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Clostridium botulinum in Scottish fish farms and farmed trout

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SUMMARY

Rainbow trout and specimens of pond mud were collected from three fish farms and examined for the presence of *Clostridium botulinum*. Two of the farms were constructed with concrete channels and one was mud-bottomed.

Cl. botulinum was isolated only from the mud-bottomed farm (24 % of muds), and the isolates were all non-proteolytic type B. The implications of the presence of *Cl. botulinum* spores in the mud of fish farms is discussed.

INTRODUCTION

Botulism has not so far presented as a serious public health problem in this country. The strict control of commercial canning and the discouragement of home canning of non-acid foods, together with a diet of fresh or well-cooked foods, probably accounts for this freedom.

Cl. botulinum is, however, widely distributed in nature, and its presence was demonstrated in Scottish soil by Leighton & Buxton (1928) during the investigation in the aftermath of the deaths from botulism at Loch Maree; and more recently in fish and coastal waters by Cann, Wilson & Hobbs (1968). Furthermore, fish farming is now well established in parts of Europe and is a developing industry in Scotland. The well-recognized association of botulism with fish and fish products (Dolman, 1957; Johannsen, 1963; Cann, Wilson, Hobbs & Shewan, 1966, 1967) and the increasing numbers of fish farms established in Scotland, encouraged us to carry out the investigations reported here. The aim was to gain further knowledge of the incidence of *Cl. botulinum* in farmed fish and pond mud and to attempt to assess the hazards to public health.

Fish cultivation is a specialized form of animal husbandry. Fertilized eggs are normally obtained from breeding farms in this country and Denmark and in the winter season from Tasmania. The eggs are hatched in fresh stream or deep well-water running through wooden boxes. Until the trout have matured from the alevin and parr stages, they are kept well separated from adult fish. When young fish have reached a length of about 2 in., mortality rates are much reduced and they are transferred to the rearing ponds or raceways. Raceways and ponds are of concrete or mud, often partitioned to facilitate ease of handling and feeding, and water flows straight through. A nitrogenous deposit of food, excreta and dead fish forms on the bottom of raceways, and they are periodically shut down and cleaned. Fig. 1 illustrates the basic fish-farm design.

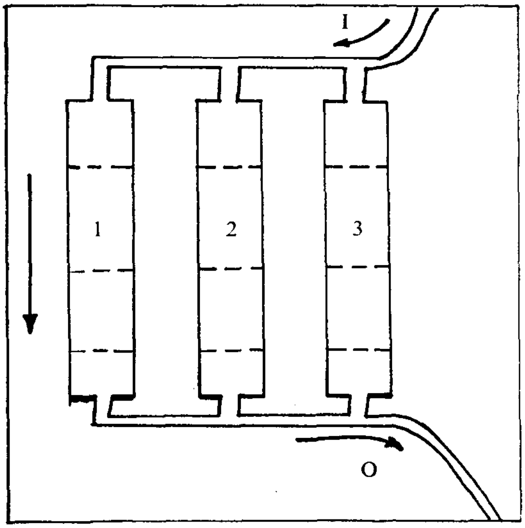

Fig. 1. Diagram of a typical fish farm. 1–3, Raceways. I, Water inlet; O, water outlet. ⟶, Direction of water flow.

MATERIALS AND METHODS

Fish farms

Three types of farms were investigated. Farm A was constructed with concrete raceways and brick catwalks. Fresh water was drawn by pump from a loch, circulated once, and expelled back into the loch at a lower level. Only rainbow trout hatched on the farm were reared.

Farm B was also constructed with concrete raceways. The farm was on the coast and different salinity levels were attained for each raceway. Water for the first raceway, which contained young trout, was fresh from a hillside stream. Some of this water was re-circulated after passing through a settling tank and a gravel filter bed. The lower raceways, towards the sea, received a proportion of sea water pumped from the firth until the raceways at the lowest level received sea water of about 18‰ salinity. Young trout hatched on the farm were graded and the larger ones placed in water of high salinity. Eventually all trout, before marketing, had spent some time in sea water. Rainbow trout and brown trout were produced.

The third farm (C) was a mud farm. Water from a stream was diverted through the mud-bottomed channels and back into the stream. At various times of the year cows, goats and hens are in the same field as the fish channels, and fish-eating birds shot on the farm were hung on a fence. All rainbow and brown trout grown were hatched on the farm.

Specimen collection

Sixty-nine fish of marketable size were netted from the three farms and placed in individual polythene bags. Forty-four mud and soil specimens, of about 10 g. were collected into universal containers using a faecal spoon. Three samples of pelleted food specimens were also tested.

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Toxicity tests

The whole fish, heat-sealed in a polythene bag, or the mud specimen in Robertson's cooked meat broth (R.C.M.B.) was incubated for 6 days at 30° C. before mouse toxicity tests were initiated. After this incubation period the sample was homogenized, centrifuged and trypsinized (Duff, Wright & Yarinsky, 1956) before injection of 0·5 ml. intraperitoneally into two mice. Control mice, injected with boiled supernatants, were included with each series.

The mice were observed over the first 30 min. for non-specific deaths due to shock, and if these occurred the inoculum was diluted in 0·1 % peptone buffer and another pair of mice injected. Mice surviving this period were observed for typical symptoms of mouse botulism and death. Such symptoms are well described elsewhere (Bott, Johnson, Foster & Sugiyama, 1968; Wenzel, Bach, Müller-Prasuhn & Glasker, 1971), but it is worth recording that they are easily recognized as rapid laboured breathing in the early stages, progressing until the mouse is gasping for air, the diaphragm is raised and the animal has an hourglass shape in its efforts to breathe. Survivors after 2 days were regarded as having received no botulinum toxin. If both mice died with typical symptoms of botulism, protection tests for typing and isolation procedures for *Cl. botulinum* were instituted.

Protection tests

The type of *Cl. botulinum* toxin present in toxic cultures was identified by mouse protection tests. After trypsinization, cultures were incubated for 1 hr. at 37° C. with specific antisera to *Cl. botulinum* type B (Wellcome Reagents) and type E (kindly supplied by Dr G. Hobbs, Torry Research Station, Aberdeen), together with antiserum to tetanus toxin, as high-titre tetanus toxin can cause symptoms similar to mouse botulism. One pair of mice was injected intraperitoneally with 0·5 ml. of each sample, and two mice received only a heated control. When *Cl. botulinum* toxin was present only the control mice and the specifically protected pair survived.

Isolation of Cl. botulinum

At the same time as protection tests were carried out, attempts were made to isolate *Cl. botulinum* from toxic cultures by further enrichment in R.C.M.B. and plating out on lactose–egg-yolk-medium (L.E.Y.) (Willis & Hobbs, 1958) and blood agar. Where appropriate toxic specimens or cultures were treated with heat (Zeller, 1964) or alcohol (Kautter & Bartram, 1966) to kill vegetative organisms, and with ether (D. C. Cann, personal communication) to control swarming. Typical colonies were picked out from either medium by removal on a piece of the agar medium, as described by A. Johannsen (D. C. Cann, personal communication), and inoculating into R.C.M.B. One toxic strain was isolated from each positive specimen.

Table 1. *Isolation of* Clostridium botulinum *from fish and fish farms*

	Farms A	Farms B	Farms C	Total
No. of fish examined	37	11	21	69
No. of fish positive	0	0	1 (4·7)	1 (1·4)
No. of muds examined	5	2*	37	44
No. of muds positive	0	0	9 (24)	9 (20)
Food specimens examined	3	—	—	3
Food specimens positive	0	—	—	0

The figures in parentheses indicate percentages.
— = not examined.
* One from filter bed, one from settling tank.

RESULTS

The results obtained are shown in Table 1. *Cl. botulinum* was found in one fish and from nine muds from farm C; all were found to be non-proteolytic *Cl. botulinum* type B.

DISCUSSION

The continuing world shortage of animal protein is likely to ensure an intensification of fish farming, and it is of some interest and importance that such production should ensure maximum safety in production methods. Although the possibility of botulism occurring under present conditions in the United Kingdom appears remote, the effect of even sporadic incidents is so disastrous that it is worth examining the production conditions in this developing industry.

If the results reported here from farm C, a mud farm, are typical of such farms, it appears that these conditions allow considerable enrichment of the numbers of *Clostridium botulinum*. A build-up of Clostridia of this nature may not be a danger as such, but Meyer (1956) considers that a relation exists between the results of soil surveys and the prevalence of botulism. In addition, the number of fish marketed containing *Cl. botulinum* spores is likely to increase. Wenzel, Bach & Müller-Prasuhn (1971), who examined fish-farm methods in Germany, following the death of three patients after eating vacuum-packed smoked-trout fillets, state that organically rich mud must be suspected of containing *Clostridium botulinum*, and that pond mud is one of the sources of fish contamination. The mud is organically enriched and rendered anaerobic by the deposition of food waste, dead fish, bird droppings, dead rodents, wild and domestic animals; and these and contaminated fish food can act as a source of spores. Although the mud farm appears to us to present a greater hazard than the concrete type of fish farm, a survey to determine whether such mud farms can be satisfactorily managed is, at present, being undertaken by Torry Research Station, Aberdeen (G. Hobbs, personal communication).

The strains isolated here were of type B, and this differs from the findings of the German group, who write that type E is the most widely distributed of the six known types in temperate soils, and that the other five types are of minor importance when considering botulism following fish consumption. However, a close relation exists between strains from the non-proteolytic group of *Clostridium botulinum* producing type B, E or F toxins (Lee & Riemann, 1970; Solomon, Lynt, Kautter & Lilly, 1971; Sugiyama & King, 1972), the type of toxin expressing only the type of lysogenic phage carried by the strain.

Botulism can also occur as a result of wound contamination, and reports of nine such cases in the United States of America are reviewed by Merson & Dowell (1973). For workers in the fish-farming industry, where injuries can occur, the possibility of protection by the incorporation of botulinum toxoid in tetanus vaccines should be considered.

We wish to express our gratitude to Dr G. Hobbs and Mr D. C. Cann of Torry Research Station, Aberdeen, for help and advice and for confirming the type of *Cl. botulinum* strains isolated by us.

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