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The low prevalence of *Clostridium botulinum* in the lakes, marshes and waterways of the Camargue

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SUMMARY

Mud samples collected in June 1975 from the lakes, marshes and waterways of the Camargue were examined for *Clostridium botulinum*. The Grand Rhône and Petit Rhône were shown to contain types B and E, but of 44 samples taken from well distributed sites on the Ile de la Carmargue, only two (4·5 %) were positive and these contained type E alone. The survey indicated a much lower prevalence of *Cl. botulinum* than any encountered in recent surveys of inland aquatic environments elsewhere.

RÉSUMÉ

Des échantillons de boue ont été recueillis en Juin 1975 dans les étangs, marais et cours d'eau de Camargue pour la recherche de *Clostridium botulinum*. Le Grand Rhône et le Petit Rhône contenaient les types B et E; par contre, sur 44 échantillons de boue prélevés dans des sites uniformément répartis sur l'Ile de Camargue, seuls deux (4·5 %) contenaient du *Cl. botulinum*, représenté par le type E uniquement.

La présente étude montre que la prédominance de *Cl. botulinum* est bien inférieure à celle qu'on a pu constater ailleurs au cours des récentes recherches sur les milieux aquatiques d'eau douces.

INTRODUCTION

As part of a study of botulism in waterfowl, Smith & Moryson (1975) found that mud from 72·5 % of the lakes and waterways of London contained *Clostridium botulinum*, type B being found most frequently, but types C and E being by no means uncommon. This high prevalence was strikingly different from that of about 4–8 % reported in earlier surveys of soil in Britain (Meyer & Dubovsky, 1922; Leighton & Buxton, 1928; Haines, 1942). The reason for the differences was not clear, but may have been that lake mud constituted a more favourable environment than soil for *Cl. botulinum*, or that the technique used was particularly sensitive. By means of identical technique, an extensive study of *Cl. botulinum* in rural and

coastal aquatic environments in Britain is being made and the results obtained so far indicate a prevalence in different areas that varies from considerable to very high (Smith, Milligan & Moryson, unpublished observation).

A similar survey has also been made in the Camargue, an area of Southern France that is of exceptional importance with regard to wild birds, both resident and migratory. Counts have indicated that in mid-winter the number of Anatidae and Coots (*Fulica atra*) may be between 150,000 and 180,000, i.e. approximately 50% of the wintering population in France. The results of the survey, reported here, indicate a prevalence of *Cl. botulinum* much lower than any encountered so far in different parts of Britain.

MATERIALS AND METHODS

Samples

The Camargue proper consists of the island that lies between the Grand and Petit Rhônes and the Mediterranean. Its area is about 757 km², of which 142 are covered by lakes and rather more by marshes. The dominant ecological influence – salt – is strongest in the lower parts that lie almost at sea level, and absent only in the northernmost part of the delta where the height above sea level is 2–3 m. (Nicholson, Ferguson-Lees & Hollom, 1957; Hoffmann, 1958).

Forty-six mud samples were collected in June 1975 from lakes (20 samples), *marshes (20), rivers (Grand Rhône 1, Petit Rhône 1), a canal (2), a ricefield (1)* and the sea (1). The various sampling sites were roughly classified according to salinity as follows: fresh or slightly brackish (11 sites), slightly brackish to brackish (8), brackish (10), brackish to salt (7), salt (10). Of the 10 sampling sites classified as 'salt', eight had a salinity of 45–100 g/l and two were industrial salt-pans with a salinity of well over 100 g/l. Sixteen of the total number of sampling sites usually become dry for a part of each summer.

Collection and examination of samples

Negative sites were not resampled. In all other respects, the methods for the collection and culture of samples and for the demonstration and typing of *Cl. botulinum* toxin in culture filtrates were exactly as described by Smith & Moryson (1975).

RESULTS

Fig. 1 shows that of the 46 mud samples collected, only four could be shown to contain *Cl. botulinum*. Samples from the Grand and Petit Rhônes each contained types B and E, and type E alone was demonstrated in a third sample from a ricefield irrigated by water pumped from the Grand Rhône; trypsinization was required before toxin could be demonstrated in any culture filtrate derived from these three samples. The fourth positive sample came from Etang du Fangassier, a salt water lake; in this instance type E toxin could be demonstrated in both trypsinized and untrypsinized culture filtrate.

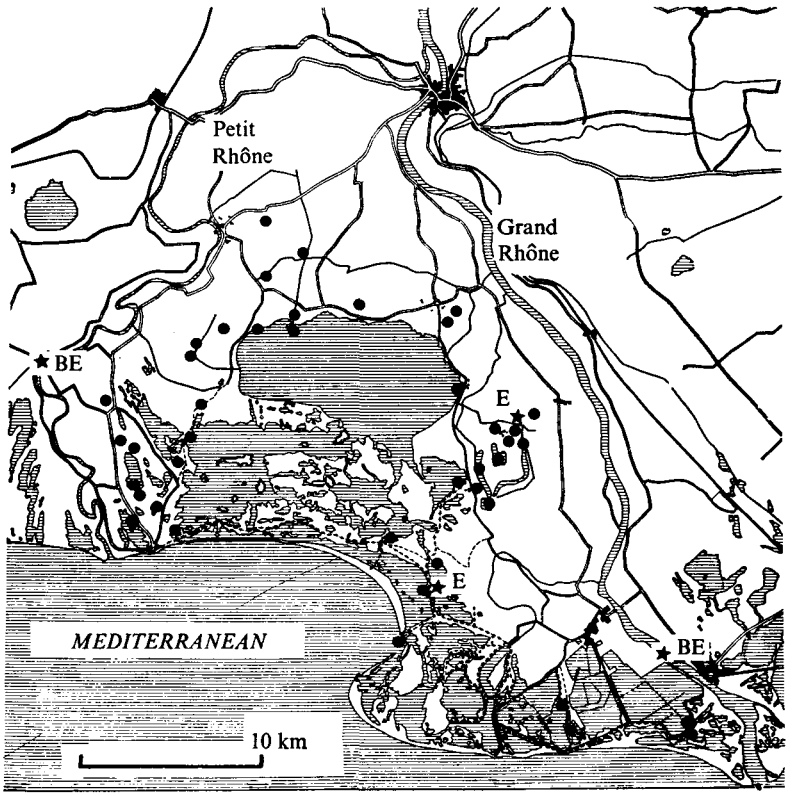

Fig. 1. *Clostridium botulinum* in the lakes, marshes and waterways of the Camargue. Sketch map shows positive (★) and negative (●) aquatic sites. The type of *Cl. botulinum* is indicated for each of the four positive sites.

DISCUSSION

In surveys of soil or mud for *Cl. botulinum*, the significance of a negative result is, for a number of reasons, impossible to assess with absolute certainty (Meyer, 1956). For example, it cannot be proved beyond doubt that a sample is free from antagonistic micro-organisms that might exert an overwhelming inhibitory influence on *Cl. botulinum* in laboratory culture; such micro-organisms might or might not exert a similar influence in the natural environment. Nevertheless, numerous surveys made over a period of more than 50 years have proved beyond doubt their value in indicating the prevalence of *Cl. botulinum* and the strikingly regional distribution of certain types (Baird-Parker, 1969).

In their survey of London lakes and waterways, Smith & Moryson (1975) found that 55% of sites were positive as judged by a single sample, but that this figure could be increased to 72·5% by resampling the apparently negative sites. The survey of the Camargue was based on single samples only and this should be borne in mind in making comparisons with the London survey. Such comparisons can be made with confidence because the technique used in the collection and examination of samples was identical in the two surveys.

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Samples from the Grand and Petit Rhônes each contained *Cl. botulinum* types B and E, but of 44 samples from well distributed sites on the Ile de la Camargue only two (4·5 %) were positive and these contained type E alone. Thus the prevalence of *Cl. botulinum* in the Camargue appeared to be considerably less than that found in the London area or, as already stated, than that found so far in an extensive survey of British rural and coastal aquatic environments. It was also considerably less than that reported in the Netherlands by Haagsma (1974), who, by means of a somewhat different technique, found that 30 % of samples and (Haagsma 1975, personal communication) about 75 % of waterways contained types B, C or E. Smith & Moryson (1975) found that for the demonstration of type E in mud from London lakes trypsinization of culture filtrates was almost always unnecessary; in the present survey, trypsinization was necessary for the demonstration of type E in three out of four positive samples.

Botulism in waterfowl is almost always due to *Cl. botulinum* type C, and our failure to demonstrate this type in the Camargue is reassuring, more especially in view of the disastrous avian mortality (Mountfort, 1973; Report, 1975), diagnosed as botulism, that occurred in 1973 in the Coto Doñana – a Reserve of exceptional importance at the mouth of the Rio Guadalquivir in Southern Spain. The low prevalence of *Cl. botulinum* of all types in the Camargue may seem surprising in view of the contamination of both branches of the Rhône with types B and E, and in view of the large numbers of migrant birds that visit the area. It seems likely that spores of *Cl. botulinum* can be transferred from one aquatic area to another in the alimentary tracts or on the external surfaces of birds (Haagsma, 1974; Smith, 1976). The fate of *Cl. botulinum* spores transferred to a previously uncontaminated lake and the subsequent establishment of the organism in the new environment probably depend upon numerous biological, chemical and other factors. It is possible that the mud of the Camargue contains factors that discourage the establishment of *Cl. botulinum* and this point would be worthy of study in relation to the epidemiology of botulism in waterfowl. It seems unlikely that the various degrees of salinity found in much of the Camargue exert any direct influence on the prevalence of *Cl. botulinum* as marine muds have frequently been shown to contain different types of the organism (Baird-Parker, 1969) including type C (Segner, Schmidt & Boltz, 1971).

The survey indicates that a high prevalence of *Cl. botulinum* is not always to be expected in the deep anaerobic mud of inland aquatic environments.

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