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FIRST REPORT OF FURUNCULOSIS IN TURBOT REARED IN FLOATING CAGES IN NORTHWEST OF SPAIN

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Aeromonas salmonicida was isolated for the first time in Galicia (NW Spain) in 1987 from rainbow trout (*Oncorhynchus mykiss*) cultured in fresh water. However, furunculosis in seawater was not detected until 1989, in Atlantic salmon (*Salmo salar*) and trout (Toranzo *et al.*, 1990), the disease originating from the transport of infected yearling brown trout (*Salmo trutta*) from central Spain. Regardless of the source of the isolates, all exhibited a great biochemical, serological and genetical homogeneity, belonging to *A. salmonicida.* subsp. *salmonicida* (Toranzo *et al.*, 1991). The incidence of furunculosis in salmonids cultured in fresh and in sea water has increased steadily in Spain in subsequent years, constituting a significant threat to other cultured fish species. However, until now, this disease had never detected in turbot (*Scophthalmus maximus*) despite the highly intensive culture of this species in our area. The production of turbot in Galicia accounts for almost the 100% of Spanish production.

In April, 1992, an outbreak of furunculosis was diagnosed for first time in turbot cultured in floating cages in Galicia. Affected fish ranged from 500g to 1kg (800g average body weight) and were stocked at a density of 15 kg/m^2. The water temperature during the outbreak fluctuated between 15-16°C.

Diseased turbot showed strong abdominal distension, haemorrhagic zones at the base of the pectoral fins and, in some cases, ulcerative lesions were present on the ventral surface. Internally, livers were extremely pale with petechiae and showed a laminar appearance. The peritoneal cavities were completely filled with ascitic fluid.

The total cumulative mortality over a month was 15% of the affected population of approximately 1,200 fish. Although the mortality rate was not very high, if one considers the size of the dead fish, this furunculosis outbreak produced important economic losses.

Samples were taken from liver, kidney, spleen and ulcers and inoculated onto Brain Heart Infusion Agar and Broth (BHIA and BHIB, Difco) and Thiosulphate Citrate Bile Sucrose (TCBS) Agar (Oxoid). After 24h-48h incubation, pure cultures consisting of typical brown pigmented colonies were obtained on BHIA plates from all the organs examined. No bacteria were recovered on TCBS Agar. The bacteria isolated from turbot (representative strain RO-61) were subjected to standard morphological, physiological and biochemical tube and plate tests and, in addition, the commercial API-20E system (Biomerieux, Spain) was used in parallel. As illustrated in Table 1, the turbot isolates exhibited the typical biochemical profile of the pigmented strains of *A. salmonicida* subsp. *salmonicida.* The main differential reactions compared to the reference *A. salmonicida* strain ATCC 33658 isolated from Atlantic salmon was a weak and delayed positive reactions exhibited by the turbot isolate for the arginine dihydrolase test and for acid production from mannitol.

Serological confirmative identification of the isolates conducted by the slide agglutination test (Toranzo *et al.*, 1987) showed that both the whole cell and the somatic "O"

Table I. Comparison of the biochemical characteristics of the turbot isolates of *A. salmonicida* with those of Reference strain ATCC 33658.

CHARACTER	Turbot isolates	ATCC 33658	CHARACTER	Turbot isolates	ATCC 33658
Diffusible pigment	+	+	ß Galactosidase	-	-
Gram stain	-	-	Urease	-	-
Oxidase	+	+	Gelatinase	+	+
Motility	-	-	Caseinase	+	+
O/F (Leifson)	+	+	Phospholipase	+	+
Gas from Glucose	+	+	Elastase	+	+
Indole	-	-	Acid from		
Voges Proskauer	-	-	Glucose	+	+
Citrate (Simmons)	-	-	Mannitol	(+)	+
Nitrate reduction	+	+	Inositol	-	-
H_2S production	-	-	Sorbitol	-	-
Thornley's Arginine	(+)a	+	Rhamnose	-	-
Moeller's Arginine	(+)	+	Sucrose	-	-
Moeller's Lysine	-	-	Melibiose	-	-
Moeller's Ornithine	-	-	Fructose	+	+
Growth on 0% NaCl	+	+	Amygdalin	-	-
Growth on 3% NaCl	+	+	Arabinose	(+)	(+)
Growth on 5% NaCl	-	-	Galactose	+	+
Growth at 15°C	+	+	Maltose	+	+
Growth at 30°C	+	+	Haemolysis (Sheep erythrocytes)	+	+
Growth at 37°C	-	-			

a(+), weak and delayed positive reaction.

antigen produced a strong agglutination with antiserum raised against the reference strain ATCC 33658.

The plasmid pattern of the *A. salmonicida* from turbot showed the distinctive pattern of 4 small plasmid bands of 4.2, 3.6, 3.5, and 3.4 Megadalton which is typical of the pigmented strains of this species (Hackett *et al.*, 1984; Bast *et al.*, 1988; Toranzo *et al.*, 1983, 1991).

Amongst chemotherapeutic agents tested, the bacteria isolated from turbot were resistant only to streptomycin, being sensitive to ampicillin, chloramphenicol, tetracycline, oxytetracycline, erythromycin, oxolinic acid, flumequine, trimethoprim-sulphamethoxazole, and nitrofurantoin. Treatment

with oxolinic acid (20 mg/kg fish/day) was effective in controlling the fish mortality.

Infectivity trials were conducted at 16°C in juvenile turbot (5g) by intraperitoneal inoculation. After a seven day period, the LD_{50} (Lethal Dose 50%) value of turbot strain RO-61 was $2x10^3$ cells. The inoculated bacterium being recovered in pure culture from all dead fish. In addition, different *A. salmonicida* strains isolated from salmonids cultured in our area also proved to be highly virulent for turbot with LD_{50}'s ranging from $1x10^3$ to $6x10^3$cells.

Although it is very difficult to determine the origin of furunculosis in a particular geographic area because the phenotypic and genetic homogeneity of the pigmented *A. salmonicida* strains (Hackett *et al.*, 1984; Bast *et al.*, 1988; Toranzo *et al.*, 1991), we believe that a possible source of the furunculosis in turbot was contaminated food. Affected turbot had been fed with trout and salmon stored frozen which had suffered furunculosis outbreaks. Although the role of the environment as a reservoir of furunculosis is a subject of controversy (Allen-Austin *et al.*, 1984; Sakai, 1986; Rose *et al.*, 1990; Morgan *et al.*, 1991), we cannot rule out the transmission of *A. salmonicida* through water, sediment particles or wild carrier fish from salmonid culture facilities located near to the floating cages of turbot.

Summary

We report the first isolation of *A. salmonicida* subsp. *salmonicida* from adult turbot reared in floating cages located near salmonid aquaculture facilities in northwestern Spain. The most evident clinical signs in diseased turbot were, a strong abdominal swelling, with accumulation of ascitic fluid, and a pale liver showing petechiae. The total cumulative fish losses in a one month period in this furunculosis outbreak was 15% of the affected population. Treatment with oxolinic acid was effective in controlling fish mortalities. Virulence tests demonstrated that this bacterium was pathogenic for turbot with an LD_{50} of $2x10^3$ cells.

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