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Clostridium botulinum in aquatic environments in Great Britain and Ireland

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SUMMARY

Mud samples from aquatic environments in many parts of Great Britain and Ireland were collected, mainly in 1975 and 1976, and examined for *Clostridium botulinum*. The samples were taken from lakes, ponds, reservoirs, marshes, mudflats, streams, rivers and canals, and the sampling areas included a number of bird refuges and reserves. Of 554 samples 194 (35·0%) were positive and 167 (30·1%), 19 (3·4%), 6 (1·1%) and 15 (2·7%) contained types B, C, D and E respectively; 13 (2·3%) contained more than one type. Each type demonstrated was found in both fresh- and salt-water environments. Type B was widespread; types C, D and E were demonstrated in widely separated areas in England and Wales, and type C was found in both the north and south of Scotland. The results were compared with those reported earlier in respect of surveys in the London area, the Norfolk Broads and the Camargue (France).

INTRODUCTION

The work of Meyer & Dubovsky (1922), Leighton & Buxton (1928) and Haines (1942) suggested that *Clostridium botulinum* type A or B occurred in 4–8% of British soil samples. Cann, Wilson & Hobbs (1968) found that 3·5% of 429 samples of bottom deposit taken around the British coast 10 miles off the mainland contained type B.

As part of a study of type C botulism in waterfowl, Smith & Moryson (1975) found that 72·5% of the lakes and waterways of London contained *Cl. botulinum*, B being the most common type, C and E less common and D uncommon. They later showed (Smith & Moryson, 1977*b*) that in the London area lake mud was a much more favourable environment than soil for *Cl. botulinum*. In the Norfolk Broads, an area in which botulism in waterfowl has recently become well known, 97·8% of samples contained *Cl. botulinum*; types B, C and E were widespread and individual samples more often than not contained two or three types (Borland, Moryson & Smith, 1977). This survey work has now been extended to cover many other aquatic areas in the British Isles and the results, are reported fully in this communication.

MATERIALS AND METHODS

Aquatic environments sampled

Most of the sampling was carried out in 1975 and 1976, but a few samples were collected in 1974 and 1977. The mud samples were taken from lakes, ponds, reservoirs, marshes, mudflats, streams, rivers and canals, and the sampling areas, which in general were chosen for their interest in relation to waterfowl, included a number of refuges and reserves. The term 'mud' is used to describe all types of sample although the deposits from which they originated varied considerably, e.g. some were deep and soft with an offensive smell whilst others were sparse and gritty with little smell.

Collection and examination of mud samples

The methods were as described by Smith & Moryson (1975) except that (1) negative sites were not resampled, and (2) on the few occasions when the depth of water made manual sampling impossible, either a long-handled metal scoop or a Jenkin surface-mud-sampler tube was used; before re-use these implements were washed clean, immersed for at least 15 min in a solution containing not less than 1000 parts/10^6 sodium hypochlorite, and thoroughly rinsed.

RESULTS

The following account excludes findings made in London, Edinburgh and the Norfolk Broads, as these have already been published (Smith & Moryson, 1975; Borland *et al.* 1977).

Of 554 mud samples 194 (35·0%) were positive and 167 (30·1%), 19 (3·4%), 6 (1·1%) and 15 (2·7%) contained types B, C, D and E respectively; 13 (2·3%) contained more than one type. Each type demonstrated was found in both fresh- and salt-water environments and type B was common in all areas sampled. Types C, D and E were demonstrated in England and in Wales, and type C in Scotland. The detailed account given below should be studied in conjunction with the summarized results (Table 1) and sketch map (Fig. 1).

Suffolk, Essex and North Kent coasts

Many of the 49 samples collected were from saline or brackish environments. Type B occurred in 22 samples, type C in one (St Lawrence Bay) and type E in two (Tilbury and Isle of Grain).

Romney Marsh

Of 26 samples seven contained type B. Type B occurred in one of six samples that were otherwise negative from the Royal Society for the Protection of Birds (RSPB) reserve near Dungeness. Type E was found in three of seven samples from well-separated sites in the Royal Military Canal and in a canal near Dymchurch and a lake near Winchelsea.

Table 1. *Distribution of* Cl. botulinum *in aquatic environments in Great Britain and Ireland*

Area sampled	Sketch map reference (see Fig. 1)	Number of mud samples collected	that contained *Cl. botulinum* of: any type	Type B	Type C	Type D	Type E	more than one type*
Suffolk, Essex and North Kent coasts	8J, 8K, 9J	49	23	22	1	0	2	2 (BE)
Romney Marsh	8K	26	11	7	0	0	5	1 (BE)
West Sussex, Isle of Wight and Dorset	5K, 6K, 7K	45	12	8	2	2	0	0
Devon, Somerset, Wiltshire, Avon and Gloucestershire	4K, 5J, 5K, 6H, 6J	59	17	17	0	0	0	0
Lancashire and Lake District	5E, 6E, 6F	42	22	21	1	0	1	1 (BC)
Co. Durham, North Yorkshire, Humberside and Lincolnshire	6F, 7D, 7E, 7F, 8F, 8G	66	20	17	3	1	0	1 (BD)
East Anglia	7H, 8G, 8H	60	28	26	5	0	0	3 (BC)
Bedfordshire, Hertfordshire, Surrey and Middlesex	7J, 8J	32	16	11	2	2	5	4 (2 BE, 2 BD)
Scotland	3C, 5A, 5B, 5C, 5D, 6A, 6B, 6C	77	20	18	2	0	0	0
Wales and Shropshire	3H, 4G, 4H, 5G, 5H, 5J	43	15	10	3	1	2	1 (BE)
Ireland	1E, 2D, 2E, 2F, 3D, 3E	55	10	10	0	0	0	0
Totals		554	194	167	19	6	15	13

* The types present in each mixed sample are shown in parentheses.

West Sussex, Isle of Wight and Dorset

Forty-five samples were collected, many from saline or brackish coastal sites. Fifteen samples from the recently established Wildfowl Trust (WT) refuge at Arundel and two samples from the Isle of Wight were negative. Of 28 samples from Dorset eight contained type B; type C occurred in two brackish or saline lagoons, one near Studland and one on Brownsea Island, and type D was found in two lakes, one on Brownsea Island and one in Weymouth (Radipole Lake).

Devon, Somerset, Wiltshire, Avon and Gloucestershire

Type B occurred in 17 of 59 samples, but no other type was found. The samples included 18 from the WT refuge at Slimbridge, of which five were positive.

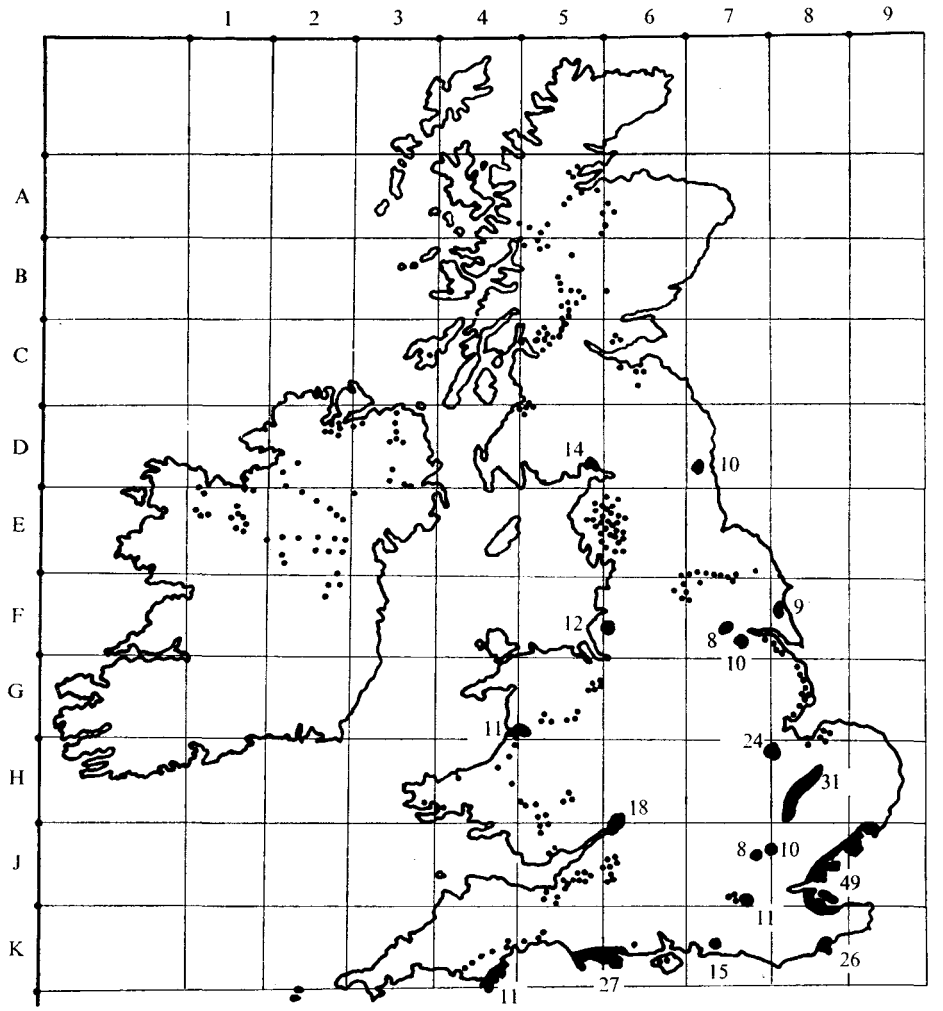

Fig. 1. The sketch map illustrates the aquatic sites from which 550 mud samples were collected for examination for *Cl. botulinum*; a further four samples (not shown) were collected in the Shetland Isles. Each small black spot represents a single sample. Each larger black area represents numerous samples (number shown) from a single locality.

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Lancashire and the Lake District

The 42 samples included 12 from the WT refuge at Martin Mere, five of which contained type B. Of 30 samples from 16 Cumbrian lakes, 16, 1 and 1 contained types B, C and E respectively. Bassenthwaite Lake, Blelham Tarn, Coniston Water, Ennerdale Water, Esthwaite Water, Grasmere, Lowes Water, Thirlmere, Ullswater, and Windermere all contained type B, and Grasmere contained C and E in addition. In Grasmere, B and E occurred in mud in shallow-water areas, and B and C in mud at a depth of 16 m.

County Durham, North Yorkshire, Humberside and Lincolnshire

The 66 samples included ten from the recently established WT refuge at Washington, all of which were negative. Of 16 samples from North Yorkshire seven, three and one contained types B, C and D respectively; type C occurred near Raskelf in a stream, a pond and the river Swale, and type D occurred in a reservoir. Type B was found in each of the RSPB reserves at Hornsea Mere, Fairburn Ings and Blacktoft Sands but in only four of 27 samples; it was also found in six of 13 further samples from sites near the coast in Humberside and Lincolnshire.

East Anglia

Sixty samples were collected. Nine of 24 samples from the WT refuge at Peakirk contained type B, and of five samples from the Snettisham area one – collected from an RSPB reserve – contained type C. A further 31 samples were taken from the Ouse Washes and surrounding area; of these, 17 contained B and four C. Three of the samples that contained C came from the RSPB reserve in the Ouse Washes, and the fourth from the WT refuge at Welney.

Bedfordshire, Hertfordshire, Surrey and Middlesex

Thirty-two samples were collected. Of ten from Whipsnade Park (Zoological Society of London) one contained type B, and of eight from Tring reservoirs three contained B and one C. A series of six artificial lakes at Hampton Court proved to be particularly interesting: of 11 samples, 7, 1, 2 and 2 contained types B, C, D and E respectively, and four samples contained more than one type. Three samples taken from the depths of two nearby reservoirs each contained type E.

Scotland

Type B was found in 18 of 77 samples, including those from Lochs Bá, Chon, Levan, Lochy, Lomond, Oich, Rusky and Venachar. Type C occurred in a sample from a saline mudflat on the Moray Firth and in a loch at Linlithgow. The samples included 14 from the WT refuge at Caerlaverock, of which six contained type B. Four samples from the Shetland Isles (Unst 2, Fetlar 2) and one from Islay were all negative.

The demonstration of type B in samples collected to the north of Glasgow did not usually require trypsinization of culture filtrates – the ratio of samples that did not require such treatment to those that did was 2:1. A strikingly different

ratio (approximately 1:8) was found in respect of samples from all parts of the British Isles south of Glasgow.

Wales and Shropshire

Of 43 samples, ten contained type B. The samples containing B included two of 11 taken in or near the National Nature Reserve and RSPB reserve on the Dovey estuary. Type C was found in two samples from Ellesmere and in a lake sample from Llangorse. Type D occurred in a sample from a mudflat near Caldicot on the Severn estuary, and type E in samples from a canal at Welshpool and the river Usk at Sennybridge.

Northern Ireland and Eire

Fifty-five samples were examined and type B was demonstrated in ten, including those from the following Loughs: Upper Erne, Lower Erne and Neagh (Northern Ireland); Bofin, Brittas, Ennell, Errit and Manalla (Eire). No samples were collected in the southern part of Eire.

DISCUSSION

It is of interest to compare the present survey with surveys made in the Norfolk Broads (Borland *et al.* 1977), the London area (Smith & Moryson, 1975) and the Camargue (Smith & Moryson, 1977*a*); the methods remained constant throughout all these investigations, except that in the London area alone apparently negative sites were resampled once and this increased the positive results from 55% to 72·5%. The percentages (to the nearest whole numbers) of samples found to contain (1) *Cl. botulinum* of any type, (2) type B, (3) type C, (4) type D, (5) type E, and (6) more than one type, were respectively: 98, 62, 51, 0, 60, 58 (Norfolk Broads survey); 72, 45, 17, 1, 14, 6 (London survey); 35, 30, 3, 1, 3, 2 (present survey); 5, 0, 0, 0, 5, 0 (Camargue survey). Thus the present survey showed a prevalence of *Cl. botulinum* in mud that, although strikingly high by comparison with the prevalence found in British soil (Meyer & Dubovsky, 1922; Leighton & Buxton, 1928; Haines, 1942) and marine deposit (Cann *et al.* 1968), was considerably lower than that in mud in the London area and Norfolk Broads. The possible reasons for the very low prevalence in Camargue mud have already been discussed by Smith & Moryson (1977*a*).

The Loch Maree tragedy (Leighton, 1923) was caused by the toxin of *Cl. botulinum* type A, and type A has been found in British soil (Leighton & Buxton, 1928; Haines, 1942). Our failure to find type A in any mud sample is therefore surprising, though it is similar to that experienced by Haagsma (1974) in the Netherlands. Smith (1975) has described strains of *Cl. perfringens*, *Cl. sporogenes* and *Bacillus cereus* that occurred in certain soil samples and interfered with growth and toxin production of *Cl. botulinum* type A in laboratory culture. However, it is difficult to see how such organisms could account for our uniformly negative results in respect of type A.

A high prevalence of type B was found in all areas examined but, as already stated, samples taken north of Glasgow differed from those taken to the south in

respect of the need to trypsinize culture filtrates in order to obtain positive results. This may have been because of differences in the type B strains concerned, or because of indirect causes such as differences in other components of the bacterial population of the mud samples. It seems probable that such components would themselves be influenced by variations in the chemical and physical properties of the environment in different geographical areas. Certainly it was noticeable that, to the north of Glasgow, the mud was often sparse and gritty, instead of deep and soft.

Type C, the organism almost always responsible for botulism in waterfowl, was much less common than B. It is possible that intensive searches of those areas in which demonstrations of type C appeared to be clustered might reveal localized foci of heavy contamination.

Type D, which differs from type C only by virtue of the different bacteriophage that it carries (Eklund & Poysky, 1974), was rarely found, yet the six positive samples represented areas as widely separated as North Yorkshire, Wales, Dorset and Middlesex.

As in the survey of the London area (Smith & Moryson, 1975), type E occurred about as frequently as C. Surveys of aquatic environments carried out from this Institute have revealed only one area – the Norfolk Broads – in which the prevalence of B was rivalled by that of C and E (Borland *et al.* 1977).

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