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CITROBACTER FREUNDII: THE CAUSE OF GASTRO-ENTERITIS LEADING TO PROGRESSIVE LOW LEVEL MORTALITIES IN-FARMED RAINBOW TROUT, *ONCORHYNCHUS MYKISS* WALBAUM, IN SCOTLAND

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Introduction

During July 1992 at a site in Scotland, rainbow trout (*Oncorhynchus mykiss* Walbaum) (average weight = 100g) developed gastro-enteritis leading to progressive low level mortalities (20-30 mortalities/tank/day). Externally, there was no evidence of any disease signs. Yet internally the digestive tract was badly swollen with watery/mucoid contents. The organs, viz. heart, kidney, liver and spleen, appeared to be normal.

Materials and methods

Kidney, liver and intestinal samples were collected aseptically from diseased animals, and inoculated onto plates of tryptone soya agar (TSA; Oxoid, Basingstoke) supplemented with 1% (w/v) sodium chloride with incubation at 15°C for up to 7 days. Cultures were purified, as necessary, by streaking and re-streaking onto fresh media, and identified as far as possible by the examination of key phenotypic traits and by means of the API 20E rapid identification system (API Laboratories, Basingstoke). A representative isolate (M7ANAL) was examined for pathogenicity in groups containing 10 rainbow trout (average weight = 4g), which were maintained in aerated static fresh water at approximately 15°C. The fish were injected intraperitoneally or intramuscularly with 0.05 ml of a bacterial suspension containing ca. 10^7 cells/ml in 0.9% (w/v) saline. All infected fish were examined daily for 7 days, and dead and moribund animals were examined bacteriologically, as before.

Results

Virtually pure, dense bacterial growth was recovered from the liver and intestinal samples of four fish on TSA. The cultures comprised Gram-negative asporogenous, fermentative motile rods, which produced catalase, β-galactosidase and H_2S, but not arginine dihydrolase, lysine and ornithine decarboxylase, indole, oxidase, or tryptophane deaminase. The Voges Proskauer reaction was negative. Gelatin was degraded, but not so urea. Sodium citrate was not utilised. Acid was produced from amygdalin, arabinose, glucose, mannose, rhamnose and sorbitol, but not from inositol or saccharose. From these results, the cultures were identified as *Citrobacter freundii* (Sakazaki, 1984). Moreover, the API 20E profile of 1404513 corresponds to *C. freundii* (=excellent identification). The isolates were sensitive to nalidixic acid (30µg), oxytetracycline (25µg) and potentiated sulphonamide (25µg).

Disease signs and mortalities (40%) followed within 72 h of injecting (intramuscularly or intraperitoneally) the fish. There was evidence of melanosis, haemorrhaging in the eyes, internal haemorrhaging, pale spleen, gastro-enteritis and the presence of ascitic fluid in the peritoneal cavity. Within 24 h of intramuscular injection, pronounced muscle necrosis was observed at the site of injection. *C. freundii* was recovered from all dead and moribund fish. Moreover, the pathogen was recovered from the kidney of all survivors at the end of the experiment.

Discussion

From previous work, there has been some indication that *C. freundii* may cause dis

ease in fish. Yet, definitive evidence was not forthcoming until the work of Sato *et al.* (1982), when the organism was recovered as a pathogen of sunfish, *Mola mola*, in a Japanese aquarium. Subsequently, *C. freundii* has been implicated with disease in Atlantic salmon and rainbow trout in Spain and the USA (Baya *et al.*, 1990; Sanz, 1991) and from carp in India (Karunasagar *et al.*, 1992). This is the first evidence of the presence of *C. freundii* as a fish pathogen in the UK. Although *C. freundii* is common in eutrophic freshwater (Allen *et al.*, 1983), it is considered possible that, in this outbreak, the source may well have been from a leaking septic tank on neighbouring property. Before chemotherapy could be instituted, an outbreak of enteric redmouth, caused by *Yersinia ruckeri* type I, was diagnosed among the fish stocks. Thereafter, *C. freundii* could not be found among diseased fish.

Summary

Citrobacter freundii has been recovered as the causal agent of gastro-enteritis, leading to progressive low level mortalities, in farmed rainbow trout (*Oncorhynchus mykiss*, Walbaum) in Scotland. This is the first indication of *C. freundii* as a fish pathogen in the UK.

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