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Landfill sites, botulism and gulls

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SUMMARY

Botulism due to *Clostridium botulinum* type C causes considerable mortality in gulls in the UK, and refuse disposal sites are suspected as a major source of toxin. *C. botulinum* types B, C and D were each found in 12 (63·2%) of 19 landfill sites examined. Type E was detected in only one (5·2%) and types A, F and G were not found. The prevalence of type C spores was much higher than that demonstrated in the UK environment by earlier surveys. The presence of these spores, together with the rotting organic matter and generated heat associated with landfill sites, undoubtedly leads to bacterial proliferation and toxigenesis. This is likely to result in botulism in scavenging gulls unless skilled landfill management prevents the ingestion of toxic material. Type D spores were previously shown to be rare in the UK environment and their high prevalence on landfill sites was therefore surprising. Four composite samples of refuse collected before distribution on a landfill gave negative results for *C. botulinum* and it seems likely that the gulls themselves play a major role in introducing contamination.

INTRODUCTION

The tendency of animals of many species to ingest rotting organic matter accounts for the much higher incidence of botulism in animals (wild and agricultural) than in man. Of all the microbiological diseases of free-living birds, botulism probably takes the heaviest annual toll. Mortality, sometimes on a huge scale, occurs each year in waterfowl on lakes, mudflats and marshes in many parts of the world, particularly in the western USA, where the disease was first recognized in about 1910, being known as 'western duck sickness' until its aetiology was established in the early 1930s [1].

Botulism in waterfowl in the UK was first diagnosed in 1969 [2, 3] and has been reported on numerous occasions since. The birds affected are often ducks in public parks, but a substantial outbreak in the Norfolk Broads has also been described [4].

Since about 1975 it has become increasingly clear that botulism causes many deaths in gulls in Britain and Ireland. In that year a major outbreak affecting several species of the genus *Larus* occurred between June and October around the Firth of Forth, causing at least 2000 deaths [5], and the disease was also confirmed in Northern Ireland, Anglesey and the Wirral [6]. Subsequent reports referred to

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outbreaks on Walney Island (Cumbria) [1], the Mersey Estuary [7], Motherwell (Scotland) [8], the Bristol Channel [9, 10], Dublin Bay [11] and elsewhere.

Botulism in gulls, as in other wild birds, is almost always caused by the toxin of *Clostridium botulinum* type C, though on the shores of Lake Michigan type E botulism is known to occur in gulls and loons [12, 13]. Type C organisms will not grow, still less produce toxin, at temperatures of 10 °C and below [14], and it is not surprising therefore that duck botulism is a disease of warm weather. Occasional cases that occur in the colder months are almost certainly due to persistent toxin formed during the previous summer [8]. Gulls, too, are mainly affected in the summer, but some outbreaks occur outside the duck botulism season [1]. An outbreak in Motherwell [8] during an unusually cold December (mean maximal air temperature, 3·5 °C) is of particular interest as it suggests that the toxin was formed in a heated environmental site. Such observations, together with the known scavenging habits of gulls and their propensity to feed on refuse, have led to a suspicion that landfill sites, parts of which generate heat, are a common source of toxin for these birds. The demonstration of type C spores by one of us (G.R.S.) on a refuse disposal site in Midlothian in 1975 [5] and on two such sites in West Wales in 1985 and 1986 [unpublished observations] during investigations on gull botulism support this hypothesis.

The purpose of the present study was to determine the prevalence of *C. botulinum* (types A–G) on landfill sites in the UK, with particular reference to type C, and to set the findings within the context of earlier environmental studies. In these studies type C spores were found rarely in British soil [15, 16] and in only 3% of mud samples from aquatic environments in the UK and Eire (554 samples) [17] other than those from London's lakes and waterways [18] and the Norfolk Broads [4], 17 and 51% of which, respectively, contained *C. botulinum* type C.

MATERIALS AND METHODS

Samples from landfills

In all, 182 samples (111 of soil, 65 of mud and 6 of water) were collected from 19 landfill sites (Fig. 1, Table 1). The sampling method was based on that described previously [18]. For each soil sample several handfuls of surface and subsurface material were taken, with plastic disposable gloves, from four or five sites *c.* 1–3 m apart. As a rule, mud samples, each weighing *c.* 20–30 g, were collected as described above from shallow peripheral areas of lakes, lagoons or streams. Some mud samples, however, were collected from deeper water by means of a sterilized plastic beaker fixed to a long pole. Soil and mud samples were placed in plastic bags. Water samples (*c.* 1 litre) were collected in autoclavable plastic screw-capped bottles. Precautions were taken to avoid cross-contamination during sampling, in transit, and in the laboratory. The samples were stored at −20 °C until examined.

Refuse samples

On arrival by rail at landfill no. 2, containers, each holding *c.* 13·5 tons of North London refuse, were removed from the railhead by lorry and tipped on to the site. Four composite samples were collected from separate container loads as follows. Before the tipped-out refuse was disturbed and compacted, a composite sample

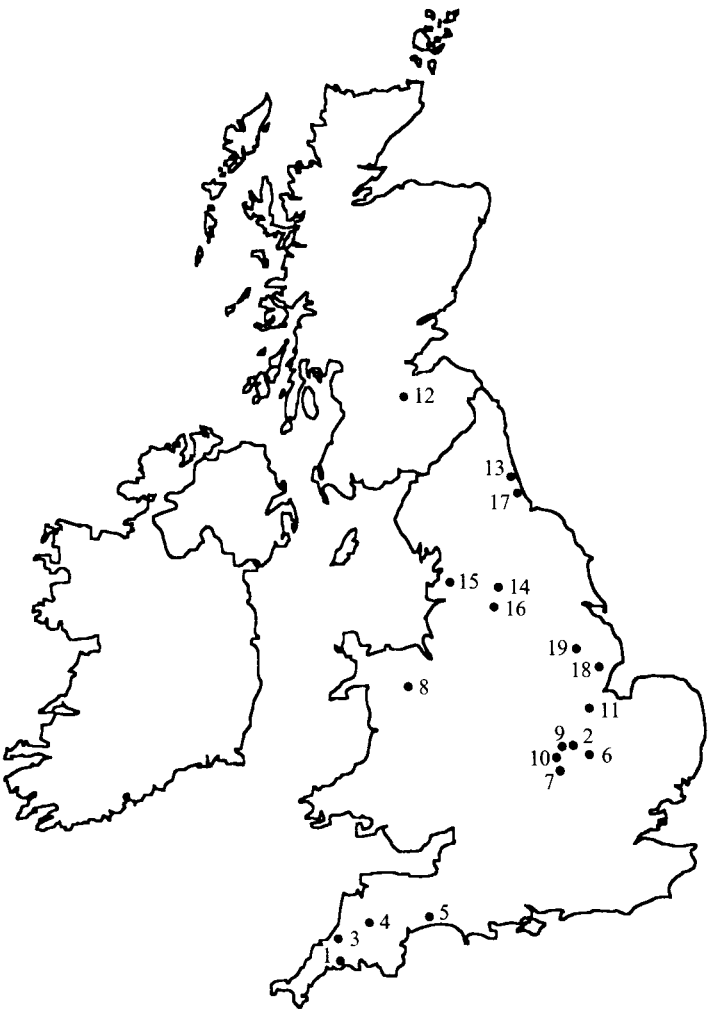

Fig. 1. Approximate location of the 19 landfill sites examined.

consisting of *c.* 60 sub-samples, each weighing *c.* 20–30 g, was collected with plastic disposable gloves, taking great care to avoid material in contact with the landfill, which might have led to a false positive result. The material collected consisted mainly of organic matter such as meat, bones, vegetables, fruit and canned food remnants. Each composite sample was placed in a plastic bag, taken to the laboratory, and stored at −20 °C.

Examination of samples

The methods were based on those used previously for mud [18] and soil [19].

Briefly, fine debris containing bacterial spores was washed from a 50-g sample of soil or mud and centrifuged at 5000 g for 30 min. The deposit was then cultured in cooked meat medium, with and without preliminary heating at 60 °C for 1 h. After incubation at 30 °C for 6–8 days, the culture filtrates were tested, with and without trypsinization, for their ability to produce botulism in mice. Any toxin present was then typed by a neutralization test with specific antitoxins.

Each 1-litre water sample was allowed to thaw and sediment overnight at room

Table 1. *Prevalence of* C. botulinum *of various types in 19 landfill sites*

Landfill no.	Number of samples	Number of samples containing *C. botulinum* of any type	type B	type C	type D	type E	unknown type*
1	11	5	2	3	0	0	0
2	12	7	0	2	4	0	1
3	8	4	0	1	2	0	1
4	10	1	1	0	0	0	0
5	10	4	1	2	1	0	0
6	10	4	1	1	2	0	0
7	11	4	0	2	0	0	2
8	10	2	1	0	1	0	0
9	8	2	1	1	0	0	0
10	11	4	0	0	1	1	2
11	10	9	1	1	5	0	2
12	7	6	0	2	4	0	0
13	10	0	0	0	0	0	0
14	10	2	1	1	0	0	0
15	5	4	0	1	3	0	0
16	12	9	3	0	3	0	3
17	10	1	1	0	0	0	0
18	11	3	1	0	2	0	0
19	6	5	1	1	2	0	1

* Toxin in culture filtrate too weak to be typed.

C. botulinum types B–E occurred in the following percentages, shown in brackets, of samples (B, 8·2; C, 9·9; D, 16·5; E, 0·5) and landfill sites (B, 63·2; C, 63·2; D, 63·2; E, 5·2).

No type A, F or G organisms were demonstrated.

temperature; 250 ml of supernate were then distributed into 10 Universal containers and centrifuged at 5000 g for 30 min. The deposit was subsequently examined as described above.

Each of the four composite refuse samples was thawed overnight at room temperature and thoroughly mixed through the plastic bag by hand. At least two pieces (*c.* 1 g each) of each identifiable sub-sample were placed in a mortar and if necessary chopped with scissors. After grinding and mixing with a pestle, a 50-g portion of this sample was weighed out and 100 ml of phosphate buffer, pH 7·0, were added. After further mixing with a mortar and pestle the material was transferred to a tall container (measuring cylinder) and allowed to sediment for 30 min. The fine debris contained in the supernate was then deposited by centrifugation and examined by the method used for fine debris from soil, mud and water samples (above). For purposes of confirmation, the examination of each of the four composite refuse samples was repeated once.

RESULTS

Examination of landfill samples

Samples of mud (65), soil (111) and water (6) were collected from a total of 19 landfill sites. Of the mud samples, 38 (58·4%) contained *C. botulinum* of one type or another; of the soil samples 35 (31·5%); and of the water samples, 3 (50%).

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As shown in Table 1, *C. botulinum* types B, C and D were each found in 12 (63·2%) of the 19 landfill sites, and type E in 1 (5·2%). Of the total number of 182 samples, 15 (8·2%) contained type B spores, 18 (9·9%) type C, 30 (16·5%) type D, and 1 (0·5%) type E.

Trypsinization of culture filtrates was not needed for the demonstration of type C or D toxin in any of the positive culture filtrates. It was needed, however, for 9 of the 15 type B-positive filtrates and for the single type E-positive filtrate. None of the samples examined contained more than a single type of *C. botulinum*, and types A, F and G were not found.

Examination of refuse samples

Four composite samples of refuse from landfill site no. 2 gave negative results on examination for *C. botulinum*.

DISCUSSION

Surveys made from this Institute during the 1970s and early 1980s showed that, in general, 30% of mud samples ($n = 554$) from British and Irish aquatic environments contained *C. botulinum* type B, 3% contained type C or E, and 1% type D [17]. However, in certain areas the prevalence was higher. Thus in London's lakes and waterways and in the Norfolk Broads the percentages of positive samples were, respectively: type B, 45 and 62; type C, 17 and 51; type D, 1 and 0; and type E, 14 and 60 [4, 18]. With the exception of the site of the former Metropolitan Cattle Market, London, which was heavily contaminated with *C. botulinum* types B, C, D and E [16], soil was much less rich than mud as a source of *C. botulinum* [15, 19], only 5% of samples being contaminated and type B alone being identified.

Against this background, and assuming that the geographical distribution and prevalence of the various types of *C. botulinum* have not changed much during the past 10–20 years, the findings of the present study are of considerable interest. Spores of *C. botulinum* type C, the cause of botulism in gulls, were present in $> 60\%$ of the landfill sites examined. This was a much higher prevalence than that found in the earlier surveys of mud and soil (see above). The presence of these spores, together with rotting organic matter, undoubtedly leads to type C toxigenesis and to botulism in the gulls almost invariably to be found scavenging on landfill sites. The frequent demonstration of *C. botulinum* type D, with a prevalence similar to, if not greater than, that of type C, was particularly surprising in view of the low prevalence found in earlier surveys.

The examination of four composite samples of raw refuse immediately before its distribution on a landfill site gave negative results for *C. botulinum*. This would indicate that the main source of contamination of landfill sites is probably not the refuse itself. *C. botulinum* spores are probably transferred from place to place by birds, either in the alimentary tract or on their external body surface [20]. It seems likely, therefore, that gulls and other scavenging birds are often responsible for contaminating sites to which they are attracted, including 'landfills', with *C. botulinum* from similar sites elsewhere. The gulls are thereby exposed to an increased risk from botulism and after death their rotting carcasses may

contribute to the available toxin in the environment. These risks are reduced if skilled landfill management minimizes the ingestion of rotting organic matter.

The propagation of *C. botulinum* organisms, with concomitant toxigenesis, is dependent on the presence of a suitable growth substrate, anaerobic conditions, and a suitable temperature. Refuse disposal sites satisfy these requirements, by virtue of their abundant content of rotting organic matter and of the heat which they generate. Landfill sites therefore represent foci where *C. botulinum* can proliferate and, given adequate vectors, be a source of contamination for other sites. For this reason it would seem prudent to attempt to deny gulls access to landfill sites by whatever practical means are available. This would have the additional advantage of reducing the mortality from botulism in gulls and thereby avoiding the public concern to which such mortality often gives rise.

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