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A comparison of the distribution of *Clostridium botulinum* in soil and in lake mud

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SUMMARY

In 1975, 25 soil samples were collected from the London area. Of these, 20 were obtained 200–300 yards from 20 lakes that had been shown in 1974 to contain mud contaminated with one or more of types B, C, D and E of *Clostridium botulinum*. By means of a technique comparable with that used for the examination of mud, the 20 soil samples were found negative. The remaining 5 soil samples, obtained from sites that were not in close proximity to lakes, were also negative except for one that contained type B.

INTRODUCTION

It is not certain whether lake mud provides a more favourable environment than soil for *Clostridium botulinum* (Smith & Moryson, 1975). This point has been studied in an investigation that may be of interest in relation to earlier surveys of soil and mud for the organism.

MATERIALS AND METHODS

Source of samples

During October and November 1975, 25 soil samples were collected from the London area within a radius of 11 miles from Charing Cross. Of these samples, 20 were taken from points 200–300 yards distant from 20 lakes examined during 1974 by Smith & Moryson (1975) and found to possess mud containing one or more types of *Cl. botulinum*. These 20 lakes were well distributed over the London area and all except two had given a positive result on the basis of a single mud sample; the two exceptions had given positive results on resampling.

The 20 soil samples referred to above came from sites classified as park grassland (9 samples), rough grassland (5) and woodland (6). Five additional soil samples came from sites that were not in close proximity to lakes; these sites were classified as park grassland (3), rough grassland (1) and woodland (1).

Method of sampling

As far as possible the method was made comparable with that used by Smith & Moryson (1975) for sampling lake mud, but negative sites were not resampled. Each soil sample consisted of material taken with a trowel from different depths up to

30 cm and from different sites within an area of a few square metres. Samples were placed in plastic bags and thoroughly mixed before being stored at – 20° C. Trowels were washed and autoclaved before re-use.

Examination of samples

The method was identical to that used by Smith & Moryson (1975) for mud samples except that each 50 g soil sample was mixed with 100 ml – not 50 ml – of phosphate buffer. This more than compensated for the lower water content of soil and therefore increased the sensitivity of the method; it is important to bear this point in mind as it constituted a bias against the results that were obtained.

RESULTS

The 20 soil samples taken 200–300 yards from 20 lakes all gave negative results. Mud samples collected from the lakes during the previous year had given the following positive results (Smith & Moryson, 1975): *Cl. botulinum* type B (7 lakes); type C (5 lakes); type D (1 lake); type E (4 lakes); types B and E (1 lake); types B, C and E (2 lakes). Of the 20 lakes, only two – both containing type B alone – had appeared negative until a second sample was examined.

Of five additional soil samples from sites that were not in close proximity to lakes, one was shown to contain *Cl. botulinum* type B; trypsinization was necessary for the demonstration of toxin in culture filtrate. Thus of 25 soil samples, only one (4%) could be shown to contain *Cl. botulinum*.

DISCUSSION

Smith Moryson & Walmsley (1976) commented that the high prevalence of *Cl. botulinum* recently found in lake mud from the London area (72·5%) and other parts of Britain, and from the Netherlands, was strikingly different from the prevalence of 4–8% found by three surveys of soil in Britain between 1922 and 1942. It seemed possible that lake mud might provide a more favourable environment than soil (Smith & Moryson, 1975).

Soil samples for the present investigation were collected from sites close to lakes previously shown to contain mud contaminated with *Cl. botulinum*. Examination of the soil samples showed that, under the conditions of the investigation, mud clearly differed from soil in respect of the occurrence of *Cl. botulinum*. Although certain modifications in technique were unavoidable because of the physical difference between soil and mud, care was taken to ensure that they did not invalidate the results. The soil and mud surveys were made in 1975 and 1974 respectively, but it seemed reasonable to assume that the comparison between the two was nonetheless reliable.

Botulism in waterfowl is almost always due to *Cl. botulinum* type C. The numerous factors that are associated with explosive multiplication of this organism and consequent outbreaks of disease have been reviewed by Smith (1976). They include protracted periods of hot weather, enlarged areas of shallow stagnant

water, alkalinity, an abundance of aquatic invertebrates, and oxygen depletion associated with large amounts of decaying vegetation. Multiplication may occur in sludge or rotting vegetation, or within the favourable microenvironment afforded by particles of decaying animal matter (Bell, Sciple & Hubert, 1955). It seems possible that the same factors might also stimulate, to some extent at least, the multiplication of certain types of *Cl. botulinum* other than type C. *Cl. botulinum* might be expected to spread easily in aquatic surroundings and to be transferred to other aquatic environments by waterfowl that move from place to place (Smith, 1976). For these reasons it may not seem surprising that the present investigation indicated a much higher prevalence of *Cl. botulinum* in mud than in soil.

Nevertheless, it should be stressed that our investigation was concerned with soil and mud only in the London area and whilst it seems probable that similar findings might often be obtained elsewhere, it would be unwise to assume that they are universally applicable. The recent survey (Smith, Moryson & Walmsley, 1976) of *Cl. botulinum* in mud from the lakes, marshes and waterways of the Camargue indicated an unusually low prevalence (4·5%); the reasons were not clear, but unsuitability of the mud for proliferation of the organism was a possible explanation. On the other hand, for reasons that once again are not clear, the prevalence of *Cl. botulinum* in the soil of California is known to be unusually high (Meyer, 1956), Meyer & Dubovsky (1922) having found that 30% of Californian specimens of soil, vegetables, fruit, feeds, manure and sewage gave positive results. Viewed against the background of these earlier findings, the present investigation, while answering some questions, raises still others.

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