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EFFECTS OF BOTULISM ON DUCKS DRINKING SALINE WATER

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ABSTRACT: Mallard (*Anas platyrhynchos*) ducklings (2 wk old) were given water from natural saline wetlands or fresh water as drinking water for 1 or 2 wk prior to, and after, receiving material containing *Clostridium botulinum* type C toxin. Water with conductivity ranging from 3,460 to 6,690 μmhos/cm had no detectable effect on the occurrence or severity of clinical signs of botulism. Ducks drinking water with conductivity of 7,130 μmhos/cm for 1 wk prior to receiving toxin had more severe clinical signs and greater mortality than did birds drinking fresh water. Ducks given the same water for 2 wk prior to receiving toxin did not differ from the controls in response to toxin. Fewer ducks in groups drinking the most saline water tested (conductivity = 13,500 μmhos/cm) had clinical signs of botulism than in groups drinking fresh water.

Key words: Mallard, *Anas platyrhynchos*, saline water, botulism, interactions, experimental, *Clostridium botulinum* type C.

INTRODUCTION

Botulism caused by *Clostridium botulinum* type C in waterfowl has been associated with saline wetlands for many years. "Alkali poisoning" was proposed as a cause for the disease (Wetmore, 1918) prior to discovery of its bacterial etiology by Kalmbach and Gunderson (1934). Shaw (1929) reported that ducks with botulism had elevated plasma chloride levels. Cooch (1964) suggested that birds drinking saline water may die of a sublethal dose of botulinum toxin because of impaired function of the salt gland, with resulting inability to control plasma osmolarity. This appears reasonable as the avian salt gland is stimulated by the release of acetylcholine (Hokin and Hokin, 1959) and botulinum toxin is thought to act presynaptically by preventing release of acetylcholine (Simpson, 1984). This paper reports observations on the effects of botulinum toxin in ducks drinking water from several Saskatchewan saline wetlands.

MATERIALS AND METHODS

Toxicity trials

Mallard (*Anas platyrhynchos*) ducklings received the day after hatching from a commercial supplier (Whistling Wings, Hanover, Illinois 61041, USA) were raised in a large indoor pen for 14 days. Infrared heat lamps were used, and Saskatoon city tap water (conductivity < 400 μmhos/cm) and commercial duck starter containing 0.5% salt (Federated Cooperatives Ltd., Saskatoon, Saskatchewan, Canada S7N 1Z3) were supplied ad libitum. On day 15, birds were weighed, marked with a numbered tag and assigned randomly to either a control or principal group. Each group of 20 birds (Table 1) was placed in an individual indoor pen with an area of 4.4 m. Birds in control groups continued to receive Saskatoon city tap water, while birds in the principal groups received water collected immediately prior to the trials from saline wetlands near Saskatoon (Tables 1, 2). On day 22, 10 ducks from each group were weighed, marked with a leg band and given material containing type C *Clostridium botulinum* toxin by esophageal intubation. The toxic material was an aqueous suspension of fly maggots collected from duck carcasses during a natural outbreak of botulism at Eyebrow Lake, Saskatchewan (50°57'N, 106°10'W) in 1984. The maggots had been held at −20 C between collection and use, and were ground with approximately four volumes of water in a blender to produce a suspension that contained approximately 15,000 mouse minimum lethal doses (MLD) of type C botulinum toxin/ml, as determined by the method of Duncan and Jensen (1976). Each bird was given 0.5 ml of the suspension (approximately 7,500 MLD toxin)/kg body weight (Table 3). Birds given toxin (the "1" subgroup within each group) were then returned to the pen with the remaining birds in the group and observed several times daily for clinical signs of botulism (paresis, ataxia, paralysis) during the next week. At the end of 1 wk the birds surviving among those given toxin were killed and the other 10 birds in each group (subgroup "2") were weighed, given toxin at the same dosage rate (0.5 ml/kg), and observed for 1 wk. Because of space limitations, the trial was conducted in two parts sequen-

TABLE 1. Experimental groups of mallard ducks, containing 10 mallards each, used in experimental trials to determine effect of saline water on botulism.

Group	Water source (location)	Weeks on saline water prior to receiving botulinum toxin
Control 1	Saskatoon city tap water	0
Control 2	Saskatoon city tap water	0
A1	Wetland A (51°55'N, 106°16'W)	1
A2	Wetland A (51°55'N, 106°16'W)	2
B1	Wetland B (51°54'N, 106°16'W)	1
B2	Wetland B (51°54'N, 106°16'W)	2
Control 3	Saskatoon city tap water	0
Control 4	Saskatoon city tap water	0
C1	Wetland C (52°14'N, 106°22'W)	1
C2	Wetland C (52°14'N, 106°22'W)	2
D1	Wetland D (51°56'N, 106°12'W)	1
D2	Wetland D (51°56'N, 106°12'W)	2
E1	Wetland E (51°55'N, 106°12'W)	1
E2	Wetland E (51°55'N, 106°12'W)	2

tially. Water from wetlands A and B was tested with control groups, and then water from wetlands C, D and E was tested with additional control groups.

The Student's t-test was used to test differences between means for weight, weight gain and dose of toxin, and a Chi-square test was used to test differences in occurrence of clinical signs or death among groups. Statistical significance was established at $P \leq 0.05$.

Serum osmoregularity determination

Eight 5-wk-old ducks that had received tap water since hatching were given water from wetland A as drinking water for 1 wk. On day 7, 1 ml of blood was collected from the brachial vein of each duck, and then four of the birds were given toxin at the dosage rate used in prior trials. One or more additional blood samples were collected from these birds when they had severe clinical signs of botulism (stage II or III, as defined by Hunter et al., 1970). A second blood sample was also collected after 96 hr from each of the four ducks that had not received toxin. Serum was separated from these samples and osmolality of the serum was determined (Osmometer model 3W11, Advanced Instruments Inc., Needham Heights, Massachusetts 01294, USA) and serum total protein was measured (American Optical Goldberg Refractometer, Fisher Scientific Company, Edmonton, Alberta, Canada T5B 1S3).

Effect of saline water on intestinal passage

Ducks drinking saline water pass voluminous fluid excreta (Mitcham and Wobeser, 1988a, b), and it was thought that rapid intestinal passage might influence absorption of toxin. Ten 5-wk-old ducks reared on tap water were divided into two equal groups. Five birds continued to receive tap water; the other five received water from wetland B. After 1 wk, the birds were transferred to individual 1 × 1-m wire-bottomed cages and allowed to acclimate to these cages for 2 days, while continuing to receive either tap water or water from wetland B. On

TABLE 2. Chemical and physical characteristics of water used in experimental trials.[a]

Wetland	mg/liter							Conductivity (μmhos/cm)
	Na	Mg	K	Ca	Cl	CO_3	SO_4	
Saskatoon city tap water	22	14	3	32	7	—	—	<400
Wetland A	775	550	53	442	265	26	4,540	7,130
Wetland B	2,260	950	85	538	622	24	9,040	13,500
Wetland C	866	222	112	223	1,990	74	603	6,690
Wetland D	538	510	95	327	120	—	3,500	5,700
Wetland E	276	324	118	119	99	110	1,900	3,460

[a] Analyses by Analytical Laboratory, Saskatchewan Research Council, Saskatoon, Saskatchewan, Canada S7N 0W0.

TABLE 3. Body weight, weight gain, while exposed to saline water, and toxin dose administered to ducks in experimental trials.

Group	Exposure to saline water (wk)[a]	Weight (g)[b]		Weight gain (%)[c]		Toxin dose (mouse MLD)	
		$\bar{x}$	SD[d]	$\bar{x}$	SD	$\bar{x}$	SD
Control 1	0	634	46	59.0	6.6	4,755	340
A1	1	614	48	57.9	6.5	4,605	360
B1	1	552**	56	41.5**	9.8	4,140**	420
Control 2	0	715	66	91.6	11.3	5,360	500
A2	2	726	66	84.1*	10.9	5,445	550
B2	2	553**	74	40.8**	12.6	4,140	570
Control 3	0	554	45	56.8	5.0	4,155	336
C1	1	556	61	61.1*	8.6	4,170	454
D1	1	526	58	56.3	6.5	4,100	265
E1	1	547	35	58.7	10.5	3,945	435
Control 4	0	714	59	124.0	14.2	5,355	445
C2	2	714	69	106.9	18.9	5,355	515
D2	2	704	64	111.8	15.3	5,280	478
E2	2	670	46	113.2	13.4	5,025	344

[a] Weeks prior to being given toxin.
[b] Weight at time toxin was given.
[c] Change in body weight during time receiving saline water prior to receiving toxin, i.e., 1 or 2 wk.
[d] Standard deviation of the sample mean.
* Significantly different from control ($P < 0.05$).
** Significantly different from control ($P < 0.01$).

day 10, each bird was given 10 ml of a 2% aqueous suspension of chromic oxide by esophageal intubation as a method of measuring intestinal passage. Excreta passed by each bird was collected each hour for 10 hr, weighed, dried, reweighed and analyzed for content of chromic oxide by the method of Fenton and Fenton (1979).

RESULTS

Toxicity trials

The saline waters used in these trials were within the sublethal range of salinity for mallard ducklings (Mitcham and Wobeser, 1987a, b), but ducks given water from the two most saline wetlands gained weight at a significantly lower rate than did the control birds (Table 3).

Some birds within each group developed clinical signs of botulism after receiving toxin, but in only two instances was the frequency of occurrence of clinical signs different from that of the appropriate control group (Table 4). Ducks that received water from wetland B (the most highly saline water tested) had a significantly lower incidence of clinical signs of botulism than did ducks in control groups 1 and 2 ($\chi^2 = 46.94$, $P < 0.01$). Ducks given water from wetland B had droplets of fluid about the external nares both prior to and after receiving toxin, indicating that salt gland secretion was occurring and that botulinum toxin did not inhibit secretion completely.

Significantly more ducks died within the group given water from wetland A for 1 wk prior to intoxication, than within control group 1 ($\chi^2 = 20.25$, $P < 0.01$). The former birds had obvious salt gland secretion about the nares prior to and after receiving toxin. The birds that survived within this group also had more severe clinical signs than did the controls. During the course of the trial each of the survivors in group A1 had severe ataxia/paresis and was unable to walk more than a few steps. In contrast, eight of the nine affected birds in control group 1 had only mild transient ataxia. Ducks given water from wetland A for 2 wk prior to intoxication did not

have obvious salt gland secretion, and the mortality and the severity of clinical signs in this group did not differ from that of control group 2.

Serum osmoregulation

Serum osmolality was not significantly different in ducks given water from wetland A together with toxin and birds given the same water without toxin ($\bar{x}$ = 316.5, SD = 8.7 mM/kg; $\bar{x}$ = 317.7, SD = 6.7 mM/kg; respectively), although the former had severe signs of botulism. The serum protein concentration was significantly higher in the intoxicated birds than that in the nonintoxicated birds ($\bar{x}$ = 51.2, SD = 3.3 g/liter; $\bar{x}$ = 46.1, SD = 4.5 g/l; respectively).

Intestinal passage

Ducks given water from wetland B passed significantly more excreta than did the ducks given fresh water on both a wet weight ($\bar{x}$ = 194.3, SD = 76.9 g versus $\bar{x}$ = 57.7, SD = 17.3 g) and dry matter basis ($\bar{x}$ = 9.8, SD = 3.4 g versus $\bar{x}$ = 2.8, SD = 0.5 g). During the 10-hr period, ducks given saline water and those receiving fresh water passed approximately 70% of the administered dose of chromic oxide. However, a much greater proportion of the dose was passed during the first 3 hr by the ducks given saline water (27.8, 36.2 and 51.8%) than by those given tap water (0.1, 8.4 and 22.6%, respectively).

TABLE 4. Occurrence of clinical signs of botulism and of death among ducks exposed to various types of water and given type C botulinum toxin.

Group	Exposure to saline water prior to toxin (wk)	Clinical signs[a]	Death[b]
Control 1	0	9/10	0/10
A1	1	10/10	5/10*
B1	1	2/10*	1/10
Control 2	0	8/10	0/10
A2	2	10/10	1/10
B2	2	1/10*	0/10
Control 3	0	5/10	0/10
C1	1	3/10	1/10
D1	1	2/10	0/10
E1	1	6/10	0/10
Control 4	0	3/10	0/10
C2	2	4/10	1/10
D2	2	3/10	0/10
E2	2	3/10	1/10

[a] Number with clinical signs of botulism/number in group.
[b] Number dead/number in group.
* Significantly different from appropriate control group (χ^2, $P < 0.01$).

DISCUSSION

The results of these trials differ markedly from those reported by Cooch (1964); however, differences in experimental design make direct comparison difficult. Cooch (1964) used mallards and pintails (*Anas acuta*) of unspecified age (presumably adult) that were given botulinum toxin either orally or by intraperitoneal injection, simultaneously with one or more oral doses of highly saline water (5 or 10% NaCl, or water from a hypersaline wetland with conductivity of about 71,000 μmhos/cm). The prior history of the birds was not specified, so it is unclear if they had "activated" salt glands at the time they were challenged with toxin and salt. A standard dose of botulinum toxin was given to all birds, and this was not related to the body weight of the birds. Under these experimental conditions, ducks given salt water developed clinical signs of botulism more rapidly, and had greater mortality than did those given the same amount of toxin, but no salt. Cooch (1964) concluded that death occurred as a result of incapacity to reduce the osmolarity of the plasma, although plasma electrolytes were not measured. He observed that the salt gland "was capable of excreting fluid at all levels of intoxication below the LD_{50}," but that the duration of flow from the gland was inversely proportional to the dose of toxin.

Mallard ducklings were used in the present experiments because birds of this species and age are involved during midsummer outbreaks of botulism in Saskatchewan, and because this allowed the use of birds with a known history of exposure to

saline water. A period of 1 or 2 wk exposure to saline water prior to administration of botulinum toxin was chosen because the salt gland becomes active within 4 days in ducks of this age exposed to saline water (Mitcham and Wobeser, 1988a). The waters used in the current trials were much less saline than those used by Cooch (1964), but were representative of wetlands with a history of confirmed outbreaks of botulism in Saskatchewan (Wobeser et al., 1987). The average conductivity during July 1985 of 14 such wetlands was 2,670 μmhos/cm (range 750–5,500). The effect of saline water on the growth of ducklings in these experiments was similar to that we have observed in earlier studies (Mitcham and Wobeser, 1988a, b).

Water from wetlands C, D and E which had conductivity similar to that found in wetlands with a history of botulism did not appear to have any influence on the frequency of occurrence or the severity of clinical signs of botulism. The results of trials with water from the two more highly saline wetlands (A, B) are difficult to interpret. Ducks exposed to water from wetland A for 1 wk had more severe clinical signs of botulism and more died than among the controls. This suggested an interaction similar to that found by Cooch (1964). These birds had obvious drops of fluid about their nares both before and after receiving toxin, suggesting that salt was being secreted by the salt glands, and that the toxin had not caused complete cessation of secretion. Ducks exposed to this same water for 2 wk (group A2) did not differ from the control group in their response to botulinum toxin, and did not have obvious salt gland secretion either before or after receiving toxin. This suggested that some other mechanism, perhaps renal excretion, had become effective with prolonged exposure to the saline water, and that the salt glands were not active. The trial to measure the effects of intoxication on serum osmolality was very limited, but the birds with severe clinical signs of botulism did not develop hyperosmolality although they had elevated serum protein. The latter was probably a result of dehydration because of difficulty in reaching water.

In contrast, ducks given water from wetland B for 1 or 2 wk prior to receiving toxin had a significantly lower rate of occurrence of clinical signs of botulism than did the control birds given fresh water. Birds given water from wetland B had obvious droplets of fluid about the nares at all times, indicating that salt gland secretion was occurring. Two factors must be considered in evaluating this response. The ducks in groups B1 and B2 were smaller than the controls, so although they received a similar dose of toxin on a body weight basis, they were given a smaller absolute amount of toxin (Table 3). This might account for the difference in response. Another possible explanation is that the birds given water from wetland B may have absorbed less of the toxin administered. In the small ancillary trial, ducks given water from wetland B passed significantly more excreta, and began to pass the chromic oxide marker more rapidly than did ducks given fresh water. These are probably only crude indicators of intestinal transit time; the relative movement of botulinum toxin and chromic oxide through the intestine is unknown, but the observation suggests another possible mechanism to explain the results.

The results of these trials suggest that there may be relatively little additive or synergistic interaction between botulinum toxin and salinity in most wetlands where botulism occurs in Saskatchewan. However, botulinum toxin might interfere with salt gland function as suggested by Cooch (1964), and this could be important in birds that become intoxicated and hence entrapped on more highly saline wetlands.

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