FIRST ISOLATION OF FLEXIBACTER PSYCHROPHILUS, AS CAUSATIVE AGENT OF RAINBOW TROUT FRY SYNDROME (RTFS), PRODUCING RAINBOW TROUT MORTALITY IN CHILE

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Introduction

Flexibacter psychrophilus (Cytophaga psychrophila) was first isolated from Coho salmon in USA and Canada (Bullock, 1972; Holt, 1987). Since 1984, in many European countries such as United Kingdom (Rangdale, 1994; Bruno, 1992; Santos et al., 1992), Italy (Sarti et al., 1992), Finland (Austín et al., 1988), France (Baudin-Laurencin et al., 1989; Bernardet et al., 1988), Germany and Switzerland (Rangdale, 1994) and Spain (Toranzo et al., 1993), this bacteria has been recognised as cause of a completely different disease in Rainbow trout fry (Oncorhynchus mykiss). Several septicaemic infections have been diagnosed in Rainbow trout fry and fingerling in hatcheries and net-cages in lakes in Chile from 1993 onwards (more frequently among imported fish).

These presented the same alterations described from European RTFS (FMS, BFA or Visceral Myxobacteriosis), causing difficulties in therapy as well as high mortalities. Most of the RTFS cases were observed in sites on Chilo island and near Puerto Monti (both in the Xth Region).

This report describes and documents the first appearance of the RTFS disease in Chile.

Materials and Methods

In September 1993, imported Rainbow trout specimens of 10 - 18 g body weight were collected from a severe outbreak of mortality located in a lake pen-culture site belonging to private fish farm.

The clinical signs were evaluated and additional examinations which were performed including histology (H&E), Gram, Giemsa and Fuchsian stains on tissues taken from various organs, hemocytocrit, virological analysis to eliminate a possible viral source and/or contaminant virus (CHISE-214 and EPC). Bacteriological cultures were taken from different organs and tissue lesions (TSA, Cytophaga, 2% horse foetal serum enriched Cytophaga, SW, MAOA, McConkey and Sabouraud). Serological (Western blot and Slide Agglutination Tests) and enzymatic (API-ZYM) analysis were performed once bacterial isolates were obtained. Biochemical and drug sensitivity tests (in MAOA and Müller-Hinton medium) using Erythromycin, Trimethoprim-Sulphadoxine, Oxolinic acid, Phosphomycin, Danofloxacin, Oxytetracycline, Flumequine, Sipramycin and Penicillin were also performed.

Finally, to satisfy the Koch's postulates reproduce the disease in healthy Rainbow trout (10 - 20 g B.W. 9-10°C), following intraperitoneal inoculation of 0.1 ml, of a bacterial suspension with 1-100 x 10^7 cell concentration and using a negative control group (isotonic solution) was assessed.

Results

Clinical signs: The affected fish showed lethargy, superficial swimming, anorexia and darkening. Ventral oedemas, even subsequent wall perforation and partial eversion was observed externally. Pale gills with haemorrhage, exophthalmia, fin base haemorrhage and in chronic cases occasional abdominal swelling were also observed.

Severe spleen inflammation and petechiae, in many cases an enlarged liver and kidneys as well, gastroduodenitis, gastro-enteritis, petechiae in pyloric caecae and visceral fat, and in some fish cardiac dilatation, brain haemorrhages and auctiae were found internally.

Histology: Multiple tissue alterations in spleen, liver, kidney, brain, skeletal muscle, gills, pancreas and pyloric caecae were evidenced. These lesions, to a greater or lesser extent, revealed degenerative and necrotic processes.

Virology: A virus implication was discarded, since no cytopathic effect was seen in the cell lines used (15°C for 28 days).

Hemocytocrit: Different degrees (frequently severe) of anaemia was one of the most common characteristics of this disease.

Bacteriology: Pure cultures of the presumptive F. psychrophilus in Cytophaga, enriched Cytophaga (PCS2%), TSA and MAOA were obtained. MAOA (15°C for 12 days) showed greater efficiency and consistency, specially those cultures taken from spleen, but growth was also achieved in cultures taken from gills, liver, kidney, skin ulcers and brain. The colonies were yellow, smooth, brilliant, convex, circular and, catalase and oxidase positive. The isolated bacteria were long thin Gram-negative rod, staining mildly with Giemsa and more intensely with Fuchsian.

The bacteria obtained from direct smears of various organs had the same stain properties.

Serological confirmation: The Western blot technique results, tested with antisemur of Flexibacter psychrophilus, showed that the protein bands and the reaction with the specific antisemur were identical to other F. psychrophilus. A Slide Agglutination Test indicated and confirmed the identity of the isolated strain. The strain reacted with antisemur obtained from European strains, but not with the American antisemur. The API-ZYM test corroborated these results, where the organism exhibits enzyme production patterns similar to European strains.

The strain under study, corresponding to European serotypes (specifically Spanish) would belong to different antigenic group than that of type strain NCMB 1947 of F. psychrophilus, characteristic of "Cold water disease".

Drug Sensitivity: The various isolates were sensitive to the majority of the drugs used in vitro under standard sensibility concentrations, excepting Phosphomycin, Sulfaphenazole and Penicillin.

Confirmation of Koch's postulates: Following inoculation with F. psychrophilus, results indicated a 73.3% mortality rate in 22 days of study. The negative controls showed no mortality. Symptoms of RTFS were observed and the same agent isolated in all death cases.

Discussion

The first appearance of RTFS in Chile was confirmed by the findings and investigation performed.

The characteristics of the disease are very similar to those described in Europe, with no concomitant virus or parasite. While the bacteria showed high drug sensitivity in vitro, field observations indicate a series of difficulties in therapeutic control: Flumequine and oxolinic acid produced limited to no effect, and only occasional success with high doses of oxytetracycline (100 - 300 mg/kg weight for 21 days) in severe cases.

RTFS has caused important losses in Chile (up to 30% in one week, 19,700 fish) and is considered an insidious and erosive disease with outbreaks common in colder seasons.
It is very difficult to determine the first date of the appearance RTFS in Chile due to the difficulty of its isolation and identification, insufficient information available and over-estimation of rainbow trout resistance to disease.

Presumably, the disease originated from eggs brought in from infected European countries, as it appears mainly in imported trout. However, is hard to determine decisively.

Abstract

An investigation was performed to determine the causative agent of the high losses occurring in freshwater culture of rainbow trout in southern Chile. According to this study and by isolation, the first appearance of RTFS (Rainbow Trout Fry Syndrome) was confirmed. The characteristics of the disease are very similar to those described in many European countries, with no concurrent virus or parasite.

Whilst the bacterium *F. psychrophila* showed high drug sensitivity in vitro, field observation differed strongly, indicating masked problems in therapy. The study indicated that the bacterial strains isolated from uninformed obtained from European strains, but not with the American aetiology, belonging to a different antigen group than that of type NCMB 1947, characteristic of "Cold water disease".

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