Clostridium botulinum in British soil

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(Received 18 February 1980)

SUMMARY

Soil samples from various parts of Britain were examined for Clostridium botulinum by a sensitive technique comparable with that recently used for mud samples from British aquatic environments. The results showed beyond doubt that in Britain the prevalence of the organism in soil is much lower than in mud. Of 174 samples from all sites examined only 10 (5.7%) could be shown to contain Cl. botulinum; this finding was consistent with the results of surveys made by less sensitive techniques in 1922, 1928 and 1942. No type other than B was found. The evidence suggested that in certain localized areas the prevalence was likely to be high.

Three sites associated for many years with animals were included in the survey; at the Zoological Society's premises at Regent's Park and Whipsnade, and at the Market paddocks, Gorgie, Edinburgh, the prevalence of Cl. botulinum was either very low, or nil. In an earlier survey of the redeveloped site of the former Metropolitan Cattle Market, London, 25% of soil samples gave a positive result and no less than four types (B, C, D and E) were demonstrated.

INTRODUCTION

Surveys by Meyer & Dubovsky (1922), Leighton & Buxton (1928) and Haines (1942) suggested that Clostridium botulinum (type A or B) was present in only 4.8% of British soil samples. Recent studies at this Institute showed a much higher prevalence in mud from British aquatic environments. Thus, 72.5% of the lakes and waterways of London contained Cl. botulinum (Smith & Moryson, 1975); of 554 mud samples from various parts of England, Wales, Scotland, Ulster and Eire, 196 (35%) contained Cl. botulinum (Smith, Milligan & Moryson, 1978); of 45 mud samples from the Norfolk Broads, 44 (98%) contained Cl. botulinum (Borland, Moryson & Smith, 1977). In general type B was found most commonly, types C and E less commonly, and type D rarely; the Norfolk Broads were unusual in that the prevalence of type B was rivalled by that of types C and E (Smith, 1978), and the samples often contained more than one type.

In the early soil surveys excessive preliminary heating of samples may have prevented the demonstration of the non-proteolytic strains of types B, C, D, E and F (Meyer, 1956; Cann, Taylor & Hobbs, 1975). It therefore seemed possible that the apparent difference between the prevalence of Cl. botulinum in soil and mud in
Britain was spurious. On the other hand, in a small survey in London made by a sensitive technique, only one of 25 soil samples could be shown to contain *Clostridium botulinum* (Smith & Moryson, 1977). The single positive sample came from the redeveloped site of the former Metropolitan Cattle Market, London; examination of a further 60 samples from this site (Smith & Milligan, 1979) showed that 15 (25%) contained *Clostridium botulinum*, and that no less than four types (B, C, D and E) were present. Although not proved, it seemed likely that the striking prevalence of the organism was due to faecal contamination by a small proportion of the many millions of farm animals brought to the market before its closure in 1939.

The purposes of the present study were (1) to confirm that in Britain the prevalence of *Clostridium botulinum* in mud from aquatic environments is higher than in soil, (2) to reassess the prevalence of *Clostridium botulinum* in British soil by means of a sensitive technique, and (3) to investigate further the possible influence of animals on the distribution of *Clostridium botulinum* in soil.

**MATERIALS AND METHODS**

**Method of sampling**

Sampling was carried out between January and September 1979. Each soil sample consisted of material taken with an autoclaved trowel from three different sites within an area of a few square metres, and from different depths up to 30 cm. Each sample was placed in a plastic bag, thoroughly mixed, and stored at ambient temperature until examined.

Certain samples (see below) were collected in pairs. Each pair consisted of two samples collected 30–100 m apart.

**Source of samples**

*England, Wales and Scotland.* Eighty samples were collected in 40 pairs as follows: Bedfordshire 1 pair, Berkshire 1, Cornwall 2, Dorset 3, East Sussex 1, Essex 2, Hampshire 2, Kent 4, Norfolk 1, Northamptonshire 1, Salop 1, Wiltshire 9; Gwynedd 1, Powys 4; Berwickshire (Border Region) 1, Caithness (Highland Region) 4, Sutherland (Highland Region) 2. The 40 pairs of samples were from the following types of land: arable land 3 pairs, common land 3, garden 0, hill 0, orchard 1, pasture 14, woodland 7. The collections were made with the assistance of colleagues at the Institute of Zoology, as and when opportunities arose; it was not possible to achieve an even distribution of sampling over the country.

*Zoological Society of London (Whipsnade).* Thirty-eight samples were collected in 19 pairs from sites well distributed across the animal paddocks. Whipsnade Park has, since 1931, been used to accommodate part of the Society's collection of animals under semi-natural conditions.

*Zoological Society of London (Regent's Park).* Twenty-eight samples were collected from sites well distributed among the animal enclosures. The Society's collection has been housed at Regent's Park since 1827.

*Market paddocks, Gorgie, Edinburgh.* Twenty-eight samples were collected from sites well distributed across the paddocks. The paddocks have been in use since 1910 for cattle and sheep.
Table 1. Clostridium botulinum in British soil

<table>
<thead>
<tr>
<th>Source of samples</th>
<th>Number of samples</th>
<th>Number (and %) of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>England, Wales and Scotland</td>
<td>80*</td>
<td>8† (10)</td>
</tr>
<tr>
<td>Zoological Society (Whipsnade)</td>
<td>38†</td>
<td>1 (2·6)</td>
</tr>
<tr>
<td>Zoological Society (Regent's Park)</td>
<td>28</td>
<td>1 (3·6)</td>
</tr>
<tr>
<td>Market paddocks, Edinburgh</td>
<td>28</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>174</td>
<td>10 (5·7)</td>
</tr>
</tbody>
</table>

* Forty pairs of samples (see Materials and Methods).
† Nineteen pairs of samples.
‡ Five pairs of samples supplied the eight positive results; thus in three instances both members of a pair contained Cl. botulinum. The positive samples all contained Cl. botulinum type B.

Examination of samples

The method was identical with that used by Smith & Moryson (1977).

RESULTS

The results are given in Table 1. Of 174 samples from all sites only 10 (5·7%) could be shown to contain Cl. botulinum; no type other than B was found.

Of the 80 samples (40 pairs) from England, Wales, and Scotland 8 (10%) contained Cl. botulinum. In three instances both members of a pair of samples gave positive results. Of the three pairs concerned two were collected, about 0·8 km apart, from a pasture and a garden in Powys; the third pair came from a garden in Essex. The remaining two positive samples came from pasture land in Dorset and Kent.

Although Cl. botulinum type B was detected on the Zoological Society's premises at Regent's Park and Whipsnade, the prevalence was very low. Cl. botulinum could not be detected in 28 soil samples from the Market paddocks in Edinburgh.

DISCUSSION

Despite the uneven distribution of sampling sites over the country, and the modest number of samples, the survey confirmed beyond doubt that in Britain the prevalence of Cl. botulinum in soil is much less than that in mud from aquatic environments. This conclusion was established despite the examination of soil by a technique (see Smith & Moryson, 1977) that was even more sensitive than that used earlier for the examination of mud (Smith & Moryson, 1975; Borland et al. 1977; Smith et al. 1978). The proportion of positive samples was consistent with that found in the early British soil surveys. Leighton & Buxton (1928) and Haines (1942) found both types A and B. Like Meyer & Dubovsky (1922), we found only type B. Type A has not been found in any survey of mud or soil made from this Institute. Type A botulism in man is, however, known in Britain; thus, the Loch Maree tragedy (Leighton, 1923) and a recent case of infant botulism (Turner et al. 1978) were both caused by Cl. botulinum type A.
The collection of many soil samples in pairs revealed that in certain localized areas the prevalence of *Clostridium botulinum* was likely to be high. Thus, although the great majority of samples gave negative results, two pairs collected approximately 0.8 km apart supplied four positive results, and a third pair on the opposite side of the country two further positive results.

Smith & Milligan (1979) demonstrated a striking prevalence of *Clostridium botulinum* at the redeveloped site of the former Metropolitan Cattle Market, Islington, London. No such distribution was found on the Zoological Society's premises at Regent's Park and Whipsnade, or at the Market paddocks, Edinburgh. A firm explanation cannot be offered. These sites, although associated with animals for many years, differed in numerous respects from the Islington site. Among the factors that conceivably affect the prevalence of *Clostridium botulinum* are type of soil, disturbance of soil by human agencies, climate, and the size and rate of change of animal populations responsible for faecal contamination.

For assistance in the collection of soil samples thanks are due to the following: colleagues at the Institute of Zoology; members of the staff of keepers at The Zoological Society of London; Mr P. L. F. Smith, B.A., M.R.C.V.S.; Mr W. T. Forrest, M.R.C.V.S., Environmental Health Department, Edinburgh.

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