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ENTEROTOXEMIA OF WILDFOWL DUE TO *Cl. perfringens* TYPE C

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Abstract: Clostridium perfringens Type C has been found as a possible cause of high mortality in waterfowl and wild birds in a 12 hectare (40 acre) area of Lake Okeechobee, Florida.

INTRODUCTION

On June 29, 1971, the Audubon Warden[1] in the Lake Okeechobee area was making routine observations on Everglades kites that had migrated to the lake as their home areas became dry. He noted dead and dying ducks and wading birds in the Fish-eating Creek Bay area of the lake. The warden notified the Fishery Biologist[2] and an immediate survey was made of the area. The affected birds were confined to an area of approximately 12 hectares of stagnant water between the island and a spoil bank (see Figures 1 and 2).

A total of 398 dead birds of 19 species, identified by the Audubon Warden, were recorded, as in Table 1.

Prior to the die-off, there was a colony of 41 roseate spoonbills in the area, and, though only 26 bodies were counted, none are in the area now, leading to the belief

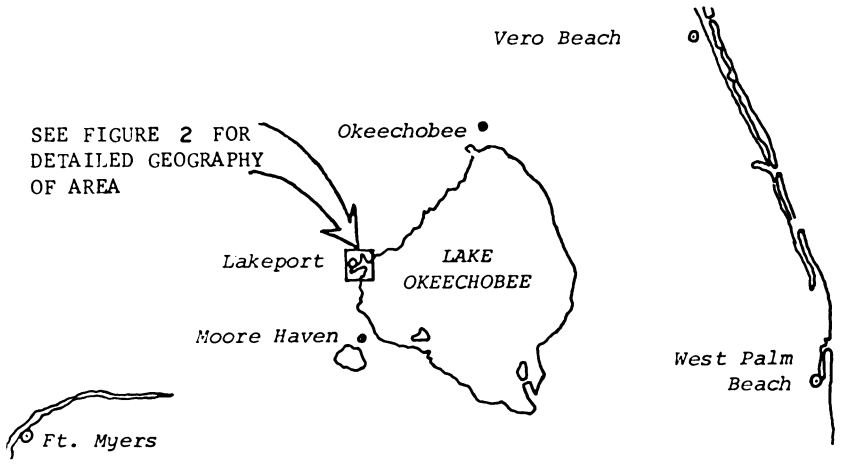


FIGURE 1. Location of Lake Okeechobee

[1] Rod Chandler, Audubon Wildlife Warden, Audubon Society
[2] Lothian Ager, Fishery Biologist, Florida State Game & Fresh Water Fish Commission

This paper was presented at the Annual Conference of the Wildlife Disease Association, Colorado State University, Fort Collins, Colorado, U.S.A., August 25-27, 1971.

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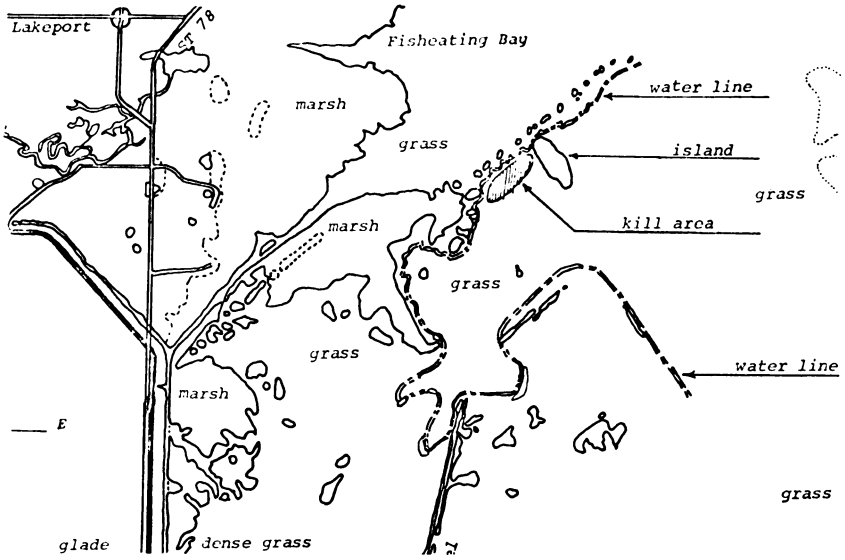


FIGURE 2. Detailed geography of waterfowl kill area.

TABLE 1. Species of birds involved*.

Species	Number
Mallard *(Anas platyrhynchos)*	184
Glossy ibis *(Plegadis falcinellus)*	58
Coot *(Fulica americana)*	35
Roseate spoonbill *(Ajaia ajaja)*	26
Black-necked stilt *(Himantopus mexicanus)*	25
Black tern *(Childonias niger)*	16
Black skimmer *(Rynchops nigra)*	16
White-faced ibis *(Plegadis chichi)*	11
Snowy egret *(Leucophoyx thula)*	6
Ring-necked duck *(Aythya collaris)*	4
Blue-winged teal *(Anas discors)*	2
Gallinule *(Gallinula chloropus)*	2
Gull-billed tern *(Gelochelidon nilotica)*	2
American egret *(Casmerodius albus)*	1
Pied-bill grebe *(Podilymbus podiceps)*	1
Dowitcher, long-billed *(Limndromus griseus scolopaceus)*	1
Great blue heron *(Ardea herodias)*	1
Louisiana heron *(Hydranassa tricolor)*	1
Lesser yellowlegs *(Totanus flavipes)*	1

* From "Complete Field Guide to American Wildlife" by Henry Hill Collins, Jr. 1959.

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that the remainder became ill and died in some other location. No Everglades kites were found dead and it is of interest that their main item of diet is a large fresh water snail of the genus *Pomaceae*. One otter was found dead in the area, but not examined to determine cause of death. There were many avocets in the area, but none were found dead. There is no conclusive evidence that all dead birds were killed by the same agent.

The last dead bird was found on July 10, 1971, at which time the water level was about 30 cm higher than on June 29 when the first dead birds were found.

Two groups of the dead and dying birds were submitted to our laboratory to determine if the loss might be due to botulism, which has been found from time to time affecting ducks, geese and swans in Florida. The two groups of birds were submitted in the last week of June and 1st week of July[2].

The first group consisted of 1 mallard, 2 glossy ibis, 1 coot and 1 mature black stiltneck. The second group consisted of 2 skimmers, 4 glossy ibis, 3 mallard, 2 black terns and 1 black stiltneck. A few of the birds were dead on arrival. Live birds were very lethargic, showing symptoms of weakness in legs and neck. All showed considerable depression, but, when aroused, some of the birds could walk and attempted to fly. Typical limberneck, as seen in botulism, was not in evidence. The living birds were killed and at necropsy materials were obtained for chemical, parasitologic, histopathologic and bacteriologic examinations.

PRELIMINARY FINDINGS

Necropsy revealed considerable congestion of subcutaneous tissue, lungs and liver. Most of the birds had a mucoid necrotic enteritis.

The biological fly test[3] applied to a composite specimen of liver and intestines was negative for insecticides. Direct smear examination of intestinal material from all birds was negative for parasite ova.

Histopathologic examination of various tissues revealed acute hepatitis, hemorrhagic congestion of the lungs with some areas of focal lymphocytic infiltration, and acute hemorrhagic enteritis.

Aerobic cultures of the heart, liver and lungs on differential media showed the presence of *E. coli, Proteus,* gram-positive non-hemolytic cocci and *Pseudomonas* sp. from some of the tissues. Intestinal cultures revealed a predominance of *E. coli, Enterobacter* sp., and a few colonies identified as *Edwardsiella tarda,* but no *Salmonella* organisms.

MATERIALS, METHODS AND RESULTS

Since botulism was suspected, a composite of heart blood, liver and intestinal tissue from the first group of birds was macerated in a mortar and pestle with alundum[4] and sterile physiological saline in equal proportions. The resulting slurry obtained was then centrifuged at 6000 rpm for 30 minutes. The supernatant fluid from this was re-centrifuged at 6000 rpm for another 30 minutes.

This clarified supernatant fluid was then combined at a ratio of 4 parts to 1 part each of *Cl. botulinum* antitoxins A, B, C, D, E, and F[5]. Simultaneously, a small amount of the supernatant fluid was boiled for 5 minutes.

After the mixtures of antitoxin and supernatant fluid had been allowed to react for 1 hour at room temperature, mice were inoculated intraperitoneally with 0.5 ml quantities of each. All mice were dead within 18 hours, except those injected with the boiled preparation (see Table 2). Examination of gram-stained preparations of the sediment showed a high concentration of organisms resembling *Cl. perfringens.* As a result, further

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[3] Kuhns, L. J. Insecticide biotest. Personal communication. Animal Disease Laboratory, Kissimmee, Florida.

[4] Alundum-Norton Co., Worcester, Mass.

[5] From NCDC, Atlanta, Ga.

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TABLE 2. Mouse Neutralization Test Results

Bird species	*Cl. botulinum* antiserum A	B	C	D	E	F	Heated control	*Cl. perfringens* antiserum A	B	C	D	E	Serum control	Results
Composite tissues, 1st group	D	D	D	D	D	D	S	D	S	S	D	D	D	Protected by Types B & C *Cl. perfringens* antiserum
Skimmer	D	D	D	D	D	D	S	D	D	S	D	D	D	Protected only by Type C *Cl. perfringens* antiserum
Black tern	D	D	D	D	D	D	S	D	S	D	D	D	D	Protected only by Type B *Cl. perfringens* antiserum
Stiltneck	S	S	S	S	S	S	S	S	S	S	S	S	S	Non-toxic
Mallards	D	D	D	D	D	D	S	D	S	S	D	D	D	Protected by Types B & C *Cl. perfringens* antiserum
Glossy ibis	D	D	D	D	D	D	S	S	S	S	D	D	D	Protected by Types A, B & C *Cl. perfringens* (probably Type C)

D = Dead
S = Survived

combinations of the supernatant fluid with *Cl. perfringens* antiserum[6] Types A, B, C, D, and E in the ratio of 4 parts to 1 part antisera were made. After antiserum neutralization for 1 hour at room temperature, mice were inoculated intravenously with 0.4 ml quantities of these mixtures. Mice protected with Type B and C antiserum survived. Those receiving Types A, D & E and the control antisera died, indicating that the toxin present was produced by Type B or C *Cl. perfringens.*

Smears prepared from the original tissues were stained with fluorescein isothiocyanate conjugates of *Cl. botulinum* Types A, C & E[6] antisera, and *C. perfringens* Types A, B, C, D & E[7], as well as *Cl. septicum, Cl. hemolyticum* and *Cl. novyi* Types A and B. A faint fluorescence was noted in a few preparations stained with fluoresceinisothiocyanate-conjugated Type E *Cl. botulinum* antiserum.[5] Significant fluoresence was noted with the *Cl. perfringens* conjugates. No fluorescence was observed in preparations stained with *Cl. septicum, Cl. hemolyticum,* and *Cl. novyi* A & B conjugates.

The second group of birds received were treated in the same manner except that the individual tissues were kept separate. Table 2 shows the results obtained on mouse inoculation.

Composite tissue specimens from the birds submitted were inoculated into chopped meat medium.[5,10,11] After 24 hours incubation at 35 C, the predominant organisms microscopically resembled *Cl. perfringens* and *Micrococcus* sp. Subcultures were heated at 80 C for 20 minutes and again incubated. The major-

[6] Burroughs-Wellcome (USA), Tuckahoe, N.Y.
[7] Sylvana Co., Milburn, N.J.

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ity of the organisms in these subcultures morphologically resembled *Cl. perfringens*.

These subcultures were then streaked on 5% sheep blood agar plates, overlaid with trypticase soy agar and incubated in Brewer anaerobe jars overnight at 35 C. From these plates, double-zoned beta hemolytic lenticular colonies were selected and transferred to chopped meat medium. Twenty subcultures were tested by neutralization procedures. Of this number, three isolates were characterized as Type C *Cl. perfringens* by neutralization tests.

After centrifugation of the cultures (at 6000 rpm for 60 minutes), trypsin[8] was added to the clarified supernatant fluid to a final concentration of 0.25%. The mixture was then heated at 60 C for 1 hour and brought to neutral pH. This material was found to be non-toxic for mice when quantities of 1.0 ml were injected intravenously, confirming the presence of Type C *Cl. perfringens*.

Biochemically these organisms showed beta hemolysis and elaborated a lecithinase by production of opacity on egg yolk agar. Acid was produced in dextrose, lactose, maltose and sucrose; salicin was not fermented. Indol was not produced, nitrate was reduced to nitrite and H_2S was weakly produced after prolonged incubation. In litmus milk, the organism produced stormy fermentation and subsequent peptonization.

Subcultures of the isolates made were referred to Dr. Lillian Holdeman at the Virginia Polytechnic Institute Anaerobic Laboratory, Blacksburg, Virginia, and there they were confirmed as typical Type C *Cl. perfringens*. All cultures were negative for *Cl. botulinum*.

DISCUSSION

C. perfringens Type C was probably first described as a toxigenic microorganism by McEwan and Roberts,[7] in a disease of adult sheep known as "Struck". The organisms first found in the intestines later invaded other body tissues after death. The toxin produced in the intestine caused rapid death through absorption of the beta toxin. In some cases, the only symptom noted was the final tetanic spasms.

Griner and Bracken[3] reported a rapidly fatal hemorrhagic enteritis of young calves due to growth of *Cl. perfringens* Type C in the intestines. The toxemia caused rapid prostration and death in 2 to 4 hours without diarrhea. Although first seen in the western U.S.A. in calves and lambs, the disease is now considered widespread and is often found in young feedlot cattle.

A rapidly hemorrhagic necrotic enteritis of young pigs in Minnesota was described by Barnes and Moon[1] and was attributed to a toxemia by *Cl. perfringens* Type C. These pigs died within a few hours and exhibited diarrhea with a typical scalding of the perineal area.

Parish[6] reported a necrotic enteritis of chickens associated with severe liver lesions in the form of focal areas of degenerative necrosis from which he isolated *Cl. perfringens*, classified as Type F. Brooks *et al.*[2] regarded *Cl. perfringens* Type F as a probable subtype of *Cl. perfringens* Type C.

A necrotic enteritis of broiler chickens in Western Australia has also been described by Nairn.[5] This disease is characterized by rapid death in 2 to 7 week-old broilers showing a yellowish-brown necrotic enteritis of the lower intestine. Isolates of the organisms involved have been tentatively identified as Type C *Cl. perfringens*.

It is evident that *Cl. perfringens* Type C has been associated with enteric disease of domestic livestock and poultry, and from findings reported herein, that it can be the cause of considerable losses in wildfowl populations as well. No attempt has been made to locate the source of the infection.

Since many toxemias of waterfowl and

[8] Difco, Detroit, Mich.

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birds showing similar symptoms (limberneck, weakness, depression) have been clinically diagnosed as botulism, it may be well to investigate such losses further by neutralization of blood and intestinal contents with the antisera of the *Cl. perfringens* group as well as mouse prottection tests for botulism.

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