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AN OUTBREAK OF BOTULISM IN WATERFOWL AND FLY LARVAE IN NEW YORK STATE

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ABSTRACT: In October 1982 the death of approximately 1,500 wild ducks, mostly mallards (*Anas platyrhynchos*), and about 100 shore birds including greater yellowlegs (*Tringa melanoleuca*) was observed in the New York State Oak Orchard Wildlife Management Area. The lack of gross pathology, the signs exhibited by the moribund ducks, and the ecologic conditions indicated possible botulinal intoxication. *Clostridium botulinum* toxin type C was demonstrated in duck serum (approximately 5×10^4 mouse intraperitoneal LD_{50} of toxin per ml of serum) and in an extract from fly larvae (*Lucilia* spp.) taken from the same area (approximately 1×10^6 mouse intraperitoneal LD_{50} of toxin per gram of larvae).

INTRODUCTION

Botulism in waterfowl has been reported in North America, particularly in the western region, and in Central and South America, Europe, South Africa, Australia, and Japan (Forrester et al., 1980; Wobeser, 1981; Ono et al., 1982).

The presence of *Clostridium botulinum*, organisms and toxin type C, in the soil around aquatic areas appears to have some role in the spread of botulinal intoxication (Smith, 1978). Detection of *C. botulinum* in aquatic birds and in fly larvae, as well as the presence of larvae in the stomach contents of moribund birds, is a strong indication of intoxication of waterfowl by consumption of toxin-bearing fly larvae (Duncan and Jensen, 1976; Wobeser, 1981).

Outbreaks of avian botulism in New York State have occurred previously: an outbreak which killed over 8,000 ring-necked pheasants (*Phasianus colchicus*) at a game farm in Delmar (Albany County) was reported previously (Cheatum et al., 1957), and another outbreak of botulism involving 1,200 ducks occurred at Ellicott Creek, in the town of Amherst (Erie County) in August 1972 (unpubl. data). About 95% of these were mallards and 5% were wood ducks (*Aix sponsa*).

The present study describes an outbreak of botulism involving 1,500 wild ducks and about 100 shore birds in a wildlife management area in New York State during October 1982. *Clostridium botulinum* type C toxin was demonstrated in serum from moribund ducks and in an extract of fly larvae collected from the same area.

STUDY AREA

The Oak Orchard Wildlife Management Area is a 1,012-ha wetland site in Orleans and Genesee counties in western New York (Fig. 1). During migration over 40,000 waterfowl were in the area. The water level in several marshes in the area was purposely lowered during the summer of 1982, and there was unusually warm weather in the fall when the outbreak occurred.

Approximately 80% of the 1,500 waterfowl affected were mallards (*Anas platyrhynchos*). Others included the American wigeons (*Mareca americana*), wood ducks, pintails (*Anas acuta*), green-winged teal (*Anas carolinensis*), blue-winged teal (*Anas discors*), gadwalls (*Anas strepera*), black ducks (*Anas rubripes*), Canada geese

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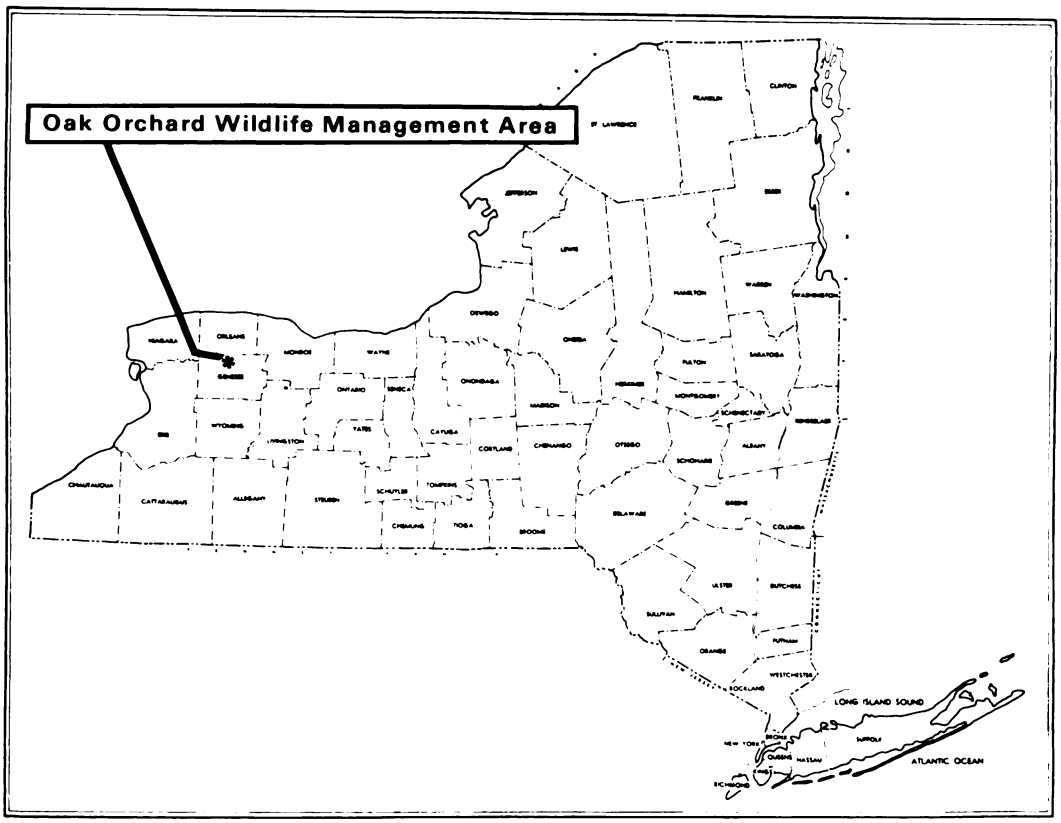

FIGURE 1. Location of outbreak of botulism in waterfowl in New York State.

(*Branta canadensis*), and about 100 shore birds, including the greater yellowlegs (*Tringa melanoleuca*).

Clinical signs varied from leg and wing weakness to flaccid paralysis. At necropsy the ducks were generally in good condition, with no specific gross pathology. The alimentary canals were usually empty, and fly larvae (*Lucilia* spp.) were found rarely.

MATERIALS AND METHODS

Samples of duck blood and feces and specimens of fly larvae were tested for toxins of *C. botulinum*. Feces and fly larvae were also cultured for *C. botulinum*.

Sample preparation: Blood samples were centrifuged for 10 min at 1,000 *g* at 5 C, and the serum was withdrawn. Feces and fly larvae were diluted 1:1 (w:v) with cold 0.2% gelatin diluent and homogenized either by inversion (feces) or by grinding with a mortar and pestle (fly larvae). The fecal homogenate was refrigerated for 18 hr at 5 C; a portion was centrifuged at 12,350 *g* for 20 min at 5 C; and the supernatant was drawn off. A portion of the fly larvae homogenate was centrifuged in the same manner for 10 min and the supernatant drawn off.

Culture: The uncentrifuged portions of the fecal and fly larvae homogenates were cultured on egg yolk agar plates and incubated at 37 C for 48 hr in an anaerobic chamber (Coy Laboratories, Ann Arbor, Michigan 48103, USA). Lipase-positive colonies were subcultured in thioglycolate broth and identified by standard biochemical and gas/liquid chromatographic methods. Since both the fly larvae and fecal homogenates had high populations of competing organisms, the homogenates were treated with alcohol and heat to reduce the number of nonsporebearing organisms as follows (Dowell

and Hawkins, 1978): 1 ml of 95% alcohol and 1 ml of the homogenate were mixed and incubated at room temperature for 1 hr. This preparation was used to inoculate four tubes of prereduced cooked meat broth medium. One tube received no further treatment; the remaining three tubes were incubated for 10 min in a 70 C water bath. Two of these tubes were then placed in an 80 C water bath for 10 min. One of the tubes heated at 80 C was then placed in boiling water for 10 min. All four tubes were cooled to room temperature, incubated anaerobically for 48 hr in a GasPak jar (BBL Microbiology Systems, P.O. Box 243, Cockeysville, Maryland 21030, USA) at 30 C, and then subcultured to egg yolk agar plates, as above. Lipase-positive colonies were identified.

Toxin test: Serum samples, supernatant from the fecal homogenate, and supernatant from the fly larvae homogenate were used directly in the toxin test. Cooked meat broth cultures of the alcohol and heat-treated homogenates were centrifuged as described above, and the supernatants were used directly in the toxin test.

Culture isolates were incubated in a GasPak jar at 30 and 37 C in the anaerobic chamber in both cooked meat broth and peptone yeast broth with 1% glucose. After 2, 7, and 14 days of growth each broth was centrifuged (12,350 *g* for 10 min at 5 C), and the supernatant was tested for toxin of *C. botulinum*.

To investigate the possible presence of any type of *C. botulinum* pro-toxins, the supernatants of the fecal and fly larvae homogenates, as well as the supernatants of the culture isolates, were trypsinized by mixing 4.5 ml of the supernatant with 0.5 ml of 1% trypsin (Difco Laboratories, P.O. Box 1058, Detroit, Michigan 48232, USA) (1:250) and incubating at 37 C for 45 min. The trypsinized samples were then tested for botulinal toxin. The toxin neutralization test was performed in mice by intraperitoneal injection of one international unit each of *C. botulinum* type A, B, C, D, E, or F, or polyvalent (ABCDEF) antitoxin (provided by the Centers for Disease Control, Atlanta, Georgia 30333, USA). Each of the seven antitoxins was injected into pairs of albino NYa:NYLAR mice (18–22 g) (provided by the New York State Health Department's Laboratory for Veterinary Science, Empire State Plaza, Albany, New York 12201, USA). Two uninjected mice were included as controls. After 30 min each set of 16 mice received one of the preparations intraperitoneally (0.2 ml of serum or 0.5 ml of extract). The mice were observed for 5 days for death or signs of botulism. Death of the unprotected mice and survival of the protected mice was considered a positive toxin test. The signs of botulism appeared in mice several hours after inoculation, and death occurred after 18 hr with individual duck serum and homogenized fly larvae, and after 2–3 days with dilutions made with fly larvae preparation or with pooled duck serum. The toxin titer was calculated on the basis of one international unit of type C antitoxin neutralizing 10^4 mouse intraperitoneal LD_{50} of type C toxin (Centers for Disease Control, 1980; Hatheway et al., 1981).

RESULTS AND DISCUSSION

Five samples of serum taken during 1 wk were examined for toxin of *C. botulinum*. Type C toxin was demonstrated in one serum pool (3 mallard and 1 wigeon) at approximately 5×10^4 mouse IP LD_{50} of toxin per ml. *Clostridium botulinum* toxin was also present in serum from a mallard but could not be typed due to insufficient sample size. Two other serum pools (from 3 mallards) and one serum specimen from an individual mallard were negative.

Preformed botulinal toxin could not be detected in the duck feces, even after trypsinization. Culture of feces for *C. botulinum* organisms was negative and remained negative after alcohol and heat treatment. The cooked meat broth cultures of the alcohol and heat-treated fecal homogenate were also negative for botulinal toxin.

Clostridium botulinum type C toxin was detected in the extract of fly larvae at approximately 1×10^6 mouse IP LD_{50} of toxin per gram. Heating of the extract for 10 min at 100 C destroyed the toxic activity.

A portion of the fly larvae extract was treated with alcohol and heat. This portion when inoculated into cooked meat broth, produced type C toxin after 48 hr of incubation. Culture of the broth yielded an organism which resembled *C. botulinum* morphologically and biochemically but which failed to produce toxin.

Recovery of a high level of toxin in maggots has also been reported by Dun-

can and Jensen (1976). They estimated that as little as 0.05 to 0.25 g of such maggots contains a lethal dose for an adult duck.

In our study the *C. botulinum* toxin was identified in serum of moribund ducks but not in their feces. This finding is in agreement with Jensen (1981), who could detect toxin of *C. botulinum* in sera of experimentally intoxicated ducks but rarely in the feces of affected ducks.

The presence of toxin of *C. botulinum* in duck serum and fly larvae in this outbreak suggests that the maggots in the area may have been contaminated with botulinal toxin. The source of *C. botulinum* organisms may have been decaying organic matter, which is a favorable anaerobic environment, or the larvae may have fed upon the carcasses of birds or other animals that died as a result of botulinal intoxication.

The outbreak ended when the carcasses were removed from the area and seasonably cool autumn weather reduced the populations of adult flies, larvae, and bacteria.

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