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Clostridium botulinum in the lakes and waterways of London

By G. R. SMITH and CAROLINE J. MORYSON
Nuffield Institute of Comparative Medicine, The Zoological Society of London, Regent's Park, London NW 1

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SUMMARY

Mud samples collected during 1974 from a large proportion of the lakes and waterways of London were examined for *Clostridium botulinum*. Of 69 such sites, 50 (72·5%) contained at least one type of the organism. Of the 50 positive sites, 31, 12, 1 and 10 contained, respectively, types B, C, D and E. Most of the demonstrations of type B required trypsinization of culture filtrates. An examination of 7 lakes in Edinburgh, made for the purpose of comparison, showed that 4 contained type B and one type C.

An analysis of the results gave quantitative information on the value of (1) resampling apparently negative lakes, (2) the use of both heated and unheated culture inocula, and (3) trypsinization of culture filtrates.

INTRODUCTION

Meyer & Dubovsky (1922*b*) examined 64 soil samples from England and Wales and found that 5 (7·8%) contained *Cl. botulinum* type B; a further 4 samples yielded weak unidentified toxin. Leighton & Buxton (1928) found *Cl. botulinum* type A or B in 4 of 100 Scottish soil samples. Haines (1942) reported that of soil samples from 106 localities mainly in south-east England, four contained type A and one type B. Cann *et al.* (1965) and Baird-Parker (1969) commented upon the probable rarity of type E in the British Isles.

Meyer (1956) considered that a telluric incidence of *Cl. botulinum* such as that found in California was exceptionally high; of 624 Californian specimens of soil, vegetables, fruits, feeds, manure and sewage, Meyer & Dubovsky (1922*a*) had found that 30% contained *Cl. botulinum*. Haagsma (1974) examined Dutch inland waters and found that of those not known to be associated with botulism in waterfowl, 30% contained *Cl. botulinum* types B, C or E, the latter occurring most frequently.

The Loch Maree tragedy (Leighton, 1923) resulted in the deaths of eight people who had ingested type A toxin. The most recent outbreak of human botulism reported in Britain occurred in 1955 – two non-fatal cases resulted from the ingestion of fish, pickled in Mauritius and containing type A toxin (Mackay-Scollay, 1958). Outbreaks of botulism in birds and mammals in Britain due to *Cl. botulinum* type C have occurred from time to time (Roberts, Keymer, Borland & Smith, 1972).

As part of a study of botulism in waterfowl, we have investigated the prevalence of *Cl. botulinum* in mud samples from a large proportion of the lakes and waterways of London. The results of this survey are now given.

MATERIALS AND METHODS

Lakes and waterways sampled

Mud samples were collected from aquatic sites, numbered 1–69, in London within a radius of 11 miles from Charing Cross. These sites consisted of 61 lakes, 1 disused reservoir, 1 river backwater, 1 canal, 4 well-separated locations on the river Thames, and 1 creek leading into the Thames. Further information on the nature and location of sites 1–69 is given in Table 1 and Fig. 1. For purposes of comparison, further samples were taken from 7 lakes in Edinburgh, within a radius of 2 miles from the General Post Office.

Unless stated otherwise, all samples were collected between 1 January and 31 December 1974. Three lakes had a known history of botulism in waterfowl due to *Cl. botulinum* type C: no. 11 was affected in 1969 and 1971 but had since been cleaned; no. 23 was affected in 1970 and 1971; no. 42 was affected in 1973, and cleaning operations began in September 1974 in that part of the lake where most deaths had occurred. All the aquatic sites except nos. 2, 23 and 67 were freely accessible to the public; many were used for angling and boating, and a few for bathing. Waterfowl were present at almost all the sampling sites, sometimes in large numbers.

For the purpose of analysing the effect of heat-treatment of cultures and trypsinization of culture filtrates, positive samples from London and Edinburgh were studied, together with additional positive samples from rural aquatic sites.

Method of sampling

In almost all instances it was possible to collect the mud samples by hand from peripheral areas of shallow water. The mud varied considerably in type, though more often than not it was soft, blackish and deep, with an offensive odour. A plastic disposable sleeve was used and each sample consisted of several handfuls of mud that included material taken from different depths and from different sites within an area of a few square yards. A small number of the mud samples consisted of material from more widely dispersed points in a single lake. On the few occasions when the depth of water made manual sampling impossible, an autoclaved metal ladle with a long handle was used. Each sample was placed in a large plastic bag and taken to the laboratory where, after thorough mixing, it was dispensed in amounts exceeding 50 g. into three smaller plastic bags. Mud was examined either after storage for not more than a few days at 4° C., or after more prolonged storage at −20° C. Usually it was necessary to examine the contents of only one of the three bags to obtain a clear result, but the other two bags were kept at −20° C. in case an equivocal result necessitated further examination. Great care was taken during sampling and at all subsequent stages to prevent cross-contamination between different samples.

Resampling

With the exception of nos. 23, 40, 42 and 50, lakes and waterways found positive for *Cl. botulinum* at the first attempt were not re-examined, but a negative result was recorded only after mud collected on two different occasions had been cultured without producing botulinal toxin.

Examination of samples

A 50 g. quantity of mud was thoroughly mixed with 50 ml. phosphate buffer, pH 7·0, in a mortar. The liquid phase was then decanted into a glass cylinder and allowed to stand for a few minutes, after which the supernatant fluid containing fine but not coarse debris was distributed in equal volumes, usually exceeding 15 ml., into two Universal containers; to produce satisfactory supernatant fluid from the small proportion of samples that contained large amounts of clay, light centrifugation (160 ***g*** for 3–5 min.) was usually necessary. The two Universal containers were placed in an angle centrifuge and spun for 30 min. at 1500 ***g***. The method thus far was based on the technique described by Kanzawa, Ono, Karashimada & Iida (1970). The two pellets were each transferred to the bottom of a 25 ml. McCartney bottle containing approximately 22 ml. of Difco Bacto Cooked Meat Medium that had just been raised to boiling point and cooled. One bottle was heated for 1 hr. in a water bath at 60° C. and both bottles were then incubated at 30° C. for 6–8 days.

After incubation, a sterile filtrate was prepared from each of the two cultures by means of cellulose acetate membranes. Each filtrate, in both the trypsinized (Duff, Wright & Yarinsky, 1956) and untrypsinized state, was made up to a final dilution of 1/4 with gelatine-phosphate buffer (Bowmer, 1963) before being tested for toxicity. The four preparations derived from a single mud sample were each injected intraperitoneally into two mice weighing 18–22 g. in doses of 0·5 ml. The mice were observed for 4 days for the development of a characteristic 'wasp-waist', usually followed by respiratory distress, paralysis and death. The initial signs of botulism rarely took as long as 48 hr. to develop. Only occasionally was a test complicated by the occurrence of other bacterial toxins in the filtrate; when such toxins interfered with the examination for botulinal toxin the difficulty could be overcome by preinjecting the mice with 0·1 ml. doses of a mixture containing equal volumes of tetanus antitoxin (12·5 i.u.), mixed gas gangrene antiserum, lamb dysentery antiserum and blackleg antiserum (Wellcome Foundation Ltd). Alternatively, the mixture could be added to the culture filtrate *in vitro* before injection.

If one or more of the four preparations derived from a mud sample appeared to contain botulinal toxin, protection tests were carried out with antisera kindly supplied by Dr J. Keppie, Microbiological Research Establishment, Porton. Only one preparation was examined in this way unless there was reason to suspect that another preparation contained a different toxin. The occurrence of weak toxin sometimes necessitated the use of increased doses of filtrate for protection tests, and only rarely was this modified procedure ruled out by the presence of non-specific toxic factors; such factors are well known to occur in the undiluted filtrates

of cultures of certain soils and they produce death within a few minutes of intraperitoneal injection. Pre-incubated mixtures of toxic filtrate and antitoxin contained *Cl. botulinum* type A, B, D or E antitoxin at the rate of 1·0 unit (0·1 ml.) per mouse dose, or type C antitoxin at the rate of 0·1 unit (0·1 ml.) per mouse dose. On rare occasions these quantities of antitoxin were insufficient and it was then necessary to dilute the culture filtrate to an appropriate degree. The usual uncomplicated test utilized one pair of mice for each of the five mixtures of antitoxin and toxic filtrate, and one pair for a mixture of normal saline and toxic filtrate; the result was shown by the deaths of all but a single pair of antitoxin-treated mice. Infrequently, owing to the occurrence of weak toxin, such a protection test gave an indication but not conclusive proof of the identity of the toxin; in such instances a further test was carried out with groups of mice large enough to give an unequivocal result.

All apparatus contaminated with mud was autoclaved at 103·5 kN./m.^{2} (15 lbf./in.^{2}) for 45 min. before re-use.

Examination of fish

Each fish was sealed inside several plastic bags and incubated at 30° C. for 5 days. A 10 % or 20% homogenate of muscle in gelatine-phosphate buffer was then prepared and centrifuged. A sterile preparation of the supernatant fluid was made with a membrane filter and tested for toxicity by the intraperitoneal injection of 0·5 ml. volumes into mice. If the mice developed botulism, the toxin was typed by means of a mouse-protection test, and simultaneously titrated. The number of viable *Cl. botulinum* per gram of muscle was estimated by making decimal dilutions of tissue homogenate and inoculating them in measured volumes into cooked-meat medium; after 6–8 days' incubation at 30° C. culture filtrates were tested for botulinal toxin.

RESULTS

Prevalence of Cl. botulinum *in London lakes and waterways*

Of 69 lakes and waterways sampled, 50 (72·5 %) contained *Cl. botulinum* (Table 1). A single sample from each of the 69 aquatic sites indicated that 38 (55 %) were positive, but on resampling the 31 negative sites a further 12 positives were found.

Table 1 shows that of the 50 positive sites, 31 contained *Cl. botulinum* type B, 12 contained type C, 1 contained type D, 10 contained type E, and 2 contained *Cl. botulinum* that could not be typed because the toxin produced in culture was weak and unstable. Three lakes and one disused reservoir were shown to contain either two or three types of the organism.

Fig. 1 indicates approximately the location of positive and negative sites and, if studied in conjunction with Table 1, it also shows the distribution of the various types of *Cl. botulinum* in the London area. It seems possible that this distribution was of a random nature, although types C and E occurred with rather greater frequency in north-east and central London respectively, than elsewhere. Samples taken north of the Thames contained more positives (79·2 %) than did those taken south of the river (47·1 %).

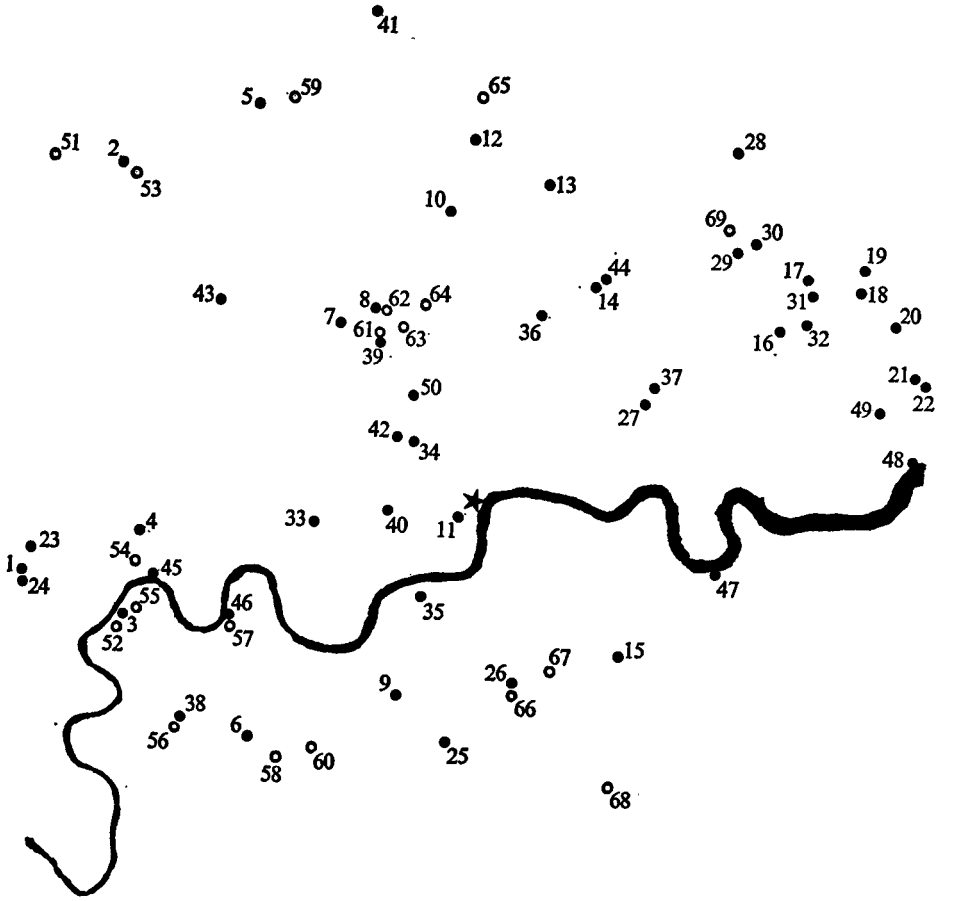

Fig. 1. *Cl. botulinum* in London lakes and waterways. Sketch map shows positive (●) and negative (○) aquatic sites in relation to Charing Cross (★) and to the river Thames. The numbering of the sites is explained in Table 1.

Table 1. Clostridium botulinum *in London lakes and waterways*

Aquatic site no.	Type of aquatic site	Type of *Cl. botulinum* demonstrated
1–22	Lake	B
23–31	Lake	C
32	Lake	D
33–37	Lake	E
38–39	Lake	Untyped*
40	Lake	B, E
41–42	Lake	B, C, E
43	Disused reservoir	B, E
44	River backwater	C
45–48	Thames	B
49	Creek off Thames	B
50	Canal	E
51–69	Lake	None

The 69 sites represent different lakes and waterways, except for nos. 45–49.
* *Cl. botulinum* toxin weak and unstable.

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For the purpose of comparison with the London survey, a small survey was made in Edinburgh. Of 7 lakes sampled, 5 (71 %) contained *Cl. botulinum*; type B was demonstrated in 4 lakes and type C in one.

Repeated sampling of selected lakes and waterways, and examination of fish

Lake no. 23, on which deaths from botulism had occurred in waterfowl in 1970 and 1971, was sampled at three different points on a single occasion in May 1974, and at a single point in the following October. All four mud samples contained *Cl. botulinum* type C. Of two bream caught in February 1975 and kept overnight in a large tank before being killed, one was shown to contain type C organisms; after incubation its muscle contained between 40 and 50 mouse lethal doses (LD) of type C toxin per gram.

Lake no. 40 was sampled at seven well-separated points as follows: four mud samples were collected on a single occasion in March 1974, two samples on a single occasion in the following July, and one sample in January 1975. All samples except one contained *Cl. botulinum* type E, and the final sample contained type B in addition.

Lake no. 42, on which deaths from botulism had occurred in waterfowl in the summer of 1973, was sampled at three points designated *X*, *Y* and *Z* and separated from each other by at least 500 yards. Point *X*, located in that part of the lake where the highest mortality had occurred, was sampled in August 1973, and in January and early September 1974; *Cl. botulinum* type C was demonstrated in the first two samples and types C and E in the third. Draining and dredging operations began in this part of the lake in late September 1974, and 1 month later types B and E were demonstrated in a mud sample taken from the middle of the lake near point *X*. Samples collected from point *Y* in August 1973 and February 1974 were shown to contain type C, but a sample taken in January 1974 was negative. A sample collected from point *Z* in March 1975 contained type B.

A canal (Table 1, no. 50) was shown in August 1974 to contain *Cl. botulinum* type E. Three fish, a roach and two perch, were obtained from the canal within 500 yards of the original sampling point, and all were shown after incubation to contain type E. One of the fish, caught in August 1974, contained after incubation between 1600 and 3200 mouse LD of toxin and $> 10^7$ viable *Cl. botulinum* per gram of muscle. The second fish, found dead in December 1974, contained between 100 and 500 mouse LD of toxin and between 10^5 and 10^6 viable *Cl. botulinum* per gram of muscle. The third fish, found dead in January 1975, contained between 2500 and 10,000 mouse LD of toxin and $> 10^6$ viable *Cl. botulinum* per gram of muscle. Seven further mud samples were taken from the canal in January and February 1975. Of these, two were collected from sites further west than that depicted in Fig. 1 (no. 50), and the remainder from sites further east. The distance between sites ranged from 0·25 to 0·5 mile, except in one instance where it was approximately 0·7 mile. All seven additional mud samples contained *Cl. botulinum* type E.

Table 2. *Observations on the techniques for examining mud*

	Types of *Cl. botulinum*			
	B	C	D	E
No. of positive samples ...	39	16	1	13
Positive results when				
NH/NT	1	11	1	7
NH/T	26	11	1	8
H/NT	4	13	0	10
H/T	34	13	0	11
NH only*	5	3	1	2
H only*	13	5	0	3
NH and H*	21	8	0	8

Data from 69 positive mud samples (London and elsewhere).
NH = culture not heated before incubation. H = culture heated to 60° C. for 1 hr. before incubation. NT = culture filtrate not trypsinized. T = culture filtrate trypsinized.
* The last three lines show the effect of heating or not heating, without regard to the effect of trypsinization.

Observations on the technique for examining mud

Table 2 shows that trypsinization of culture filtrate was almost always necessary for the demonstration of type B strains present in mud samples. Trypsinization increased only very slightly the number of demonstrations of type E in mud, and it had no effect in respect of type C. In two instances only, the presence of botulinal toxin in a culture filtrate was obscured by the action of trypsin; the toxins involved were those of types C and E.

Cultures were incubated both with and without previous heat-treatment at 60° C. for 1 hr. This procedure appeared to be worth while because, although 53·6% of 69 positive samples were positive regardless of heat-treatment, the remainder gave a positive result only in heated or in unheated cultures. In heated cultures alone and in unheated cultures alone, 15·9% and 30·4% respectively of the positive samples would have been judged negative; the difference between these two percentages was accounted for largely by mud samples containing *Cl. botulinum* type B.

DISCUSSION

It is surprising that as many as 72·5% of the large proportion of London lakes and waterways sampled should have contained *Cl. botulinum* of one type or another. Possibly the sediment in such aquatic environments is more likely to contain the organism than is soil, though Haagsma's (1974) survey of Dutch inland waters other than those known to be associated with avian botulism revealed an incidence of less than half that reported in this study. The results of our small-scale survey in Edinburgh indicate that London should probably not be regarded as exceptional in respect of the occurrence of *Cl. botulinum* in urban aquatic environments. The high prevalence found in this study may have been related to the technical methods used.

Haagsma (1974) found that *Cl. botulinum* type A occurred only rarely in Dutch aquatic areas and that type E predominated. We failed to demonstrate type A on

any occasion, but found that type B predominated; most of our type B demonstrations required the use of trypsin. Non-proteolytic *Cl. botulinum* type B strains have recently been found in Scottish fish farms (Burns & Williams, 1975). Although the demonstration of type C has in the past been confined generally to environments where botulism in birds and animals has occurred (Roberts, 1959), we frequently demonstrated this organism in lakes not known to be associated with the disease. Type D was demonstrated in a single lake; this organism is thought to be rare in the northern hemisphere, and in the course of a survey of soil and water in the U.S.S.R. Kravchenko & Shishulina (1967) demonstrated its presence in only one of 4345 samples. Type E was found in 10 London lakes or waterways and the earlier view (Cann *et al.* 1965; Baird-Parker, 1969) that this organism is rare in the British Isles must now be revised.

The extensive literature on botulism in waterfowl suggests that almost all outbreaks are due to *Cl. botulinum* type C, although there is some evidence that type E can produce the disease (Fay, 1966). Three outbreaks known to us in recent years in London were all due to type C despite the findings of the present study, namely the much more frequent occurrence of type B than type C, and the not infrequent occurrence of type E. The full explanation of this apparent paradox may well be complex, but Gross & Smith (1971) found that in gallinaceous birds type C toxin was more readily absorbed through the gut wall than type B or E toxin and that type B toxin was only slightly toxic.

Meyer (1956) considered that some relation exists between the prevalence of *Cl. botulinum* in the soil and the occurrence of botulism. Nevertheless, any risk to public health associated with the widespread occurrence of *Cl. botulinum* revealed by this study must be exceedingly low. Although most of the lakes and waterways examined are freely accessible to the public, large numbers of whom use them for recreational purposes such as angling, boating and even bathing, no case of human botulism has been reported in this country since 1955 (Public Health Laboratory Service, 1972).

Our findings may be of indirect interest in relation to fish farming; contamination of fish with *Cl. botulinum* from the environment on farms is a matter that is now attracting study (Huss & Pedersen, 1973; Burns & Williams, 1975).

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