

Clostridium botulinum in British soil

BY G. R. SMITH AND ANGELA M. YOUNG

*Nuffield Laboratories of Comparative Medicine, Institute of Zoology,
The Zoological Society of London, Regent's Park, London NW1 4RY*

(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 *Cl. 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 *Cl. 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 *Cl. botulinum* in mud from aquatic environments is higher than in soil, (2) to reassess the prevalence of *Cl. botulinum* in British soil by means of a sensitive technique, and (3) to investigate further the possible influence of animals on the distribution of *Cl. botulinum* in soil.

MATERIALS AND METHODS

Method of sampling

Sampling was carried out between January and September 1970. 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 6, hill 6, 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

Source of samples	Number of samples	Number (and %) of positive samples	
England, Wales and Scotland	80*	8‡	(10)
Zoological Society (Whipsnade)	38†	1	(2.6)
Zoological Society (Regent's Park)	28	1	(3.6)
Market paddocks, Edinburgh	28	0	(0)
Total	174	10	(5.7)

* 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 *Cl. 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 *Cl. 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 *Cl. 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.

REFERENCES

- BORLAND, E. D., MORYSON, C. J. & SMITH, G. R. (1977). Avian botulism and the high prevalence of *Clostridium botulinum* in the Norfolk Broads. *Veterinary Record* 100, 106.
- CANN, D. C., TAYLOR, L. Y. & HOBBS, G. (1975). The incidence of *Clostridium botulinum* in farmed trout raised in Great Britain. *Journal of Applied Bacteriology* 39, 331.
- HAINES, R. B. (1942). The occurrence of toxigenic anaerobes, especially *Clostridium botulinum*, in some English soils. *Journal of Hygiene* 42, 323.
- LEIGHTON, G. R. (1923). Report of the circumstances attending the deaths of eight persons from botulism at Loch Maree (Ross-shire). *Report of the Scottish Board of Health*. Edinburgh: H.M. Stationery Office.
- LEIGHTON, G. R. & BUXTON, J. B. (1928). The distribution of *Bacillus botulinus* in Scottish soils. *Journal of Hygiene* 28, 79.
- MEYER, K. F. (1956). The status of botulism as a world health problem. *Bulletin of the World Health Organization* 15, 281.
- MEYER, K. F. & DUBOVSKY, B. J. (1922). The occurrence of the spores of *Bacillus botulinus* in Belgium, Denmark, England, the Netherlands and Switzerland: *Journal of Infectious Diseases* 31, 600.
- SMITH, G. R. (1978). Botulism, waterfowl and mud. *British Veterinary Journal* 134, 407.
- SMITH, G. R. & MILLIGAN, R. A. (1979). *Clostridium botulinum* in soil on the site of the former Metropolitan (Caledonian) Cattle Market, London. *Journal of Hygiene* 83, 237.
- SMITH, G. R., MILLIGAN, R. A. & MORYSON, C. J. (1978). *Clostridium botulinum* in aquatic environments in Great Britain and Ireland. *Journal of Hygiene* 80, 431.
- SMITH, G. R. & MORYSON, C. J. (1975). *Clostridium botulinum* in the lakes and waterways of London. *Journal of Hygiene* 75, 371.
- SMITH, G. R. & MORYSON, C. J. (1977). A comparison of the distribution of *Clostridium botulinum* in soil and in lake mud. *Journal of Hygiene* 78, 39.
- TURNER, H. D., BRETT, E. M., GILBERT, R. J., GHOSH, A. C. & LIEBESCHUETZ, H. J. (1978). Infant botulism in England. *Lancet* i, 1277.