demonstrating toxin in the serum of sick birds in the protection test using mice was not employed because the number of sick terns collected was small and sufficient serum could not be obtained for the test.

RESULTS

Gas was detected in 48 hour cultures of the livers from sick terns and from killifish organs. Microscopic examination of these cultures and subsequent subcultures showed motile gram positive

rods which contained subterminal spores. The mice which had received injections of the filtered supernate of the initial culture died within 50 minutes with typical signs of botulism, indicating the presence of at least 10,000 mouse MLD per ml³. The filtered supernate from the subcultures produced a similar effect in mice.

The results of the neutralization test showed that only mice which had received supernate antitoxin C α or C β mixtures survived. Type D antitoxin prolonged the survival of mice but did not protect them, while Types A and B were not effective. The protection afforded by Type C α and C β antitoxin indicated that the agent responsible for the epornitic was *C. botulinum* Type C β . This organism, while primarily a Type C strain, possessed some antigenic characteristics of Type D strains as indicated by the prolonged survival of mice receiving Type D antitoxin. A similar organism was isolated by Prevot and Bygöo²⁰ from an outbreak of botulism among horses in France.

DISCUSSION

Two other incidents of possible botulinus intoxication in waterfowl and shorebirds occurring in estuaries or tide water have been reported. In 1934, Kalmbach and Gunderson¹⁶ reported the recovery of *C. botulinum* Type C from the liver of a single Black Duck collected on the Potomac River below Washington, D. C. Three years earlier, Austin and Austin¹ described food poisoning in Ruddy Turnstones (*Arenaria interpres morinellae*) and Sanderlings (*Crocethia alba*) from a sandpit in Massachusetts Bay. This was characterized by signs of muscular weakness, inability to fly and disturbances of equilibrium and muscular coordination. The authors suggested that the condition closely paralleled botulism or acute gastro-entero-colitis in man. No attempt was made to identify the toxin or the bacteria involved but the source of

intoxication was attributed to bacteria and toxin adhering to blowfly maggots (*Calliphora* sp.) infesting decomposed stranded Blackfish (*Globicephala melana*) and True Seal (*Phoca vitulina concolor*) carcasses.

First among the factors involved in the epizootiology of an outbreak of botulism are those which affect the growth and elaboration of the toxin by the organism. The "sludge bed" concept of Bell *et al.*² proposed that ubiquitous spores vegetate and elaborate toxin under the proper conditions of anaerobiosis, alkalinity, temperature, and humidity in the organic substrate of the marsh. In addition, a favorable microenvironment may become available in carcasses of invertebrates which may be buried in this debris. Hobmaier¹¹ Quortrup²¹, and Bel *et al.*² have experimentally produced toxic cultures of *C. botulinum* in carcasses of arthropods, mollusks and fish, while Kalmbach and Gunderson¹⁶ demonstrated this organism in larvae of hydrophiled beetles. Subsequently, Jensen and Allen¹³ in 1960 correlated botulism outbreaks in the Bear River Refuge, Utah, with a sharp decline in invertebrate bottom fauna following their population peak.

The recovery of the causative organism from the livers of morbid terns and killifish from this outbreak indicates the invasion of the host tissue. Elkin⁵ (1961) suggested that this invasion is facilitated by the ingestion of preformed toxin which overcomes the homeostatic barriers of the vertebrate host's tissue, and results in subsequent elaboration of additional toxin. Wound contamination with proliferation of *C. botulinum* has been reported in six instances in man^{4 8 9 22}.

The epizootiology of botulinus intoxication not only involves investigation of the source of the toxin, but also the manner in which it becomes available to vertebrates. It has adequately been demonstrated that botulinus toxin is in-

gested in or on preferred or available food; therefore food-chain transmission is of importance. A review of the food habits of some of the vertebrate species intoxicated in this epornitic points out the possible pathways for the spread and continuance of the intoxication once it has been triggered.

McAtee⁸¹ observed in 1918 that Black Ducks inhabiting salt marshes consumed snails, mussels, arthropods, and fish, including killifish. Four years later Palmer and Baker¹⁹ reported clinical signs of botulinus intoxication in ducks and swans which had fed on dead tadpoles and small fish; unfortunately, the causative organism was not isolated, nor was the demonstration of the toxin attempted. The Ring-billed Gull kills and feeds on ducks paralyzed by duck sickness, i.e. botulism²³. Judd¹⁵ reported in 1900 that the Black-crowned Night Heron consumes dead fish, while according to Grinnell *et al.*⁷ and Jones¹⁴, the Goot will eat duck carcasses as well as mollusks, crustaceans, fish and amphibians. Killifish feed on a large variety of food, including copepods, mosquito and fly larvae, as well as miscellaneous insect debris and particulate animal and vegetable matter¹⁰. Though the muskrat is considered to be primarily a vegetarian, reports by Lantz¹⁷, Hollister¹², and Evermann and Clark⁶ state they will feed on bodies of dead ducks, coots and fish.

The nidus of this outbreak may have been either botulinus contaminated animal or vegetable offal in the sanitary fill, or invertebrate carcasses and organic debris in the substrate of the tidal flat. The ingestion of materials containing preformed toxin and the organism by avian or pisces species conceivably could have provided further impetus for a chain reaction which spread the epornitic via the food web; each resulting death providing an additional source of toxin and organisms for other vertebrates. In this outbreak, only the trans-

mission via the food chain between the killifish and the tern has been demonstrated by the recovery of the organism from the species involved and the observed ingestion of killifish by terns. Unfortunately, toxin could not be demonstrated in either species due to the insufficiency of sera.

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ERRATA

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BOTULISM IN A TIDAL ESTUARY IN NEW JERSEY, by J. R. Reilly and D. A. Boroff. The Greek letters α and β should appear as subscripts to the capital letter C instead of superscripts in line 1 of the Abstract, page 26, in line 4 of the third paragraph of Materials and Methods on page 27, and in lines 3, 8 and 10 of the second paragraph in Results on page 27.

Page 27, line 2 of the first paragraph should read:
terns, egrets and other aquatic birds,

Page 28, line 4 of the third paragraph should read:
invasion of the host tissue. Elkin⁵ in 1961

Page 28, lines 7 and 8 of the third paragraph should read:
which overcomes the homeostatic barriers of the vertebrate host's tissue, and

Page 28, line 1 of the fifth paragraph should read:
McAtee¹⁸ observed in 1918 that Black

Page 29, line 5 of the first paragraph should read:
served ingestion of killifish by terns.

The following references were missing from Literature Cited:

2. BELL, J. F., G. W. SCIPLE, and A. A. HUBERT. 1955. A microenvironment concept of the epizootology of avian botulism. J. Wild. Mgmt. 19: 352-357.
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Page 29, reference 18, line 2 should read:
Dept. Agr. Bull. No. 720, 36 pp.

Page 29, reference 20, first line should read:
20. PREVOT, A. R. AND E. R. BRYGOO. 1950. Etude de la Premiere Souche Francaise de

Page 29, reference 23, first line should read:
23. WETMORE, A. 1921. Wild Ducks and Duck Food of the Bear River Marshes, Utah.