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The Relationship of Lake Michigan Waterbird Mortalities to Naturally Occurring *Clostridium botulinum* Type E Toxin

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ABSTRACT

Gulls were force fed *Clostridium botulinum* type E toxin. Their susceptibility varied with the strain of *C. botulinum* type E toxin, and the LD_{50} for gulls to a toxic strain was calculated to be approximately 20,000 mouse MLD. Type E botulinal toxin was present in dead alewives taken from the beach and water of Lake Michigan, and thus a vehicle for waterbird intoxication is available.

INTRODUCTION

Outbreaks of type E botulism in humans in 1960 and 1963, in which smoked fish from the Great Lakes were found to be the vehicle of intoxication[9,10,12], stimulated research into the ecology of *Clostridium botulinum* type E. Subsequent investigations have shown this organism to be widespread throughout Lake Michigan[1,4]. Late in the fall of 1963, and every year since, mortalities of fish-eating waterbirds have occurred along the Lake Michigan shore with *C. botulinum* type E toxin being demonstrated in the blood and tissues of many of the carcasses. Accounts of these mortalities and the laboratory investigations concerning them have been well documented[2,3,6,7].

The fact that *C. botulinum* type E is present in Lake Michigan and that the type E botulinal toxin has been found in the dead waterbirds presents good, but only circumstantial, evidence that type E botulism is the cause of the bird mortalities. A susceptibility level for the birds must be determined and then a source of the toxin in a form suitable for intoxication must be found in nature to be reasonably sure that botulism is causing the deaths. Kaufman, *et al*[8] studied the susceptibility of ring-billed gulls (*Larus delawarensis*) to type E botulinal toxin and although botulism was induced in them, no definite level of susceptibility was determined. Also, no direct evidence of "naturally occurring" type E botulinal toxin in the Lake Michigan ecosystem has been presented. This study presents the evidence necessary to conclude that the waterbird mortalities are indeed caused by type E botulism.

MATERIALS and METHODS

Preparation of toxin for feeding trials: The type E botulinal toxin used in the determination of the susceptibility of waterbirds was produced in pure culture using a medium consisting of 5.0% trypticase (BBL), 0.5% peptone, 0.2% sucrose and 1.0% yeast extract. Anaerobic conditions were produced in the culture by the addition of 0.1% sodium thioglycollate. The cultures were incubated in one-liter prescription-type bottles at 30° C for five days, and then held at -15° C until used in the feeding trials.

Toxin from two strains of *C. botulinum* type E was produced. These strains were VH, a "common" laboratory culture, and a strain isolated from a Lake Michigan whitefish chub and identified as 026-080 X by the Food and Drug Administration. Titration and positive identification of the toxins were determined by the injection of mice with aliquots of the toxins thawed just previous to feeding. Both cultures contained from 3,000 to 4,000 mouse MLD of toxin per milliliter.

Susceptibility of waterbirds: Gulls were chosen for the bioassay of the botulinal toxin because of the previous work done with them. Ring-billed gulls (*Larus delawarensis*) and herring gulls (*L. argentatus*) which were three to five weeks of age were taken from a gull nesting colony on northern Lake Huron. California gulls (*L. californicus*) were captured as young on the Bear River Migratory Bird Refuge (near Brigham City, Utah) and shipped to East Lansing by air express. All gulls were held until they were full-grown. Their diet consisted primarily of alewives (*Alosa pseudoharengus*) supplemented periodically with canned dog food or raw meat. A dose of thiamine hydrochloride was also given periodically to prevent vitamin deficiencies caused by thiaminases in the alewives.

The primary objectives of the bioassay of type E botulinal toxin in gulls was 1) to support evidence that gulls, as Hazen[5] found for chickens, vary in their susceptibility to toxin produced by different strains of *C. botulinum* type E[13] and 2) to determine the level of a toxic strain that affects gulls.

Differentiation of the toxicities of the two strains of *C. botulinum* type E was done by force feeding ring-billed gulls 40,000 mouse MLD toxin, four birds receiving the VH strain and four receiving the 026-080 X strain. This toxin was contained in a volume of 10 ml. Two groups of three california gulls were also fed the different strains of toxin; one bird in each group was fed 40,000, 80,000 or 120,000 MLD.

Feeding of the botulinal toxin to the waterbirds was accomplished using a syringe and blunted 1½-inch 18 gauge needle. The beak of the bird was held open, and the needle was placed into the throat so as to avoid getting fluid into the trachea. The toxin was slowly squirted into the esophagus and the bird was held with its neck outstretched for approximately one minute so that the fluid entered the intestines and could not be regurgitated. Elimination of the possibility of oral irrigation due to large volumes of fluids was accomplished by feeding the larger doses in aliquots of 10 ml at intervals of one hour.

C. botulinum type E toxin produced by strain 026-080 X was used to determine the susceptibility level of ring-billed gulls. Four groups of ten gulls were fed from 7,500 MLD to 30,000 MLD toxin. All birds received a volume of 10 ml. Control groups included five gulls fed 10 ml of the uninoculated bacterial growth medium and five gulls fed 10 ml of the gelatin-phosphate buffer which was used to bring the toxin doses to their final volume.

Several other species of waterbirds were fed doses ranging from 30,000 MLD to 150,000 MLD of the 026-080 X strain of toxin. These birds included 15 herring gulls, one great blue heron (*Ardea herodias*), one common loon (*Gavia immer*), and one horned grebe (*Colymbus auritis*).

Detection of C. botulinum type E toxin in Lake Michigan: Eight dead alewives were collected during a trip to Lake Michigan, just north of Ludington, Michigan, on June 16, 1967. While scuba diving about 300 yards off shore in 25 feet of water, two of the fish were taken from the bottom of Lake Michigan. Two fish were collected from the high water line on the bathing beach while four were picked up from the beach at the water's edge. These fish were placed in plastic bags which were then placed in ice within 20 minutes following collection.

The following day in the laboratory, each fish was squeezed and the fluids thus obtained diluted with a gelatin-phosphate buffer. Four Swiss-Webster mice, two of which were protected by antiserum specific for *C. botulinum* type E toxin, were injected intraperitoneally with 0.2 ml of each dilution of juice. The fish juices were also treated with trypsin to test for activation of the toxin. Equal parts of the juice and a 1.0% solution of trypsin (Difco, 1:250) were incubated at 40° C for one hour. This juice was tested in a manner similar to that used for the untreated fluids.

The physical condition of the fish varied. Those taken from the beach appeared to have recently died and only slight visible decay was evident. The fish from the water were considerably decayed and covered with fungus.

RESULTS

Gull susceptibility: All of the california gulls fed the 026-080 X strain of toxin developed signs of botulism and died. No signs of illness of any kind were observed in those that received the VH toxin. All of the ring-billed gulls that received 40,000 MLD of the 026-080 X toxin became obviously ill and two of them died, while only one gull fed with the VH toxin exhibited slight symptoms of botulism. None of the other ring-billed gulls fed the VH toxin became visibly ill.

Toxin feeding trials using various doses of toxin 026-080 X administered to the ring-billed gulls (Table 1) indicate that the LD_{50} is approximately 22,500 mouse MLD. However, calculation by the method of Reed and Muench[11] gives a LD_{50} of just under 20,000 mouse MLD. The level which causes 50% of the gulls to become obviously sick is calculated to be approximately 12,000 mouse MLD. One of the gulls fed gelatin-phosphate buffer as a control died but it did not exhibit signs of botulism. None of the gulls receiving the uninoculated medium became ill or died.

TABLE 1. *Results from feeding ring-billed gulls* C. botulinum *type E toxin strain* 026-080 X.

Dose fed (MLD)	No. of gulls treated	No. exhibiting botulism symptoms	No. dead
30,000	10	10	8
22,500	10	8	5
15,000	10	8	4
7,500	10	3	2
Uninoculated medium	5	0	0
Gelatin phosphate buffer	5	0	1

TABLE 2. *Results from feeding various waterbirds type E* C. botulinum *toxin strain* 026-080 X.

Species	Dose fed (MLD)	No. birds fed	No. exhibiting botulism symptoms	No. dead
Herring gull	45,000	5	3	1
Herring gull	90,000	5	4	0
Herring gull	135,000	5	5	0
Great blue heron	150,000	1	1	1
Common loon	150,000	1	1	1
Horned grebe	30,000	1	1	0

The results from feeding the other waterbirds toxin 026-080 X (Table 2) indicate that these species may vary in their susceptibility to the toxin. A much larger number of birds would be needed to determine what specific levels of toxin produce specific effects.

Toxin in the Lake Michigan ecosystem: *C. botulinum* type E toxin was found to be present in the Lake Michigan ecosystem both on the beach and in the water (Table 3). The toxin was present in a vehicle suitable for the intoxication of fish-eating waterbirds. Increased toxicity was not found after treatment with trypsin and it is concluded that the toxin was therefore present in the activated form.

TABLE 3. Clostridium botulinum *type E toxin in alewives taken from the Lake Michigan ecosystem.*

Site collected	Weight of fish (g)	Highest dilution to kill mice	MLD toxin per gm fish	Total MLD toxin in fish
Underwater	19	1:800	4,000	76,000
Underwater	26	1:100	500	13,000
Beach at waters edge	30	0[1]	—	—
Beach at waters edge	24	0	—	—
Beach at waters edge	23	0	—	—
Beach at waters edge	32	0	—	—
Beach at high water line	30	1:100	500	15,000
Beach at high water line	27	1:100	500	13,500

[1] Undiluted fresh fish juice kills mice, so if a 1:10 dilution did not kill, the sample was considered non-toxic.

DISCUSSION

Although the number of gulls tested was small, it is highly probable that gulls vary in their susceptibility to type E botulinal toxin produced by different strains. Statistical analysis by the non-parametric Fisher Exact Probability Test indicates only a 7.1% chance that the two strains of toxin tested have the same effect on ring-billed gulls. Also, this same statistical test indicates that, in doses from 40,000 to 120,000 MLD, the chance is only 5.0% that california gulls will be killed in equal numbers by the two strains of toxin tested. This study therefore substantiates the previous investigations[5 13] showing that the susceptibility of birds varies with the strain of type E toxin ingested.

This difference in susceptibility becomes of great interest when considering both the toxin found in the dead alewives and the calculated susceptibility levels of the ring-billed gulls to the 026-080 X strain of type E toxin. Although the maximum amount of toxin found in the alewives should be sufficient to sicken or kill many of the gulls that would eat the fish, the question arises as to whether this strain of toxin is toxic to gulls. This specific toxin from the fish was not fed to gulls, but Fay[2] indicated that some dead alewives picked up along the beach of Lake Michigan did kill some of the captive gulls which ate them and that type E toxin was then detected in blood from these gulls.

From the data presented, it can be concluded that type E botulism is the cause of at least part of the waterbird mortalities that occur in the Lake Michigan area. The one remaining piece of information needed is an observation of a free-living waterbird actually consuming and then becoming affected by a naturally occurring toxic fish. To obtain this information would be an extremely difficult task.

A question arises as to why half of the ring-billed gulls fed 40,000 MLD of toxin died while the calculated LD_{50} is less than 20,000 MLD. This cannot be answered, although such differences are not uncommon when working with *C. botulinum* and gulls. One possible explanation, however, is the lack of an adequate number of gulls fed the 40,000 MLD to compute an accurate LD_{50}.

It is interesting to speculate about the ecology of the type E botulinal toxin found in Lake Michigan. Although the toxin was found in only one location, there is no reason to believe that it is restricted to that geographical area or even the time of year in which it was found. The fact that the toxin was found on a bathing beach and in the water raises the possibility of accidental human intoxication. It is evident that the ecology of the *C. botulinum* type E toxin needs more investigation.

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ANNOUNCEMENTS CONTINUED FROM PAGE 69

CATALOGUE OF ARTHROPOD-BORNE VIRUSES NOW AVAILABLE

Taylor, R. M. (comp.) Catalogue of Arthropod-borne Viruses of the World. PHS Publication No. 1760. LC Catalogue No. 67-60097. First ed., 1967. U.S. Government Printing Office, Washington, D.C., 908 pages. Available from Superintendent of Documents, $5.25.

ZOOLOGICAL SOCIETY OF LONDON SYMPOSIUM

A symposium on diseases in free-living wild animals was held at the Zoological Society of London, May 9 and 10, 1968. Eighteen papers were presented. The presentations are being assembled in a Proceedings by Dr. A. McDiarmid. Announcment of availability of this publication will appear in a subsequent issue of the Bulletin of the Wildlife Disease Association.

ZOONOSES LABORATORY MOVING

On 15 June 1968 the personnel and facilities of Zoonoses Section, Ecological Investigations Program, National Communicable Disease Center, 15th Avenue and Leke Street, Building 18, San Francisco, California 94118 were transferred to Fort Collins, Colorado. After this date, please address all correspondence to: Zoonoses Section, Ecological Investigations Program, P.O. Box 551, Fort Collins, Colorado 80521.