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Source: Journal of Wildlife Diseases, 19(2): 90-94

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-19.2.90

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# AVIAN BOTULISM DURING LATE AUTUMN AND EARLY SPRING IN SASKATCHEWAN

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ABSTRACT: Two outbreaks of botulism in central Saskatchewan in which mortality of waterfowl continued into late autumn and then recurred in the same marshes the following spring are described. Small numbers of birds were involved in each instance. Dabbling ducks (predominantly mallards, *Anas platyrhynchos* and pintails, *Anas acuta*) and American coots, *Fulica americana* were affected most commonly in autumn; whereas only diving ducks (predominantly lesser scaup, *Aythya affinis*) were found to be involved in spring. Live maggots present in carcasses despite sub-freezing temperatures were the probable source of intoxication in the autumn; the source of toxin in the spring was not determined.

# INTRODUCTION

Botulism is an important disease of wild waterfowl, particularly in western North America. The disease is generally a warm weather phenomenon usually "appearing after the hottest part of summer and continuing into the cooler days preceding fall" (Enright, 1971). Biologists engaged in disease control look forward to cool autumn weather and a cessation of mortality. Outbreaks of botulism have been reported in autumn (Bossenmaier et al., 1954), winter (Rosen and Cowan, 1953; Haagsma, 1973; Graham et al., 1978; Parrish and Hunter, 1979), and spring (Wetmore, 1918; Jensen and Williams, 1964), but these occurrences are "unpredictable, seldom recorded, and remain little understood" (Enright, 1971).

This paper describes two botulism outbreaks in Saskatchewan that continued after the occurrence of freezing overnight temperatures and in which mortality occurred early in the following spring.

#### MATERIALS AND METHODS

Axe Lake and Rice Lake marsh are shallow, flatbottomed freshwater wetlands with abundant emergent vegetation located approximately 135 km southeast and 30 km southwest of Saskatoon, respectively. Axe Lake is approximately 100 ha in area, the Rice Lake marsh contains about 50 ha. Both marshes have dams on their outlet to control water levels; however, because of drought conditions in 1981, water levels were very low and extensive exposed mudflats were present on both areas. Only about 0.5 ha of water remained at Rice Lake marsh in October.

Botulism was diagnosed using serum collected from live birds with clinical signs of paralysis in a mouse protection test (Wobeser, 1981). The toxicity of maggots collected from waterfowl carcasses and of tissue from a waterfowl carcass found in the spring was assessed by intraperitoneal injection of mice with serially diluted suspensions of maggot or tissue material, as outlined by Duncan and Jensen (1976). Physiologic saline rather than phosphate buffer was used as a diluent. Only two mice were used per dilution so that the minimum lethal dose (MLD) calculated is only an approximation. Flies were reared for identification from larvae and pupae by methods previously described for fleshflies (Wobeser et al., 1982).

Weather records from the weather stations nearest to the two sites were examined (Wynyard in the case of Axe Lake, Saskatoon for Rice Lake); the values from the Saskatoon weather station are used here for both sites, because there were no major differences between the stations during the period considered.

#### RESULTS

Disease was first recognized in waterfowl at Axe Lake on 24 August 1981 and the marsh was inspected on 28 August. Sera collected from three mallards (Anas platyrhynchos) and a pintail (Anas acuta) on that date, as well as sera from two mallards, a pintail and a northern shoveler (Anas clypeata) collected on 29 August contained toxin of Clostridium botulinum type C. All of these birds were showing signs of intoxication. An intensive cleanup of carcasses was conducted on 30 August, during which approximately 1,240 dead birds were collected and buried. Mortality appeared to be very low following this cleanup and very few sick birds were evident on 7 September, so disease control efforts were diverted elsewhere.

Received for publication 27 September 1982.

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On 6 October a hunter reported many dead birds on Axe Lake. The marsh was visited on 9 October at which time the desiccated skeletons of many ducks that had been dead for several weeks were found on the mudflats around the shore. During a circuit of the shore, only two recently dead birds were found on the shore. One of these was being fed on by a bald eagle (Haliaetus leucocephalus). A small portion of the marsh was examined from a canoe, and many dead waterfowl were found floating among the emergent vegetation. These ranged from recently dead birds to extensively scavenged carcasses. The flesh of these birds, even those that appeared to have been dead for several days, was well preserved. Live maggots were present in many of the carcasses, and in one instance, live wriggling maggots were found floating within a 0.5 m radius of a carcass. The distribution of dead birds did not appear to be random and clusters of carcasses in various states of decomposition were found. Five partially paralyzed birds (three mallards, a pintail and an American coot [Fulica americana]) were captured; blood samples were collected and the birds were necropsied. Botulinum toxin was found in serum from these birds. The birds were in good body condition and no gross lesions were found. A pooled sample of maggots collected from several carcasses was found to contain approximately 5,000 MLD of botulinum toxin per gram. The number of dead and sick birds on Axe Lake was not determined because inclement weather, including freezing of the marsh on 9 October, prevented a carcass cleanup.

On 19 October 1981 dead ducks were reported on Rice Lake marsh. A preliminary check was made by Department of Tourism and Renewable Resources (DT&RR) personnel, and one paralyzed green-winged teal (Anas crecca) was collected. On 20 October, personnel from DT&RR and Canadian Wildlife Service went to the marsh with the intention of collecting and destroying all carcasses. However, this was found to be impractical because most of the carcasses were among the dense vegetation on the exposed mudflats. The small number of live birds in the area and the formation of ice on the small area of water remaining in the marsh also mitigated against a major cleanup campaign. A mallard that was in extremis and was killed and a paralyzed green-winged teal were captured. Carcasses of 10 ducks of various species were collected for examination. Live maggots were present in nine of these; the other, a desiccated specimen, contained only a few pupae. A pooled sample of these maggots contained approximately 5,000 MLD of toxin per gram. Flies reared from the maggots and pupae were identified as *Phormia regina* (Meigen) and *Bufolucilia silvarum* (Meigen).

On 21 October the marsh was revisited. At this time there was about 1 cm of snow on the ground, the mid-afternoon air temperature was -2 C and more than half of the water area was frozen. The temperature of the water was 0.5 C. Approximately 40 mallards, 25 American coots, one green-winged teal and one pintail were on the open water. The pintail, greenwinged teal and six of the American coots had difficulty in taking off or were unable to fly. The green-winged teal and four of the American coots were captured.

Several hundred carcasses, predominantly mallards, pintails and green-winged teal, were present in the portion of marsh examined. The birds appeared to have died over a period of weeks, with the majority of carcasses being only desiccated skeletal and plumage remnants. The few carcasses that had flesh remaining also contained live maggots. The temperature within or under three such carcasses was 0.5, 1.0 and 3.0 C, respectively.

Toxin was demonstrated in serum from the green-winged teal collected on each of 19, 20 and 21 October, but not in that of the three American coots tested. (The mallard collected 20 October was not tested.) All of these birds were in good condition and no gross lesions were observed at necropsy.

The weather became much colder immediately after this visit and then moderated in early November (Fig. 1). On 7 November, a small portion of the water area that had been frozen on 21 October was open and 12 mallards were present. No sick or recently dead birds were found, but one desiccated carcass that had a small amount of muscle in the shoulder region contained more than 50 live maggots. These were sluggish in the field, but became active when warmed.

The minimum daily temperature did not rise above 0 C again until 29 March 1982 and the soil temperature remained at or below 0 C until 12 April (Fig. 2).

On 28 April, Rice Lake marsh was examined

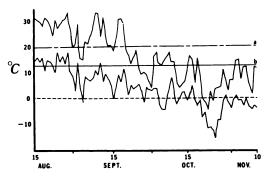


FIGURE 1. Daily maximum (upper line) and minimum (lower line) temperature at Saskatoon, Saskatchewan 15 August to 10 November 1981. The minimal temperatures for toxin production by *Clostridium botulinum* type C suggested by Segner et al. (1971) (a) and Haagsma (1973) (b) are shown. Weather data from Environment Canada (1981).

with the intention of removing any carcasses from the previous fall that still contained flesh. Four recently dead birds (2 lesser scaup, Aythya affinis, 1 each of canvasback, Aythya valisineria and common goldeneve, Bucephala clangula) and five live birds that were unable to fly (1 canvasback, 3 lesser scaup and a Franklin gull, Larus pipixcan) were observed and collected. A number of carcasses from the previous autumn, most of which had little flesh, were also collected. The sick birds were paretic or had flaccid paralysis. Botulinum toxin was demonstrated in serum from all the live birds. The water temperature was not measured on 28 April, but the following day the temperature was 12.5 C.

No live maggots were found in the carcasses that remained from the previous autumn, but intact dead maggots were present. These were not tested for toxin, but muscle tissue collected from one carcass contained at least 100 MLD of toxin per gram.

On 2 May the marsh was revisited and four birds that were unable to fly were observed. Toxin was present in the serum of the three birds captured (2 lesser scaup and a bufflehead, *Bucephala albeola*); the fourth bird, a common goldeneye, was not captured.

On 4 May a cleanup of carcasses from the previous autumn was undertaken at Axe Lake. A total of 263 carcasses was found; of these 117 had flesh remaining and were collected. Twelve birds (10 lesser scaup, 1 ring-necked duck, Ay-

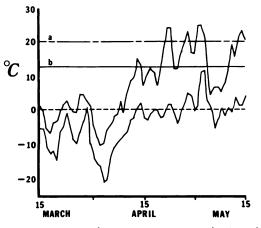


FIGURE 2. Daily maximum (upper line) and minimum (lower line) temperature at Saskatoon, Saskatchewan 15 March to 15 May 1982. Symbols as in Figure 1. Weather data from Environment Canada (1982).

thya collaris, 1 American coot) that were unable to fly were observed and three recently dead lesser scaup were found. Three of the scaup were not captured; the other birds were weak or had flaccid paralysis. The five most severely affected birds were killed and serum was collected. Botulinum toxin was present in the serum of all the birds. These birds plus the scaup found dead were in good body condition with no gross lesions visible. The only food items in the gizzard were seeds. The remaining live birds were held in captivity, given water by stomach tube until they drank voluntarily and released after 5 days, when they had regained the ability to fly. Botulism was not recognized on either marsh later in the spring and summer.

## DISCUSSION

These outbreaks of botulism were minor when compared to those that occur in summer in many areas and to the large winter outbreak in California described by Parrish and Hunter (1979); however, they may provide useful epizootiologic information. It appeared that the disease had been causing mortality at both sites for some weeks prior to detection. One possibility that must be considered is that the sick and recently dead birds found in late autumn were birds that had consumed toxin much earlier and survived for an extended period. This is unlikely as the birds necropsied were in good body condition and had no evidence of chronic illness. Predators and scavengers, including bald eagles, were active at both marshes and would probably have removed debilitated birds rather rapidly.

There is general agreement that toxin formation by C. botulinum type C proceeds best at warm temperatures, but the minimum temperature at which toxigenesis can occur is not well defined. Segner et al. (1971) found that a temperature of 20-23 C was required. Haagsma (1973) reported that three of five strains of the organism tested produced toxin at 12.5 C, although the amount of toxin produced was relatively small and production was delayed at this temperature. At 15 C all eight strains of the organism tested by Haagsma (1973) produced detectable toxin within 15 days. These suggested minimal temperatures for toxigenesis are shown in relation to the ambient temperature during the autumn of 1981 and spring of 1982 in Figures 1 and 2. Several factors must be considered in extrapolating laboratory data to the field situation. One is that the microclimate within a duck carcass exposed to the sun may be considerably warmer than that of the air, so that toxigenesis might occur even at cold air temperatures. Another factor is that the laboratory studies were based on constant rather than fluctuating temperatures. Haagsma (1973) reported that toxin was not detectable until after 56 days incubation at 12.5 C. The minimum daily temperature was consistently below that level after 10 September (Fig. 1), so that this temperature would be reached only for a portion of some days prior to 19 October and was not reached again after that date until 2-3 November.

Rosen and Cowan (1953) and Parrish and Hunter (1979) suggested that winter botulism outbreaks were due to persistence of toxin formed during warm weather, but the method of transfer of this toxin to birds was not identified. Haagsma (1973) and Graham et al. (1978) have demonstrated that toxin can persist under field conditions for at least several months in cool weather. Scavenging invertebrates, particularly larvae of blowflies, are generally accepted as a major method of transfer of toxin from carcasses to healthy birds during summer outbreaks (Hunter, 1970; Duncan and Jensen, 1976). Our observations suggest that live toxinbearing maggots can persist in carcasses despite freezing temperature and probably represent the source of toxin for continuation of outbreaks into late autumn. The carcass may provide some protection against the cold, but the temperatures recorded within three carcasses on 21 October suggest that this effect is not great. The live maggots found on 7 November are particularly interesting in that the maximum daily temperature did not rise above freezing on 21, 22 and 24 October and the soil was frozen to at least 10 cm depth on 23 and 25 to 27 October (Environment Canada, 1981). The temperatures reported here are lower than those during the botulism outbreak in California (Parrish and Hunter, 1979), in which the minimum temperature was -6 C.

Toxin-bearing maggots become non-toxic at the time of pupation (Duncan and Jensen, 1976) and carcasses decompose rapidly at warm temperatures. Cold temperature may preserve both the toxin-bearing carcasses as well as inhibit development, pupation, and hence detoxification of the fly larvae. It seems probable that toxinbearing carcasses could be preserved over winter and, if repopulated by maggots, could initiate an outbreak the following spring.

In the present cases, no source of toxin was detected in the spring. Live maggots were not found in any of the carcasses from the previous autumn, and the few recently-dead birds had not been populated by fly larvae. Dead maggots, presumably from the previous autumn, were present within some of the "old" carcasses, and toxin was present within the flesh collected from one such carcass. One possibility (suggested by J. Bowman, Ducks Unlimited [Canada]) is that birds may have ingested toxinbearing maggots that had sunk to the pond bottom. The ducks affected in the spring were entirely diving species, whereas dabbling ducks comprised over 90% of the affected birds identified during the autumn outbreaks, although both types of ducks were present in both spring and fall.

#### ACKNOWLEDGMENTS

We thank the field staff of Ducks Unlimited (Canada), Saskatchewan Department of Tourism and Renewable Resources, and Canadian Wildlife Service for help with various parts of this investigation. P. Mason, Department of Biology, University of Saskatchewan identified flies reared from maggots. 94 JOURNAL OF WILDLIFE DISEASES, VOL. 19, NO. 2, APRIL 1983

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