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WATERBIRD MORTALITY FROM BOTULISM TYPE E IN LAKE MICHIGAN: AN UPDATE

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Botulism as a food poisoning is acquired from ingestion of material containing a potent neurotoxin produced by the bacterium *Clostridium botulinum*. Under aerobic conditions, this microorganism exists in a spore stage. In the presence of a suitable nutrient source, favorable temperature, and in the absence of oxygen, vegetative growth can occur. It is in the vegetative stage that botulinal toxin is synthesized within metabolizing cells, and is released upon autolysis of the cell (Rosen 1971). Seven types of botulinal toxins are currently known: types A through G. Botulism outbreaks in humans are usually caused by toxin types A, B, E, and more recently F; outbreaks usually result from improperly prepared or stored food products (Dowell et al. 1970). Suspected cases of botulism type G have recently been reported (Sonnabend et al. 1981). Botulism type D usually occurs in cattle.

Avian mortality from botulism occurs essentially worldwide, most frequently among waterfowl and shorebirds, and is usually associated with type C toxin (Rosen 1971). Annual mortality to botulism type C in North America alone has been in the hundreds of thousands (Rosen 1971). Naturally occurring botulism type E was not reported in birds until 1963 and 1964, when Herman (1964) and Kaufman and Fay (1964) associated type E toxin with extensive die-offs of Common Loons (*Gavia immer*) and gulls (*Larus* spp.) on the Indiana-Michigan shores of Lake Michigan. Mortality was estimated at 7720 in 1963 and 4920 in 1964 (Fay et al. 1965). Another die-off of similar nature in 1965 was reported by Fay (1966); additional mortality attributed to botulism type E in Michigan has since been reported (Monheimer 1968, Graikoski et al. 1970, Stuht et al. 1977). In the present paper, we report three additional outbreaks of botulism type E in birds in Lake Michigan occurring in 1976, 1980, and 1981, and we present evidence of the natural consumption by loons and Ring-billed Gulls (*Larus delawarensis*) of food items containing type E toxin.

MATERIALS AND METHODS

Carcass examination.—Necropsies were conducted at the National Wildlife Health Laboratory (NWHL) on selected fresh avian carcasses found during epizootics reported herein; carcasses were submitted either chilled (4°C) or frozen. Moribund birds were bled in the field and sacrificed; blood samples were chilled and submitted with the carcasses. Upon

necropsy, lesions were noted and appropriate diagnostic tests were conducted to determine cause of morbidity or mortality. Tests included routine bacterial and viral studies, mouse toxicity tests, and parasite examinations of selected tissues. Tissues from several specimens were analyzed for organochlorine content at the U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center. Mercury levels were determined by the Wisconsin Alumni Research Foundation (now Raltech, Inc.), Madison, Wisconsin.

Demonstration of botulinal toxin.—Serum was separated from blood samples collected from moribund birds and from heart blood of dead birds to test for the presence of type C and type E botulinal toxins. Standard mouse toxicity tests were employed, involving intraperitoneal (IP) injection of 0.5 ml of serum each into unprotected mice (*Mus* sp.) and into mice protected with type-specific antitoxin. Demonstration of toxin was based on death of unprotected mice and survival of protected mice. Death of unprotected mice and survival of mice protected with type E antitoxin and mice protected with type C antitoxin provided evidence that both C and E toxins were present in the serum sample, though each alone at levels sublethal to mice. This interpretation is based on studies by Jensen and Gritman (1966), who suggest an adjuvant (synergistic) effect between sublethal levels of types C and E toxin when present together in causing mortality in Mallards (*Anas platyrhynchos*).

Fish samples removed from alimentary tracts of moribund and dead birds were processed by using the extraction method described by Foster et al. (1974). The presence of botulinal toxins was determined by IP injection of 0.5 ml of extract into mice, as described above.

RESULTS

1976 epizootic.—Mortality involving Common Loons along the southeast shore of Lake Michigan was first reported on 22 October 1976 to the Michigan Department of Natural Resources (MDNR). By 4 November, reports were received of dead birds on Michigan beaches from Union Pier to Benton Harbor in Berrien County. On 8 November, an aerial survey conducted by MDNR from the Indiana-Michigan border to Muskegon (about 145 km) disclosed 592 dead loons, 6 moribund loons, and 6 dead gulls (J. Aldrich, pers. comm.). Carcasses were most heavily concentrated along shorelines in Berrien (N = 455) and Van Buren (N = 118) counties; snow cover on beaches north of Van Buren County caused counts in those areas to be incomplete.

Between 26 October and 19 November, 342 avian carcasses (330 Common Loons, 1 Red-throated Loon [*G. stellata*], 6 unidentified gulls, 1 Oldsquaw [*Clangula hyemalis*], 2 grebes—an Eared Grebe [*Podiceps caspicus*] and an unidentified individual, 1 Redhead [*Aythya americana*], and 1 White-winged Scoter [*Melanitta deglandi*]) were collected from Lake Michigan shores in Michigan. Searches for additional carcasses were discontinued after 22 November. Total mortality in Michigan was estimated to be between 600 and 1000 birds. An additional 157 dead Common Loons and one gull carcass were also collected along the Indiana shores of Lake Michigan between 2–15 November.

Botulinal type E toxin was demonstrated in blood from carcasses submitted to NWHL and to the MDNR Rose Lake Wildlife Research Center. At NWHL, 21 Common Loons, 1 Ring-billed Gull, 1 Oldsquaw, and 1

Eared Grebe were received for examination. Type E toxin was demonstrated in 8 of 18 loons tested. The gull had a mycotic air saculitis caused by *Aspergillus fumigatus*. Botulism tests on the Eared Grebe were inconclusive; the Oldsquaw was too autolyzed for examination. Other bacterial and viral studies on these specimens were negative for other etiologic agents.

Analysis of tisses from the Ring-billed Gull and seven loons examined above disclosed organochlorine levels below those considered lethal (Ohlendorf et al. 1978); polychlorinated biphenyl (PCB) residues were slightly elevated (ranging from <0.2–2.5 ppm wet weight in brain tissue, 1.1–14.0 ppm in muscle tissue), but below levels known to have serious effects on birds. Mercury analyses of kidney tissues from two loons also were below levels known to be significant (0.75 and 1.35 ppm). Type E toxin was demonstrated in the remains of an unidentified fish removed from the ventriculus of one loon.

1980 epizootic.—A die-off of about 60 Ring-billed Gulls was investigated during 10–25 June 1980 on a small island in southern Green Bay, near Green Bay, Brown Co., Wisconsin. About 1000 gulls were nesting in a colony on this island. Mortalities were composed of about 64% subadult and adult gulls, and 36% gull chicks. In addition, one moribund Black-crowned Night-Heron (*Nycticorax nycticorax*) was found.

During the die-off, reports were also received of mortality among alewives (*Alosa pseudoharengus*) and suckers (Catostomidae) along the eastern shores of Green Bay. The cause of that mortality was not investigated. Surveillance of other areas of Green Bay for additional avian mortality was conducted by the Wisconsin Department of Natural Resources. Since no additional dead birds were found, it appeared that mortality was limited to the nesting colony.

Type E botulinal toxin was demonstrated in blood samples from one of five moribund adult gulls, but not in two moribund gull chicks or the Black-crowned Night-Heron. No other pathogens were isolated from the examined birds. The mercury level in the kidney of the Black-crowned Night-Heron was below the lethal level (0.3 ppm), as were PCB (8.6 ppm) and other organochlorine levels in the brain.

Type E toxin was demonstrated in the ventricular contents of two moribund adult gulls. Unfortunately, blood samples from these birds were not tested for presence of the toxin. One dead alewife found on the island was negative for type E toxin.

1981 epizootic.—On 3 November 1981, a report was received by the Illinois Department of Conservation (IDOC) of six dead Common Loons found along a 90-m section of Lake Michigan shoreline near Kenilworth, Cook Co., Illinois. Additional dead Common Loons and Herring Gulls (*Larus argentatus*) were also reported by the city of Winnetka, Illinois,

and the Metropolitan Sanitary District of Greater Chicago (MSD) on 4–6 November on a 24-km-section of Lake Michigan between Evanston and Lake Forest. A total of 69 dead Common Loons, 8 Herring Gulls, and 17 other birds (reported to be loons and gulls) was collected within this region.

An aerial survey was conducted on 17 November by IDOC to assess the distribution and magnitude of any additional mortality. About 100 km of Lake Michigan shoreline in Illinois were surveyed; in addition, a 1.6-km zig-zag pattern in open water adjacent to the shoreline was surveyed. One unidentified dead bird was observed. Live loons and diving ducks were easily visible from the aircraft.

Six Common Loons and one Herring Gull were examined at NWHL. A pooled sample of heart blood from three additional loons was also received. Types C and E toxin were found in the heart blood of three of the six Common Loons and in the pooled sample from the three additional loons. Only Type E toxin was found in blood samples from one of the six loons and one Herring Gull.

No other pathogens were isolated from carcasses examined at NWHL. PCB levels in three loons were determined independently at the Illinois Department of Agriculture Laboratory in Centralia, and were below those considered lethal (H. Shivaprasad, pers. comm.). Gross and histologic examinations of tissues failed to reveal evidence of infectious or other toxic diseases.

The presence of an oil slick in Lake Michigan off of Evanston during this die-off led to speculation that oiling may have been responsible for the deaths observed. Although one carcass found by the MSD appeared oiled, there was no evidence of petroleum residues on any of the carcasses examined at NWHL.

DISCUSSION

The presence of botulinal toxin in the carcass of a bird does not necessarily mean that the bird died from botulism. Postmortem invasion of tissues by *C. botulinum* present in intestinal tracts of birds occurs readily; toxin thus produced under anaerobic postmortem conditions could result in a false positive diagnosis. Additional evidence needed to substantiate a diagnosis of botulism includes demonstration of the toxin in blood samples from moribund birds known to be susceptible, and identification of the source of the toxin.

In the present investigations, type E toxin was demonstrated in blood from 8 of 18 Common Loon carcasses examined in 1976 and five of six loons and a Herring Gull in 1981. In 1980, one of five moribund Ring-billed Gulls had type E toxin in its blood. All three species are susceptible to experimental intoxication by type E toxin (Monheimer 1968). The negative

botulism tests among some dead birds in these studies could have resulted from: (1) insufficient amounts of toxin in samples to produce mortality in unprotected mice; (2) destruction of toxin by proteolytic activity of other postmortem invaders or destructive environmental factors (most carcasses were in poor postmortem condition); or (3) other factors as the cause of death. No other causes of mortality, however, were found in carcasses examined, other than one gull in 1976 which had a mycotic air saculitis. Negative tests in some of the moribund gulls examined in 1980 could be a result of levels of toxin too low to be detected. Fay et al. (1965) likewise found that toxin could seldom be demonstrated in blood from sick birds which remained alive for several days; they suggested this was due to low (undetectable) levels of ingested toxin. Jensen and Gritman (1966), however, found that type E toxin disappeared from the bloodstream of experimentally inoculated Mallards rapidly (by 8 h); thus birds showing clinical signs of botulism may not have detectable levels of circulating toxin.

Type E toxin was demonstrated in fish remains found in the ventriculus of a dead loon and two moribund gulls. It is possible that the toxin in the fish from the loon was formed after the bird had died; however, type E toxin in the fish remains from the moribund gulls confirms the natural ingestion of material containing toxin. Toxin from the fish remains found likely caused the observed morbidity, since clinical signs of botulism can occur in gulls within 3 h of ingestion (Kaufman and Crecelius 1967). This information adds an important link to the epizootiology of botulism type E in the Great Lakes. Although type E toxin has been demonstrated in fish carcasses from the Great Lakes (Monheimer 1968, Graikoski et al. 1970), and the feeding of dead Lake Michigan fish to captive gulls has caused botulism type E mortality (Fay 1966), observations of free-flying gulls and loons consuming material containing preformed toxin and becoming sick have not been previously reported.

With these factors in mind, we believe that mortality in loons and gulls reported herein was due to botulism type E; during the 1981 epizootic, type C toxin was also present and may have enhanced the effects of type E toxin (Jensen and Gritman 1966). To our knowledge, this is the first evidence of concomitant presence of types C and E toxin in naturally occurring mortality. The common documentation of botulism type C mortality in the Great Lakes (Herman 1964; Graikoski et al. 1970; NWHL, unpubl.) leads one to wonder whether the concurrent presence of type E toxin might be obscured during routine testing for type C toxin (Jensen and Gritman 1966). Mortality in species other than loons and gulls found during epizootics cannot be attributed to botulism type E with the present data.

Evidence suggests that botulism type E in gulls and loons is acquired through ingestion of fish containing the toxin. Since fish have been shown

susceptible to type E toxin (Huss and Eskildsen 1974), birds may have consumed intoxicated fish or fish carcasses containing toxin formed after the fish's death. The widespread presence of *C. botulinum* type E spores in Great Lakes sediments and in a variety of fish species has been well documented (Bott et al. 1964, 1966, 1968; Graikoski et al. 1970; Sugiyama et al. 1970). We would thus expect avian mortality to botulism E to be likewise widespread. The three epizootics reported here give further evidence of its widespread occurrence.

Before 1980, reports of avian mortality to botulism type E were largely limited to autumn occurrences; however, the die-off of Ring-billed Gulls in Green Bay demonstrated that it could also occur in early summer. S. Schmitt (pers. comm.) also reported summer (July) 1981 mortality of 13 loons in Lake Superior in which type E toxin was demonstrated. We do not know whether mortality occurs during other seasons, but it is possible that continual low level losses could occur unnoticed or unreported.

SUMMARY

Three outbreaks of botulism type E occurring in waterbirds on Lake Michigan since autumn 1976 are discussed. Natural ingestion of food containing type E toxin by Ring-billed Gulls (*Larus delawarensis*) and the presence of type E toxin in blood from moribund gulls were demonstrated. Concurrent presence of type C and type E botulinal toxins was found in a die-off of Common Loons (*Gavia immer*). In combination with previous reported outbreaks, these incidents suggest that this disease is geographically widespread in Lake Michigan, and that environmental conditions conducive to type E botulinal toxin production and consumption occur in both summer and autumn.

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