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OCCURRENCE OF TOXIGENIC *CLOSTRIDIUM BOTULINUM* TYPE C IN THE SOIL OF WETLANDS IN SASKATCHEWAN

G. Wobeser,¹ S. Marsden,¹ and R. J. MacFarlane²

ABSTRACT: Mouse-lethal toxin identified as that of *Clostridium botulinum* type C by antitoxin neutralization was present in cultures of 38.0% of 326 soil samples collected from 28 wetlands in Saskatchewan. There was no difference in prevalence of toxicity between samples collected in spring and summer, and no relationship was evident between the occurrence of toxicity and water salinity, marsh type or water depth. There was a strong association between the prior occurrence of avian botulism in a marsh and the presence of toxin in cultures from soil; 59.2% of soil samples from marshes with a known history of botulism produced toxin, whereas only 6.2% of soil samples from marshes with no history of the disease produced toxin. Eight of the 10 soil samples collected from a marsh that had been dry for several years, and from another marsh that had not had a recognized outbreak of botulism for 11 yr produced toxin, indicating a long residual effect after a botulism outbreak. The results suggest that any wetland with a history of botulism is likely to suffer repeated occurrences because of heavy contamination of the soil with spores, and should be managed to control the disease.

INTRODUCTION

Botulism caused by the ingestion of toxin produced by *Clostridium botulinum* type C is an important disease of wild waterfowl in many areas of the world, particularly in western North America. Although the cause has been known for more than 50 yr, aspects of the ecology of the disease remain speculative. The basic requirements for an outbreak include the presence of a toxigenic strain of *C. botulinum*, a suitable substrate for bacterial growth and toxigenesis, suitable environmental conditions (temperature, oxygen concentration, etc.) for bacterial growth, a method of transferring toxin from substrate to birds, and a population of susceptible birds. Most of these are present in any marsh used by birds, as the carcass of either vertebrates or invertebrates dying in the marsh provides a good substrate with its own microenvironment (Kalmbach and Gunderson, 1934; Bell et al.,

1955; Wobeser and Galmut, 1984), and larvae (maggots) of sarcophagous flies are a suitable method of transferring toxin from substrate to birds (Hunter et al. 1970; Duncan and Jensen, 1976). However, despite the occurrence of these factors in most marshes, the disease does not occur ubiquitously. In Saskatchewan certain wetlands have severe outbreaks almost annually, whereas many other superficially similar wetlands have no history of the disease. One possible explanation for this distribution might be related to the prevalence of spores of toxigenic *C. botulinum* in marshes. Surveys in Great Britain, Europe and Asia suggest that there are differences in the prevalence of spores among wetlands (Table 1). Only one report is available describing the occurrence of spores of *C. botulinum* type C in marshes in North America (Marion et al., 1983) and that dealt only with artificially created phosphate-mine settling ponds. If spores are not ubiquitous in marsh soil, it might be possible to choose wetlands with few spores for development as intensively used waterfowl areas, or to use the characteristics of "spore-free" marshes as a guide for manipulation of contaminated wetlands to reduce the prevalence of

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TABLE 1. Reported occurrence of *Clostridium botulinum* type C in wetland soils.

Location	Wetland type	No. samples with toxin per no. samples examined	Reference
Botswana	Freshwater	1/8	Smith (1982)
England			
London	Freshwater	12/69	Smith and Moryson (1975)
Norfolk Broads	Salinity not specified	23/45	Borland et al. (1977)
Many sites	Fresh and marine	14/379	Smith et al. (1982)
Mersey Estuary	Brackish	19/98	Smith et al. (1977)
France			
Camargue	Freshwater	0/44	Smith and Moryson (1977)
Holland	Freshwater (area with history of botulism)	184/237	Haagsma (1977)
	Freshwater (area with no history of botulism)	6/141	Haagsma (1977)
Indonesia	Freshwater	1/50	Haq and Sukadi (1981)
	Marine	2/46	Haq and Sukadi (1981)
Ireland	Fresh and marine	0/55	Smith et al. (1978)
Japan	Freshwater	129/230	Serikawa et al. (1977)
River Nakagawa	"Sludge"	42/108	Itoh et al. (1978)
Mauritius	Freshwater	5/17	Smith (1982)
Nigeria	Freshwater	5/18	Smith (1982)
Scotland			
Edinburgh	Freshwater	0/7	Smith and Moryson (1975)
Many sites	Fresh and marine	2/77	Smith et al. (1978)
United States			
Florida	Phosphate-mine settling ponds	26/467	Marion et al. (1983)
Wales	Fresh and marine	2/43	Smith et al. (1978)

spores. This report describes a survey of the occurrence of spores of *C. botulinum* in Saskatchewan wetlands.

MATERIALS AND METHODS

Twenty-eight wetlands in southern Saskatchewan were chosen for examination. Most were in the grasslands vegetation zone, with a few in the parkland and two near the margin of the mixed-wood forest zone (Fig. 1). These wetlands varied greatly in size, type, salinity (Table 2), permanency and suitability for waterfowl. All were of interest to Ducks Unlimited Canada either as existing or potential waterfowl habitat projects. Wetlands, managed as a project, generally had been modified to manipulate water levels through construction of dams, dikes and channels. In some instances, waterfowl nesting sites were increased through construction of small earthen islands. Some of these projects consisted of several basins or compartments that were sampled separately. Potential projects were

generally in a natural undisturbed state except for agricultural uses such as haying or grazing.

Soil samples were collected on two occasions from each wetland; during the spring (21 May–6 June) and summer (16–21 July) of 1985. On the first occasion, a sampling area that was readily accessible and thought to be generally representative of the marsh was selected in each wetland, or compartment. The shoreline at the site was marked with stakes, and samples were collected into the wetland along a line perpendicular to the shore. The first sample was taken at the water's edge (0 cm), and other samples were collected at water depths of 15, 30, 60 and 90 cm, wherever water depth was sufficient. In very shallow wetlands five samples were taken at approximately equal intervals from the shore line to a point about 100 m from shore.

At sites that were dry in the spring, the first sample was taken at the high water mark, and other samples were taken along a line extending 100–200 m across the dry basin. Water levels receded considerably in most wetlands between

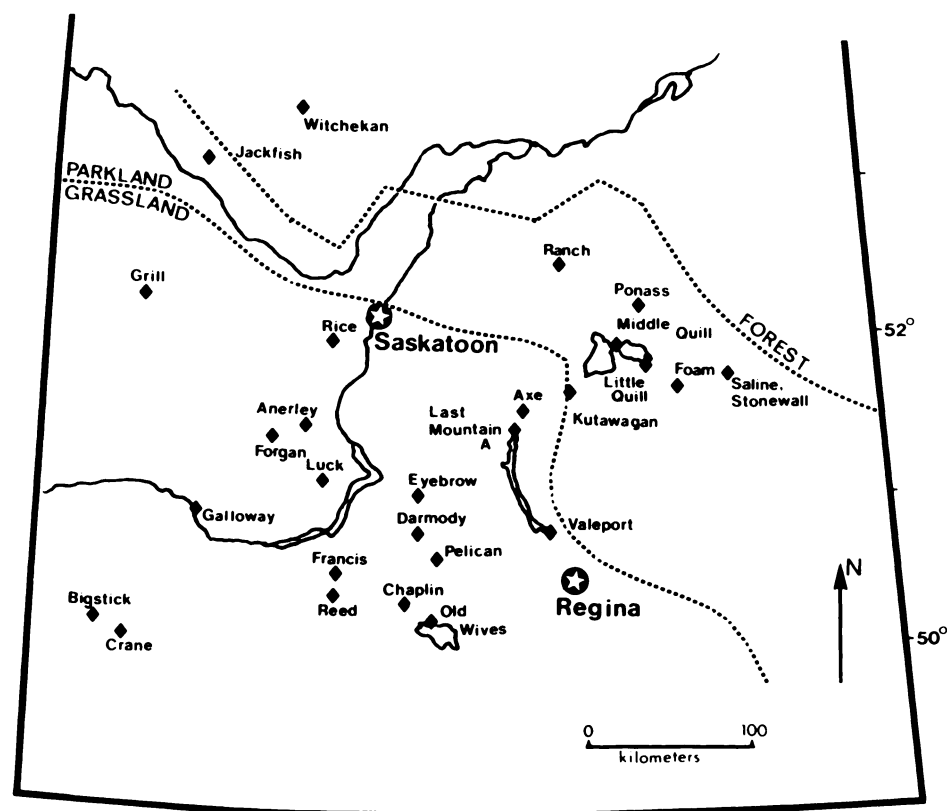


FIGURE 1. Location of wetlands sampled for spores of *Clostridium botulinum* type C in southern Saskatchewan, 1985.

the two sample collection dates, and some wetlands were totally dry by July. Sampling in July was done along the line established in the spring.

Individual soil samples were collected by forcing a previously autoclaved hollow plastic cylinder (1.5 cm diameter $\times$ 8 cm long, constructed by removing the end of a serum tube) approximately 5 cm into the soil. The tube with enclosed soil core was placed immediately in a sterile screw-cap vial, labelled, placed on melting ice in a cooler for transport, and frozen at -10°C upon return to the laboratory. Samples were held frozen until cultured.

A number of methods have been used for culturing *C. botulinum* type C from wetland soils. We initially used the method of Marion et al. (1983) with modifications. Briefly, 1 g of soil was suspended in 5 ml sterile phosphate buffered saline (PBS) (the original technique used "broth"), centrifuged (365 g for 3 min) to remove larger particles, and the supernatant was filtered through a sterile 0.45 μm membrane filter (Millipore Corporation, Bedford, Massa-

chusetts 01730, USA). After filtration, the filter pad was removed aseptically and placed into 10 ml of Cooked Meat Media (CMM) (Difco Corporation, Detroit, Michigan 48232, USA) (the original method used chopped meat carbohydrate media). The inoculated medium was heat-treated in a waterbath at 60°C for 30 min prior to inoculation. The original method used 80°C for 20 min, but we chose the lower temperature because Haagsma et al. (1972) reported that spores of *C. botulinum* were killed when heated at 80°C for 30 min. The inoculated medium was then incubated at 35°C for 5 days and frozen for 24 hr. After thawing, the free broth was filtered through a 0.45 μm membrane filter prior to use for mouse injection. This method was used for all samples collected in spring, but because no toxin was found in any of the 168 samples tested, we changed to a more simple technique that was found to be effective in pilot trials with material known to contain *C. botulinum* spores. In this technique 0.5 g of soil from each sample was inoculated directly

TABLE 2. Selected characteristics of wetlands in Saskatchewan sampled for the occurrence of spores of *Clostridium botulinum* type C in spring (21 May–6 June) and summer (16–21 July) 1985.

Wetland	Area (ha)	Type ^a	Conductivity (μ mho/cm)		pH ^b	History of botulism (date) ^c
			Spring	Summer		
Anerley Lake	60	Shallow open water	4,600	6,200	10	—
Axe Lake Project ^d	125	Open water marsh	1,320	3,800	9	+ (1981–1983) ^e
Bigstick Lake Extension Project ^d	205	Open water marsh	2,500	Dry	NM ^f	+ (1979, 1980)
Chaplin Marsh Project ^d	1,172	Shallow marsh and wet meadow	1,400	2,470	9	—
Crane Lake ^d						
Enclosure	1,140	Shallow marsh (diked)	670	1,020	8	—
Periphery	910	Shallow marsh (saline)	17,300	43,250	9	—
Darmody Flats	251	Dry valley floor with intermittent stream	Dry	Dry	NM	—
Eyebrow Lake ^d						
Basin A	160	Open water marsh	1,400	3,450	8.5	+ (1981–1984)
Basin B	246	Emergent deep marsh	2,650	4,900	8.5	+ (1981–1984)
Basin C	386	Emergent deep marsh	1,500	3,550	8.5	+ (1981–1984)
Foam Lake ^d	1,652	Open water marsh	1,070	1,300	6	+ (1984)
Forgan Flats	220	Shallow marsh	2,000	3,300	10	—
Francis Marsh	440	Shallow marsh	710	Dry	NM	—
Galloway Bay	809	Intermittently flooded bay of South Saskatchewan River	Dry	Dry	NM	+ (1981)
Grill Lake Project ^d	76	Emergent deep marsh	670	1,750	9	—
Jackfish Marsh	543	Emergent deep marsh	2,100	2,150	7	—
Kutawagan Lake Project ^d	997	Open water marsh	4,250	5,450	7	+ (1974)
Last Mountain Lake ^d	244	Open water marsh	3,600	4,450	8	+ (1983)
Project, Basin A						
Little Quill Restriction Project ^d	440	Open water marsh	4,520	5,500	8	+ (1972, 1980)
Luck Lake	2,516	Shallow open water wetland	6,300	Dry	NM	—
Middle Quill Lake Project ^d	1,315	Shallow open water wetland	2,250	3,000	8	+ (1979)
Old Wives Lake, Wood River Delta	1,730	Intermittently flooded open water marsh, grazed in 1985	Dry	Dry	NM	+ (1970, 1979)
Pelican Lake Project ^d						
Mallard Bay	388	Open water marsh	1,350	2,000	8	+ (1984, 1985)
Mallard Bay Ext.	157	Open water marsh	920	1,580	9	+ (1984, 1985)
Pelican Lake Ext.	99	Open water marsh	1,600	2,500	9	+ (1984, 1985)
Pelican Lake Proposal	410	Wet meadow	2,250	Dry	NM	—
Ponass Lake Project ^d						
Basin A	750	Open water marsh	1,100	1,600	6	—
Basin B	212	Open water marsh	1,600	1,930	6	—
Ranch Lake Project ^d	893	Open water marsh	670	750	8	+ (1984)
Reed Lake	2,400	Open alkali wetland	12,500	Dry	NM	—
Rice Lake Extension Project ^d	158	Open water marsh	920	1,180	6	+ (1981, 1982) ^e
Saline Lake	431	Open water marsh	880	960	6	+ (1984)
Stonewall Lake	316	Open water marsh	950	990	6	+ (1984)
Valeport Marsh Project ^d	376	Emergent deep marsh	2,050	3,000	8	—
Witcheakan Lake (southeast) ^d	242	Shallow marsh	480	830	8	—

into a tube containing 10 ml CMM. The surface of the medium was then covered immediately with a 5 mm layer of sterile mineral oil, and the inoculated tubes were incubated at 35 C for 5 days without any heat treatment prior to incubation. This method was used for all samples collected in spring and summer, and the results reported relate to this method only. (The soil samples collected in spring were refrozen following the initial attempt at culture.)

In all instances the soil for culture was taken from the "bottom" of the collection cylinder and was representative of soil approximately 3–5 cm below the bottom of the pond.

After incubation, the liquid broth was withdrawn aseptically from the culture tube, placed in a sterile vial and held at 4 C for 5–30 days until tested for toxicity. Each broth sample was tested initially for toxicity by injection of 0.1 ml intraperitoneally into each of two young adult white mice (weight ca. 30 g). Any broth sample that caused death or signs suggestive of botulism (paresis, "wasp-waist" appearance) within 4 days postinoculation (PI) was retested using four mice, two of which received 0.1 ml (5 IU) of type C antitoxin approximately 30 min prior to injection of the broth. Death within 4 days PI of both unprotected mice and survival of both mice that received antitoxin was considered a positive test for the presence of type C toxin. In four instances, one of the two mice given antitoxin died; the broth sample in these instances was then diluted 1:10 with sterile PBS, and the mouse protection test was repeated.

Types of *C. botulinum* other than type C have been isolated from aquatic soils, so an attempt was made to ensure that the mouse deaths were not due to another toxin. Polyvalent antitoxin prepared for human prophylaxis and containing 2,780 IU/ml type A, 1,090 IU/ml type B and 1,520 IU/ml type E antitoxins was obtained from Connaught Laboratories (Willowdale, Ontario M2N 5T8, Canada) and used in a mouse protection test as outlined previously. Antitoxin was limited, so only 15 broth samples, each inoculated with soil from a different wetland and known to cause mouse death, were tested. Six mice were used per sample; all received 0.1 broth, two were given 0.1 ml of

the polyvalent antitoxin, two received 0.1 ml type C antitoxin, and the remaining two did not receive antitoxin.

Chi-square analysis was used to test for differences in prevalence of spores between seasons; the *t*-test was used to test for differences related to water salinity.

RESULTS AND DISCUSSION

A variety of techniques have been used to prepare and culture mud samples for *C. botulinum*, including combinations of dilution, centrifugation, filtration and heat treatment prior to culture to reduce contamination of cultures by other organisms and to prevent oxidation of the culture media. Unfortunately, because of this, results are not comparable from one study to another. We initially used a technique similar to that of the only other study from North America (Marion et al., 1983), but, as noted earlier, none of the 168 samples collected from wetlands during May–June produced detectable toxin when prepared and cultured by this method. We then tried a more simple technique in which soil was added directly to the culture media (similar to the method of Haq and Sukadi, 1981). Cultures from 60 of the same spring soil samples caused death of mice when prepared by this method, and as will be described later, this toxicity was neutralized by type C antitoxin. We cannot account for the difference in results between the two techniques, but suspect that spores present in the soil samples were lost or inactivated in the first technique. The heat treatment (60 C for 30 min) should not have inactivated spores, as this temperature has been used successfully by others (e.g., Smith and Moryson, 1975).

The soil samples may have contained

←
* Wetland type using classification system of Millar (1976).

^b pH determined in July with indicator sticks (Colorphast, E. Merck, Darmstadt, Germany).

– = no known history of botulism outbreak; + = botulism outbreak occurred in waterfowl in years indicated.

^d Existing Ducks Unlimited Canada project with water level management.

^e Wobeser et al. (1983).

^f NM = not measured.

TABLE 3. Occurrence of *Clostridium botulinum* type C toxin in cultures of soil collected in spring (21 May–6 June) and summer (16–21 July) 1985 from Saskatchewan wetlands.

Wetland	Number of samples containing toxin per number of samples examined	
	Spring	Summer
Wetlands with no known history of botulism		
Anerley Lake	0/5	0/5
Chaplin Marsh Project	2/5	1/5
Crane Lake		
Enclosure	0/1	0/2
Periphery	0/3	1/3
Darmody Flats	0/4	0/4
Forgan Flats	0/5	0/5
Francis Marsh	0/5	0/5
Grill Lake Project	0/5	0/4
Jackfish Marsh	0/5	0/5
Luck Lake	1/5	0/5
Ponass Lake Project		
Basin A	0/5	0/4
Basin B	0/5	
Reed Lake	1/5	0/5
Valeport Marsh Project	0/5	2/5
Witcheakan Lake (southeast)	0/5	0/5
	4/68	4/62 = 8/130
Wetlands with known history of botulism		
Axe Lake Project	5/5	2/5
Bigstick Lake Ext. Project	1/5	1/5
Eyebrow Lake		
Basin A	3/5	4/5
Basin B	3/5	5/5
Basin C	1/5	2/4
Foam Lake	4/5	5/5
Galloway Bay	0/5	0/5
Kutawagan Lake Project	3/5	5/5
Last Mountain Lake Project		
Basin A	4/5	2/5
Little Quill Rest. Project	4/5	2/5
Middle Quill Lake Project	0/5	2/5
Old Wives Lake		
Wood River Delta	4/5	4/5
Pelican Lake Project		
Mallard Bay	1/5	3/4
Mallard Bay Ext.	4/5	4/5
Pelican Lake Ext.	1/5	4/5
Pelican Lake Prop.	1/5	2/4
Ranch Lake	5/5	5/5
Rice Lake Ext. Project	5/5	4/5
Saline Lake	5/5	5/5
Stonewall Lake	2/5	1/4
	56/100	60/96 = 116/196

relatively few spores, and these may have been precipitated with particulate material during centrifugation. The use of PBS, rather than broth, as a diluent may have enhanced precipitation.

We decided to use the direct method for the July samples as well, because of its simplicity and because we did not encounter problems with either oxidation of culture media as reported by Marion et al. (1983) or excessive deaths of mice from causes other than botulism. Using this technique, 35.7% of soil samples collected in the spring and 40.5% of samples collected in July caused death of mice (Table 3). Mice that died almost invariably succumbed within 24 hr PI, with most dying in less than 12 hr.

Six samples that killed mice on the initial screening were non-lethal when retested approximately 5 days later in the mouse protection test. (These are not included in the data above or in Table 3.) We feel that this "transient" toxicity was not caused by type C toxin, as we and others (Graham et al., 1978) have found type C toxin to be very stable under cool conditions. In all other instances the mouse lethal factor was stable, and mice were protected by prior administration of antitoxin to *C. botulinum* type C. In four instances undiluted broth killed mice given antitoxin; however, when the broth was diluted, unprotected mice died, whereas mice given antitoxin survived. The polyvalent antitoxin (types A, B, E) had no protective effect for mice against any of the 15 samples tested, whereas type C antitoxin was fully protective against all of these. On this basis we conclude that the toxicity was caused by *C. botulinum* type C toxin.

There was no significant difference in the overall proportion of soil samples from the spring (60/168) and summer (64/158) collections that produced toxin ($\chi^2 = 0.793$, 1 df, $P > 0.200$). There is little informa-

tion on seasonal prevalence of spores in marshes, but Marion et al. (1983) found spores that produced toxin only in the summer, with the highest prevalence in June; Serikawa et al. (1977) found the highest prevalence of toxin production in soil samples collected during October and November in Japan.

No clear relationship was evident between water depth and proportion of samples producing toxin. Smith and Moryson (1977) and Smith and Young (1980) found the prevalence of *C. botulinum* of all types to be much greater in aquatic mud than in soil collected from dry sites away from wetlands. Type C, which was found commonly in aquatic mud, was only found in soil from one non-aquatic site (Smith and Milligan, 1979) among the many sampled throughout the United Kingdom by Smith and coworkers. At Old Wives Lake, the collection site had not been flooded for several years, but eight of 10 samples collected from the dry marsh bottom produced toxin. This suggests that short-term drying of a wetland is unlikely to result in a rapid "disappearance" of spores, and hence temporary drawdown and drying of a marsh is unlikely to be an effective management technique to reduce spore contamination in a problem wetland.

Wetlands from which one or more samples produced toxin were more saline (mean conductivity = $3,463 \pm 4,108$ mho/cm) than those from which no samples produced toxin (mean conductivity $1,799 \pm 1,280$ mho/cm), but this difference was not significant ($t = 1.75$, $P = 0.091$). One sample from the most saline wetland tested (Reed Lake, conductivity in May = $12,500$ mho/cm) produced toxin. This is not surprising as *C. botulinum* type C has been identified in marine environments and growth is not inhibited until salt concentrations reach 2.5–3.0% (Segner et al., 1971).

Segner et al. (1971) found that terres-

trial strains of *C. botulinum* type C failed to grow below pH 5.62. The lowest pH measured with the relatively crude method used in this study was 6; which was found in six wetlands; samples from four of which produced toxin. Samples from Pelican Lake, pH 9, also produced toxin. No clear relationship was evident between marsh type and production of type C toxin by soil samples; however, there was a very strong association between the prior history of botulism in a wetland and the proportion of soil samples that produced toxin. More than half of the mud samples (116/196, 59.2%) from wetlands with a known history of botulism produced toxin, whereas only 6.2% (8/130) of the samples from wetlands with no known history of the disease produced toxin ($\chi^2 = 93.3$, 1 df, $P < 0.001$). Positive samples were found in every wetland with a prior history of botulism, except Galloway Bay, while no positive samples were found from nine of 13 wetlands with no prior history of the disease. Galloway Bay is distinct from all other wetlands sampled in that it is an extension of the South Saskatchewan River that is flooded each summer and is the only wetland with an active waterflow or current when flooded. The flowing water may have prevented deposition and accumulation of spores in the mud during the botulism outbreak that occurred in Galloway Bay in 1981. Encouragement of active waterflow might be possible in some wetlands as a measure to reduce the "residual" effects of a botulism outbreak.

Haagsma (1973) found type C toxin in 71.6% of cultures from 184 mud samples collected from known areas of avian botulism in the Netherlands, whereas only 4.2% of 141 samples from other wetlands produced toxin. Similarly, Smith et al. (1978) found type C toxin in 3.4% of 554 mud samples collected from wetlands with no history of botulism throughout Great Britain and Ireland, whereas 51.5% of 45 samples collected from the Norfolk Broads,

a known site for avian botulism outbreaks, produced type C toxin when cultured by the same method (Borland et al., 1977). Despite the differences in technique used by Haagsma (1973), Smith and coworkers (Smith and Moryson, 1975) and in the present study, the ratio of positive samples from known botulism areas to that from other wetlands is similar (11-17:1). Results from other studies (Table 1) suggest that in general, 1-5% of mud samples from wetlands with no history of botulism may contain type C spores. One exception to this general pattern may be the study of Serikawa et al. (1977) in which a high proportion of samples from five lakes in Japan produced toxin, although only one of the lakes was mentioned as having been the site of a botulism outbreak.

Spores of *C. botulinum* are considered very resistant, and Smith et al. (1982) stated that "mud from an aquatic environment that has given rise to an outbreak of avian botulism will remain permanently contaminated with type-C spores." The results from Kutawagan Lake support this contention, as no outbreak of botulism has been recognized there since 1974, but eight of 10 mud samples produced type C toxin. The phenomenon of heavy persistent contamination with spores following an outbreak is important for marsh management. Any wetland with a known history of the disease is probably very susceptible to repeated occurrences. Routine and regular monitoring for dead birds, and contingency planning for dealing with outbreaks, should be included in the management plan for any such marsh that is developed for intensive use by waterfowl.

No toxin type of *C. botulinum* other than type C was identified in this study. Similarly, only type C was found during surveys in Japan (Serikawa et al., 1977; Itoh et al., 1978) and in Florida (Marion et al., 1983). However, in Great Britain (Smith et al., 1978) and Holland (Haagsma, 1973), type B was found more fre-

quently than type C, and type E also was common. The methods used in our study may have discriminated against detection of types B and E, as neither heat treatment nor trypsinization was used. Smith and Moryson (1975) found that heat treatment prior to culturing increased the frequency of production of type B toxin. Most strains of type E and some non-proteolytic strains of type B produce protoxin that must be activated by trypsin (Smith, 1982), and Smith and Moryson (1975) found that trypsinization of culture broth prior to injection was usually necessary to demonstrate type B toxin. Treatment with trypsin was not done in our survey because we were only interested in type C, which is usually not influenced by trypsin. Smith and Moryson (1975) reported one instance in which trypsinization apparently obscured a culture positive for type C.

The results of this study help to explain the distribution and repeated occurrence of botulism on some wetlands in Saskatchewan. Failure to demonstrate toxin in soil cultures does not assure that spores of *C. botulinum* are not present in a marsh. Other bacteria in the soil may inhibit its growth or denature the toxin when soil is cultured in the laboratory (Graham, 1978); knowledge of the ecology of *C. botulinum* type C in soil is inadequate to know if the same phenomenon may occur in nature.

The method we used for culturing is simple and rapid and could be used for large scale surveys of wetlands. Culturing of marsh soil also could be used in the retrospective investigation of die-offs of birds when suitable avian specimens are no longer available, as suggested by Smith et al. (1982). Failure to demonstrate toxin production by any of 10 mud samples from an outbreak site was suggested to be good evidence that the die-off was not the result of botulism.

Spores of *C. botulinum* can undoubtedly be transferred in the gut or on the surface of birds to "uncontaminated" wet-

lands (Smith, 1978). However, it seems unlikely that heavy contamination of the new environment would occur unless there was extensive vegetative growth of the bacterium at the site. This might occur if birds that had ingested a large number of spores and toxin at an outbreak site were to fly to another marsh before becoming sick and dying. The spores produced in the carcass of such birds could then heavily contaminate small areas of the new marsh. This has implications for the common practice of dispersing birds from the site of botulism outbreaks.

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