***Clostridium botulinum* type C in the Mersey estuary**

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(Received 21 April 1982; accepted 7 July 1982)

SUMMARY

Nineteen of 98 samples of mud or sand taken from the Mersey estuary in 1981 contained *Clostridium botulinum* type C, the organism almost always responsible for botulism in water birds. In the Dungeon and Score Bank areas, where many dead and dying birds were found during the period September–December 1979, almost half the samples contained type C. Most of the positive samples were essentially muddy rather than sandy. The findings do not prove that botulism contributed to the 1979 mortality but are nonetheless thought-provoking, particularly because type C – unlike type B – is by no means ubiquitous in Britain. Type B was present in 12.2 % of samples from the Mersey estuary.

INTRODUCTION

Whenever mass mortality suggestive of poisoning suddenly occurs in water birds, an urgent question invariably arises. Is the disaster man-made or natural? The choice usually lies between pollution by industrial or agricultural chemicals on the one hand and botulism on the other, but it is always conceivable that both factors may be operating simultaneously.

Botulism in water birds occurs in Britain (Smith, 1982), but reliable diagnosis requires considerable knowledge and expertise both in the collection of samples and in their laboratory examination for *Clostridium botulinum* type-C toxin.

During the period September to December 1979 some 2400 birds are known to have died on the middle reaches of the Mersey estuary, mainly between Oglet and Hale Marsh on the north bank and the river Gowy and Weaver Sluices on the south (Head, D'Arcy & Osbaldeston, 1980). This part of the estuary is very shallow and at low water consists of mud and sand banks separated by channels. The mortality affected waders, gulls, and wildfowl, but approximately 56 % of the dead birds were dunlin (*Calidris alpina*). In 1980 further deaths occurred, but somewhat earlier in the year (August and September) and mainly in gulls. In the 1979 outbreak, post-mortem examinations failed to reveal a cause of death, but chemical analysis suggested that poisoning by lead, some of it in the organic trialkyl form, played a part (Head *et al.* 1980); the possibility of botulism was not, however, fully

Fig. 1. *Cl. botulinum* type C in the Mersey estuary. Sketch map shows positive (●) and negative (○) sites. The arrows indicate three samples taken from sites off the map (map references SD 293004, SD 296005, and SJ 607878).

investigated at the time. In the 1980 outbreak, and again in the summer of 1981, laboratory investigations confirmed the presence of botulism in gulls in the locality (D. F. Gibbons, personal communication).

Uncertainty as to whether botulism could have played a part in the 1979 mortality gave rise to the present study – a survey of sediment in the Mersey estuary for *Cl. botulinum* type-C spores.

MATERIALS AND METHODS

Collection of samples from the Mersey estuary

Ninety-eight samples were collected by the methods of Smith & Moryson (1975) during the period May–November 1981 (Fig. 1). All except three came from a 14-mile stretch of the estuary – not only from the shores but also from mid-channel areas exposed at low tides. A hovercraft was used where necessary. The sediments from which the samples were obtained varied; some consisted of deep, soft, often fetid mud, and others mainly of sand. Typical salinity values of the estuarine water in the locality of Speke and Garston range from 11 to 27 parts per thousand, depending on tidal state and freshwater flows.

Examination of samples

The methods have been fully described by Smith & Moryson (1975). Mud culture filtrates were tested by intoxication and neutralization tests in mice.

RESULTS

Cl. botulinum type C was demonstrated in 19 of the 98 samples (Fig. 1).

Most of the type-C positive samples came from an area off-shore from Speke (Dungeon Bank; seven positive samples) and from an off-shore area between Hoolpool Gutter and Weaver Sluices (Score Bank; six positive samples). Almost half the samples from these two areas contained type C. It is interesting that of the birds that died in the 1979 outbreak, most were found on Dungeon Bank (Head *et al.* 1980) and many on Score Bank; both areas are popular feeding grounds for water birds.

Approximately 36% of the 98 samples were essentially sandy rather than muddy. Despite this fact, as many as 16 (84%) of the 19 samples containing type C consisted of silt, and only three (16%) of sand. Type C contamination occurred as far upstream as Widnes. It was not found, however, downstream from a line drawn approximately from Garston to Hoolpool Gutter, although about 58% of the samples from this part of the Mersey consisted of silt rather than sand.

Cl. botulinum type B was found in 12 samples (12%) and suspected in a further two. The sources of the 12 positive samples were Dungeon Bank (five), a site south of Dungeon Bank (one), the shore between Garston and Oglet (three), a site downstream from Garston (one), and the Eastham area (two). The majority (8/12) of type-B positive samples consisted of mud rather than sand.

DISCUSSION

The clinical signs of botulism in waterfowl are rarely sufficiently specific to permit a firm diagnosis without supporting evidence. The usual laboratory procedure is to demonstrate, by intoxication and neutralization tests in mice, *Cl. botulinum* type-C toxin in the serum of birds that are clinically affected or have just died. The considerable difficulties that may in practice complicate this apparently simple procedure have been discussed by Smith (1982).

If the opportunity of making a laboratory diagnosis is missed, examination of mud for type-C spores can be helpful in forming a retrospective opinion as to whether botulism played any part in the mortality. The mud from an aquatic environment that has given rise to an outbreak of avian botulism will remain permanently contaminated with type-C spores. Failure to demonstrate such spores in say ten mud samples from a suspect area by a method such as that of Smith & Moryson (1975) is therefore good evidence against botulism. On the other hand, a positive result suggests that the environment was probably capable of giving rise to botulism but does not prove that it did so.

The comments that follow are relevant to the interpretation of the discovery of heavy type-C contamination on Dungeon and Score Banks – areas particularly associated with the 1979 avian mortality. The carcasses of birds that die from botulism are often found in large numbers in the locality in which the toxin was ingested. It is possible, however, for birds to fly considerable distances after having ingested a lethal dose, or for carcasses to be carried by water currents and deposited elsewhere. The rotting carcasses of birds that have died from botulism are likely to be invaded by *Cl. botulinum* type C from the gut; this will result in contamination

of the environment not only with toxin but also with massive numbers of organisms. Whether the seeding of a previously uncontaminated area results in the permanent establishment of type C will depend on microbiological, chemical and other factors. Some environments are known to be inhibitory (Sugiyama, Bott & Foster, 1970; Smith, Moryson & Walmsley, 1977; Smith, 1978). Even in generally inhospitable surroundings, however, the organism may multiply in small particles of organic matter (Bell, Sciple & Hubert, 1955). Holdeman (1970) states that outbreaks of botulism in waterfowl do not occur in waters of high salinity (7 % or greater); but that they may occur in adjacent marshlands where the salinity is lower and concentration of organic matter higher. Indeed some of the largest outbreaks ever recorded occurred on the shores of the Great Salt Lake (Kalmbach, 1935). Estuarine outbreaks, though seldom reported, have been described in New Jersey (Reilly & Boroff, 1967) and Japan (Sakai *et al.* 1975). In Britain botulism is known to occur in gulls, whose scavenging habits are probably often responsible for outbreaks (Smith, 1982). Heavy contamination with *Cl. botulinum* type C is present in certain British aquatic areas such as the Norfolk Broads, in which large outbreaks of botulism in waterfowl are known to have occurred (Borland, Moryson & Smith, 1977; Smith, 1978). Type C is, however, far from ubiquitous. Thus of 554 mud samples from lakes, marshes, reservoirs, rivers, canals, and mudflats in various parts of England, Wales, Scotland, Ulster and Eire, 30 % contained type B but only 3 % type C (Smith, Milligan & Moryson, 1978).

Our findings suggest that parts of the Mersey estuary, especially Dungeon and Score Banks where many dying and dead birds were found in the 1979 disease outbreak, are heavily contaminated with spores of the causative organism of avian botulism – *Cl. botulinum* type C. This does not prove that botulism occurred on the Mersey in 1979, but is nonetheless thought-provoking. If any further mass mortality of birds should occur on the estuary, thorough laboratory tests for botulism must be considered a matter of high priority.

We thank Dr K. Wilson, North West Water, for his interest and helpful advice. The views expressed are the responsibility of the authors alone. The paper is published with the permission of the Director of Operations, North West Water Authority.

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