

REVIEW

Salmonid rickettsial septicemia caused by *Piscirickettsia salmonis*: a review

Felipe E. Almendras, I. Carmen Fuentealba*

Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island,
550 University Avenue, Charlottetown, PEI, Canada C1A 4P3

ABSTRACT: Rickettsial diseases affecting several fish species have emerged in the last years. The most important outbreaks have been reported among farmed salmonid and non-salmonid species. The most important economical impact on the Chilean fish farming industry is probably the rickettsial pathogen *Piscirickettsia salmonis*, which is the microorganism responsible of the disease salmonid rickettsial septicemia (SRS). In the present article the authors review current knowledge on the natural history, lesions, transmission, diagnosis and control of SRS.

KEY WORDS: *Piscirickettsia salmonis* · Salmonid rickettsial septicemia · Piscirickettsiosis · Review

INTRODUCTION

Rickettsial infections in finfish have been reported in several salmonid and non-salmonid fish in fresh water and salt water since 1939 (Tables 1 & 2). However, rickettsia were not considered of economical importance until a massive outbreak was reported in Chile. In 1989, a new disease of unknown aetiology killed approximately 1.5 million market-sized (2 kg) coho salmon *Oncorhynchus kisutch* cultured in the area of Calbuco, southern Chile (Bravo & Campos 1989, Cvitanich et al. 1990, Fryer et al. 1990). The disease was later described as affecting Atlantic salmon *Salmo salar*, rainbow trout *Oncorhynchus mykiss*, and chinook salmon *Oncorhynchus tshawytscha* (Cvitanich et al. 1991). In Chile, the losses were extensive and, on certain farms, mortalities of up to 90% were reported (Bravo & Campos 1989).

The causative organism of the Chilean outbreaks has been identified as *Piscirickettsia salmonis*, a rickettsial organism belonging to the order Rickettsiales, family Rickettsiaceae, and the tribe Ehrlichieae (Fryer et al. 1992).

Piscirickettsia salmonis was the first rickettsial agent to be implicated in the aetiology of a major fish disease, affecting several species of salmonids cultured in salt water (Cvitanich et al. 1991). The systemic character of the disease motivated the proposed name salmonid rickettsial septicemia, SRS (Cvitanich et al. 1991). Today, this disease, also known as Piscirickettsiosis, is probably the most important disease affecting the salmon industry in Chile. Recently, the Chilean Salmon and Trout Growers Association reported that all cultured salmonid species may be affected by the syndrome. Coho salmon are by far the most susceptible species, accounting for approximately 68% of the outbreaks, compared with 18% for rainbow trout and 14% for Atlantic salmon (Cassigoli 1994).

ISOLATION AND GROWTH OF *PISCIRICKETTSIA SALMONIS*

Piscirickettsia salmonis is an obligate intracellular Gram-negative, spherical to coccoid, often pleomorphic, non-motile, non-encapsulated organism ranging in size from 0.5 to 1.8 µm in diameter (Cvitanich et al. 1991). *P. salmonis* is periodic acid-Schiff (PAS), acid-

*Addressee for correspondence. E-mail: cfuentealba@upeu.ca

Table 1 Rickettsial organisms reported in freshwater finfish. M: microscopic diagnosis of the organism; I: isolation of the organism in cell lines

Host species	Country	M/I	Source
Non-salmonid			
<i>Tetrodon fahaka</i>	Egypt	M	Mohamed (1939)
<i>Oreochromis mossambicus</i>	Taiwan	M	Chern & Chao (1994)
<i>Oreochromis niloticus</i>	Taiwan	M/I	Chern & Chao (1994)
<i>Oreochromis aureus</i>	Taiwan	M	Chern & Chao (1994)
<i>Tilapia zillii</i>	Taiwan	M	Chern & Chao (1994)
<i>Tilapia hornorum</i>	Taiwan	M	Chern & Chao (1994)
<i>Cichlasoma managuense</i>	Taiwan	M ^d	Chern & Chao (1994)
<i>Panaque suttoni</i>	Colombia ^b	M	Khoo et al. (1995)
Salmonid			
<i>Oncorhynchus mykiss</i>	Germany	M	Ozel & Schwanz-Pfitzner (1975)
	Chile	M	Bravo (1994)
	Chile	M/I	Gaggero et al. (1995)
<i>Oncorhynchus kisutch</i>	Chile	M/I ^a	Cvitanich et al. (1991)
	Chile	M/I	Gaggero et al. (1995)
<i>Salmo salar</i>	Chile	M/I ^a	Garcés et al. (1991)
	Chile	M/I	Cvitanich et al. (1995)

^aSusceptible in experimental infection
^bFish imported from Colombia, diagnosed in USA

fast, and Giménez-negative, but stains well with hematoxylin and eosin (H&E), Giemsa, and methylene blue (Branson & Nieto 1991, Cvitanich et al. 1991).

Piscirickettsia salmonis has been cultured in 6 fish cell lines maintained in buffered minimal essential medium (MEM) supplemented with 10% fetal bovine serum (Fryer et al. 1990, Cvitanich et al. 1991). Typically, piscirickettsial growth is determined by the gradual appearance of cytopathic effect (CPE) in cell monolayers. Characteristic CPE include cell rounding and the development of one or more large vacuoles within the cytoplasm (Cvitanich et al. 1991, Garcés et al. 1991). Four susceptible fish cell lines have been derived from salmonid species: chinook salmon embryo (CHSE-214); chum salmon *Oncorhynchus keta* heart (CHH-1); coho salmon embryo (CSE-119); and rainbow trout gonad (RTG-2). The other cell lines are from warm water fish species, epithelioma papulosum cyprini (EPC) from *Cyprinus carpio*, and the fat head minnow *Pimephales promelas* (FHM) line of epithelial cells, exclusive of the caudal fin (Fryer et al. 1990, Cvitanich et al. 1991). Conversely, 2 cell lines, i.e. brown bullhead *Ictalurus nebulosus*

posterior trunk (BB) and bluegill *Lepomis macrochirus* fry caudal trunk cells (BF-2), have failed to develop a CPE after infection with *P. salmonis* and incubation at 15°C for 30 d (Fryer et al. 1990, Cvitanich et al. 1991). The reasons for the observed differences in susceptibility among cell lines have not been studied.

PRESENTATION OF SRS

Natural SRS outbreaks in salt water

The first outbreaks appeared in 1988 in the Huito channel, Calbuco, Chile, causing massive mortalities (up to 90%) and affecting only coho salmon cultured in the area (Bravo & Campos 1989). Due to the unusual characteristics of this new disease, it was initially named U.A. (unknown agent), 'coho salmon syndrome', or 'Huito disease'

(Branson & Nieto 1991, Cvitanich et al. 1991). The disease causes substantial economic losses to the salmon aquaculture industry of southern Chile (Bravo & Cam-

Table 2. Rickettsial organisms reported in saltwater finfish. M: microscopic diagnosis of the organism; I: isolation of the organism in cell lines

Host species	Country	M/I	Source
Non-salmonid			
<i>Callionymus lyra</i>	Wales	M	Davies (1986)
<i>Dicentrarchus labrax</i>	France	M	Comps et al. (1996)
Salmonid			
<i>Oncorhynchus kisutch</i>	Chile	M	Bravo & Campos (1989)
		M/I	Fryer et al. (1990, 1992)
		M/I	Cvitanich et al. (1991)
		M	Branson & Nieto (1991)
		M/I	Garcés et al. (1991)
<i>Oncorhynchus mykiss</i>	Chile	M	Lannan et al. (1991)
		M	Cvitanich et al. (1991) Fryer et al. (1992)
<i>Oncorhynchus tshawytscha</i>	Chile	M	Cvitanich et al. (1991)
		M	Fryer et al. (1992)
		M	Evelyn (1992)
<i>Oncorhynchus gorbuscha</i>	Canada	M	Brocklebank et al. (1993)
		M	Evelyn (1992)
<i>Salmo salar</i>	Chile	M	Evelyn (1992)
		M	Cvitanich et al. (1991)
		M	Garcés et al. (1991)
		M	Fryer et al. (1992)
		M	Brocklebank et al. (1992)
<i>Salmo salar</i>	Canada	M	Evelyn (1992)
		M	Brocklebank et al. (1993)
		M/I	Brocklebank et al. (1993)
<i>Salmo salar</i>	Norway	M	Olsen et al. (1993)
		M	Rodger & Drinan (1993)

pos 1989, Cassigoli 1994). Epizootics typically occur 10 to 12 wk after healthy fish are introduced into seawater, usually from March through August, and the outbreaks may last up to 10 wk and then subside (Cvitanich et al. 1991). The disease has also been described in several species of salmonids cultured in salt water in southern Chile, including Atlantic salmon, rainbow trout, and chinook salmon (Cvitanich et al. 1991, Lannan & Fryer 1993).

The geographical distribution of this condition may be wider than previously estimated since similar pathogenic rickettsial organisms have been recently reported affecting salmonids cultured at saltwater sites in Canada (Brocklebank et al. 1992, 1993), Ireland (Rodger & Drinan 1993), and Norway (Olsen et al. 1993). The mortalities in these countries did not reach the importance and prevalence of the Chilean outbreaks, and it is not clear whether the agent producing these outbreaks was *Piscirickettsia salmonis* or not. However, isolates from the Canadian and Irish outbreaks reacted positively with a polyclonal antibody made against *P. salmonis* (Brocklebank et al. 1993, Alday-Sanz et al. 1994).

Natural SRS outbreaks in fresh water

Initially SRS was only described as occurring in salt water (Bravo & Campos 1989, Fryer et al. 1992), although subsequently natural freshwater outbreaks of SRS have been reported in Chilean rainbow trout and coho salmon cultured in a freshwater lake (Bravo 1994a, b, Gaggero et al. 1995). The lesions observed in affected fish and the *in vitro* growth characteristics of the isolate were similar to those observed previously in saltwater outbreaks (Gaggero et al. 1995).

Isolation of an apparently new rickettsia-like organism (RLO) from Atlantic salmon has recently been reported in Chile in fish held in fresh water, salt water and an estuary (Cvitanich et al. 1995). Based on their findings, the aetiologic agent of these new outbreaks appears to be different from *Piscirickettsia salmonis*. Apparently, this pathogen, U.A.2 (unknown agent 2), had important differences from the typical strain: it did not react with a rabbit anti-*P. salmonis* polyclonal antibody; presented either an extra and intracellular location; and grew in BB and BF-2 cell lines, both of which were previously reported as non-susceptible to *P. salmonis* (Fryer et al. 1990, Cvitanich et al. 1991, 1995). In Scotland, an RLO has been isolated from farmed Atlantic salmon, associated with neurological signs and mortality (Grant et al. 1996). Although the geographical distribution of freshwater outbreaks of RLO is not limited to salmonids (Chern & Chao 1994, Khoo et al. 1995), no information is available comparing *P.*

salmonis with the RLO affecting non-salmonid fish (*Tilapia* sp. and *Plecostomus* sp.).

PATHOLOGY

Clinical signs and gross external lesions

The clinical signs and gross lesions described for natural and experimentally infected fish are similar (Cvitanich et al. 1991). Reported clinical signs are lethargy, anorexia, darkening of the skin, respiratory distress, and surface swimming (Branson & Nieto 1991, Cvitanich et al. 1991). Skin lesions include perianal and periocular haemorrhages, petechia in the abdomen, shallow haemorrhagic ulcers varying in size from 0.5 to 2 cm in diameter, and firm white nodules measuring up to 1 cm in diameter (Branson & Nieto 1991, Cvitanich et al. 1991). Additionally, bilateral exophthalmia and ulcerative stomatitis have been described. However, clinical signs and external lesions are occasionally absent in affected fish (Branson & Nieto 1991, Cvitanich et al. 1991).

Gross internal lesions and histological changes

The most characteristic lesions observed in heavily infected fish are off-white to yellow subcapsular nodules, measuring up to 2 cm in diameter, scattered throughout the liver (Cubillos et al. 1990, Branson & Nieto 1991, Cvitanich et al. 1991, Garcés et al. 1991, Brocklebank et al. 1993). Macroscopic changes in other organs include ascites, peritonitis, general pallor, diffuse swelling and presence of multifocal pale areas in the kidney and spleen (Bravo & Campos 1989, Cubillos et al. 1990, Branson & Nieto 1991). In coho salmon, the renal lesions have been interpreted as chronic damage characterized by fibrosis (Cubillos et al. 1990). Petechiae and ecchymoses on the serosal surfaces of the pyloric caeca, swim bladder and caudal intestine have also been reported in Atlantic salmon (Brocklebank et al. 1993).

Histological changes, have been usually classified in the broad category of necrosis and inflammation (Garcés et al. 1991). Commonly affected organs are liver, spleen, intestine and hematopoietic tissue of the kidney (Garcés et al. 1991). Specific lesions in these organs include multifocal to generalized coagulative necrosis, presence of fibrin thrombi within small blood vessels with necrosis of the endothelium, and infiltration by inflammatory cells (Branson & Nieto 1991, Cvitanich et al. 1991).

Microscopic lesions have also been described in other organs such as brain, skeletal muscle, skin,

heart, intestine, gills and ovary (Cvitanich et al. 1991). Specific histological lesions include mild to severe pericarditis, mild endocarditis and focal hyaline necrosis of the myocardium (Cvitanich et al. 1991). Necrosis and inflammation of the lamina propria of the intestine, epithelial hyperplasia of the gill, fusion of secondary lamella, and presence of RLO within the secondary lamella blood spaces have also been detected (Branson & Nieto 1991). The same authors describe renal tubular degeneration and pyogranulomatous myositis with ulceration of the overlying epidermis. Additionally, meningoencephalitis, focal pyogranulomatous bronchitis, pyogranulomatous splenitis with acute vasculitis and haemorrhage were detected in the Canadian outbreak (Brocklebank et al. 1993).

The rickettsial organism infects a wide variety of cells, including circulating macrophages, in which they replicate within variable-sized, membrane-bound, intracytoplasmic vacuoles (Cvitanich et al. 1991). Although varying numbers of organisms are frequently observed within these intracytoplasmic vacuoles, *Piscirickettsia salmonis* has also been found extracellularly as a result of cell lysis (Branson & Nieto 1991, Cvitanich et al. 1991).

Mixed infections with *Piscirickettsia salmonis*, along with *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD), and a pathogenic microsporidian have been observed in seawater cultured salmonids in Chile (Cvitanich et al. 1991, Smith et al. 1995). Recently, the microsporidian *Enterocytozoon salmonis* has been reported as occurring in U.A.2 infected fish (Cvitanich et al. 1995).

Infection and intracellular survival

The location and distribution pattern of *Piscirickettsia salmonis* have been observed by electron microscopy in cell lines such as CHSE-214 (chinook salmon embryo) by Fryer et al. (1990) and Cvitanich et al. (1991). Using scanning electron microscopy, 24 h after inoculation, microorganisms of 1 µm in diameter were observed attached to the exterior surfaces of CHSE-214 cells, and after 8 d of incubation the organisms were spilling from ruptured host cells or in the intercellular spaces (Fryer et al. 1990). Tissues from naturally and experimentally infected fish had either individual or paired coccoid, often pleomorphic organisms, usually enclosed within membrane-bound cytoplasmic vacuoles, with some cellular debris in addition to the RLO (Cvitanich et al. 1991). Organisms undergoing binary fission were also observed (Cvitanich et al. 1991).

Two membrane layers were identified in these organisms, a closely apposed inner membrane, and an external layer (cell wall), usually separated from the

plasma membrane or with a rippled appearance (Fryer et al. 1990, Cvitanich et al. 1991). These membrane alterations may have been caused by shrinkage of the cytoplasm during fixation and embedding procedures. Many organisms contained one or more electron-lucent spherical structures (Fryer et al. 1990). Electron-dense areas containing ribosome-like structures dispersed throughout the cell, and fibrillar DNA-like material was localized in the central region (Cvitanich et al. 1991). Small electron-lucent vacuoles, variable in size and apparently lacking any membrane, were also observed in some cells (Cvitanich et al. 1991). The mechanisms by which *Piscirickettsia salmonis* infects the target cells, avoid the intracellular killing activity, and survive inside the host are not clear and further studies are required.

Pathogenesis of SRS

The pathogenesis of SRS, or that caused by any other aquatic rickettsial organisms, has not been clarified yet. Histopathological findings of naturally and experimentally infected fish have been described in advanced stages of the disease (Branson & Nieto 1991, Cvitanich et al. 1991), but no description of the sequence of histological changes and systemic dissemination of *Piscirickettsia salmonis* has been published.

TRANSMISSION OF SRS

Despite the high impact of RLOs on the Chilean salmon industry, the mode of invasion into the host and the horizontal transmission of RLO in the natural environment still remain unclear (Cvitanich et al. 1991, Garcés et al. 1991, Bravo 1994b).

Route of infection

The intraperitoneal (i.p.) route has been reported as being effective in producing piscirickettsiosis in experimentally infected coho and Atlantic salmon (Cvitanich et al. 1991, Garcés et al. 1991). Examination of tissues from experimentally i.p. infected coho salmon showed similar lesions to naturally infected fish (Cvitanich et al. 1991).

Natural outbreaks of SRS typically occur a few weeks after smolts are transferred to the sea (Fryer et al. 1990, Branson & Nieto 1991, Cvitanich et al. 1991), which suggests that the oral route might be especially important in the case of *Piscirickettsia salmonis*. Whether other routes of infection are important in the transmission of this disease is still unknown.

Horizontal transmission

Natural horizontal transmission in seawater has been reported in stocks of salmon. Mortality occurred 2 wk after their introduction into infected sites, although the mechanisms of spread are unknown (Bravo 1994b). Elucidation of the natural mode(s) of infection and transmission of *Piscirickettsia salmonis* are one research priority (Lannan & Fryer 1993). It is still unclear if transmission is direct, or through an intermediate host (i.e. sea lice, isopods) as occurs with other pathogenic rickettsial organisms (Cvitanich et al. 1991, Garcés et al. 1991). Studies in aquaria regarding horizontal transmission have shown different results. Garcés et al. (1991) did not observe horizontal transmission in a group of non-inoculated coho salmon held in the same tank with i.p. injected fish. However, Cvitanich et al. (1991) showed that horizontal transmission of the RLO can occur in coho salmon held in seawater or fresh water without parasite vectors. The presence of the pathogen was demonstrated using light microscopy and isolation of the RLO from mortalities of experimentally inoculated and non-inoculated cohabitant fish.

Abundant RLO-laden cells have been detected in the intestine of coho salmon, leading to the hypothesis that the agent could be released through the fish faeces, and survive to infect other fish (Cvitanich et al. 1991). However, *in vitro* experiments examining extracellular survival of *Piscirickettsia salmonis* from coho salmon indicated that no infectious particles could be detected immediately after exposure to fresh water. Conversely, when suspended in salt water, infectious particles of *P. salmonis* were detected after 10 to 15 d (Lannan & Fryer 1994). The capacity of *P. salmonis* to persist in salt water could be an important epidemiological factor for the transmission of the microorganism.

Intermediate vectors may also play an important role in the natural transmission of SRS, as it occurs in mammals where rickettsial diseases are mainly transmitted by ticks (Woldehiwet & Ristic 1993). External parasites, such as the hematophagous isopod *Ceratohoa gaudichaudii*, are commonly found affecting cultured salmonids in Chile (Inostroza et al. 1993, Sievers et al. 1995). Recently, *Piscirickettsia salmonis* was detected by an indirect fluorescent antibody technique (IFAT) in tissue sections of *C. gaudichaudii* (Garcés et al. 1994); however, the importance of this finding in the transmission of the disease requires further study.

Vertical transmission

Recently, vertical transmission of *Piscirickettsia salmonis* was reported in coho salmon by Bustos et al. (1994). Results of IFAT showed that 98.3% of the fin-

gerlings coming from positive broodstock were *P. salmonis* positive. In contrast, only 26.7% of the fingerlings coming from negative broodstock were positive (Bustos et al. 1994). Due to the risk of congenital or true vertical transmission, the Chilean salmon farming industry has been screening broodstocks using available detection techniques (Cassigoli 1994).

Diagnostic methods for SRS

Salmonid rickettsial septicemia is usually diagnosed from gross lesions, and with the use of histochemical stains such as H&E, Gram, Giemsa, acridine orange, Giménez, Machiavello, and PAS to detect the pathogen in smears or tissue sections (Fryer et al. 1990, Branson & Nieto 1991, Cvitanich et al. 1991, Lannan & Fryer 1991). Although these techniques are nonspecific, they are fast, and widely used for diagnosis of SRS.

More specific techniques are also available. *Piscirickettsia salmonis* can be isolated in cell lines (Lannan & Fryer 1991), but the technique is time consuming and difficult since culture has to be performed without antibiotics, which opens the possibility of other agents proliferating and contaminating the culture (Lannan et al. 1991, Bustos et al. 1994).

Immunofluorescent antibody technique and immunohistochemistry with peroxidase-anti peroxidase (PAP) using a rabbit anti-*Piscirickettsia salmonis* polyclonal antibody are alternative procedures to detect this pathogen (Lannan et al. 1991, Alday-Sanz et al. 1994). Enzyme linked immunosorbent assay (ELISA) has also been used to detect SRS (Cassigoli 1994). Nested polymerase chain reaction (PCR) methods to detect the *P. salmonis* have been recently described (Mauel et al. 1996). These PCR procedures are fast, specific and highly sensitive for detecting *P. salmonis* genomic DNA from cultured fish cells and tissues of asymptomatic carriers (Mauel et al. 1996).

CONTROL OF SRS

Chemotherapy

In vitro tests have shown sensitivity to streptomycin, gentamicin, tetracycline (Fryer et al. 1990), chloramphenicol, erythromycin, oxytetracycline, clarithromycin and sarafloxacin (Cvitanich et al. 1991), and resistance to penicillin (Fryer et al. 1990), penicillin G, and spectinomycin (Cvitanich et al. 1991). In practice, variable results have been achieved using oral antibiotics (Cassigoli 1994). Losses due to the pathogen have increased progressively and are apparently due to antibiotic resistance (Bustos et al. 1994, Cassigoli

1994) Among the antibiotics currently used, the quinolones are the most popular, oxolinic acid and flumequine being the main quinolones currently used orally to control outbreaks (Cassigoli 1994).

Although resistance to quinolones can not be discarded, there are other factors that may influence the efficacy of the treatments. A recent report showed that several aquaculture antibacterials are antagonized by seawater cations (Barnes et al. 1995). The *in vitro* minimum inhibitory concentrations of oxolinic acid, fluoroquinolones, and oxytetracycline were increased 40- to 60-fold when exposed to Mg^{2+} , and the inactivity of antibiotics in the intestine of salmon held in the marine environment may be especially important due to the large amounts of seawater ingested (Barnes et al. 1995). Variations from pharmaceutical companies regarding effective dose and period of treatment have also been observed (Cassigoli 1994).

Other quinolones, such as enrofloxacin (Bayer®) and danofloxacin (Pfizer®), are used as i.p. injections. Encouraging results have been achieved with this procedure, possibly because every fish receives a therapeutic dose (Cassigoli 1994). Oxytetracycline, spiramycine and florfenicol have also been used (Cassigoli 1994).

Strategies to decrease the vertical or congenital transmission of *Piscirickettsia salmonis* include i.p. injection of broodstock with antibiotics 30 to 60 d before spawning, and incorporation of antibiotics in the water during hardening of the eggs after fertilization (Bustos et al. 1994).

Vaccines

There is a general agreement that a vaccine would help considerably to control SRS (Cassigoli 1994), and several groups have established research programs aimed at developing a vaccine in Chile (J. Cassigoli pers. comm.) and Canada. Only one vaccine trial using a bacterin has been published (Smith et al. 1995) and an immunoprotective effect was reported in vaccinated fish compared to controls. Although the results obtained are encouraging, they must be interpreted cautiously since the natural challenge may have been low and *Renibacterium salmoninarum* was detected in experimental fish. The trial was also too short to determine if a protective effect remained active in larger fish where economic losses are more significant (Smith et al. 1995).

Other methods

Another important factor thought necessary for the development of SRS is stress (Branson & Nieto 1991,

Cassigoli 1994). Fish carrying *Piscirickettsia salmonis* are observed in sea cages with neither clinical signs nor mortalities (Branson & Nieto 1991). It would appear that other factors are needed to precipitate the massive losses which have been experienced (Branson & Nieto 1991). Outbreaks have occurred after smolt transfer, water temperature changes and severe storms (Branson & Nieto 1991). In salmonid populations with a high prevalence of *P. salmonis* infection, the decrease of unnecessary stress and general husbandry practices such as grading, sampling, and net changes have proven to be effective preventive measures for limiting outbreaks (Cassigoli 1994).

Early elimination of dead and clinically diseased fish, appropriate disposal of blood from harvested fish, reduction of stocking densities, a decrease biomass per site and region, and provision of periods of site fallowing are some management practices suggested by the Salmon and Trout Growers Association of Chile (Cassigoli 1994). Strategic measures such as examination, diagnosis of infected broodstock and removal of their eggs, and individual batch incubation of eggs are proposals made by Bustos et al. (1994) based on their observations of vertical transmission.

RICKETTSIAL ORGANISMS IN NON-SALMONID FINFISH

Salmonids have not been the only target of rickettsial organisms and several reports have been published describing rickettsial infections in non-salmonid finfish (Tables 1 & 2). Recently, a RLO ($0.86 \times 0.63 \mu m$) was identified as the causative agent of an outbreak with mass mortality among pond-reared tilapia in Taiwan (Chern & Chao 1994). Affected species included *Oreochromis mossambicus*, *O. niloticus*, *O. aureus*, *Tilapia zillii*, *T. hornorum*, and some hybrids. Fish had hepatic lesions characterized by diffuse necrotizing hepatitis, severe vasculitis, fibrin thrombi and granuloma formation. The RLO were observed inside macrophages near or in the centre of granulomas, especially in the spleen and kidney. Effacement of hematopoietic and renal tissue by inflammatory cells was often observed. In gills, proliferation of epithelial cells resulted in fusion of lamellae, and vacuolated macrophages containing the RLOs were usually seen. Low hematocrits and increased numbers of macrophages containing RLOs were also reported (Chern & Chao 1994).

The RLO responsible for the tilapia outbreaks was isolated in epithelioma papulosum cyprini (EPC) carp cell line. The disease was reproduced in fish following intramuscular inoculation, and cohabitation among 8 tilapia species and the cichlid *Cichlasoma managu-*

ense. Experimental inoculations produced higher mortalities at 15°C than at 30°C. No infection or disease were produced in 8 other cichlid species (Chern & Chao 1994).

An RLO affecting the blue-eyed plecostomus *Panaque suttoni*, a pet fish imported from Colombia, has also been described (Khoo et al. 1995). Numerous intracytoplasmic rickettsial organisms were observed in the cells of the mononuclear phagocytic system, and in macrophages of the heart, spleen, kidney, and sometimes liver (Khoo et al. 1995). The microorganisms were within membrane-bound vacuoles, measured approximately 0.5 µm, and possessed a trilaminar cell wall. Tufts of fibrin indicative of endothelial damage were present within the interstitium of the kidney and spleen. Granulomatous lesions, necrosis and effacement of the hematopoietic elements were frequently observed. Other parasites such as trypanosomes, nematodes and embryonated eggs were also observed in the liver, kidney, spleen and heart (Khoo et al. 1995).

Recently, a RLO infecting juvenile sea-bass *Dicentrarchus labrax* reared in floating sea cages at 12 to 15°C in the Mediterranean coast of France has been described (Comps et al. 1996). Focal necrosis of mesencephalic tissues associated with an inflammatory reaction, and RLO contained within vacuoles were seen in affected fish (Comps et al. 1996).

CONCLUSIONS

In the past 8 years reports of rickettsial diseases affecting several finfish species have increased around the world. Due to the intracellular location of this organism, the control of these pathogens through the use of antibiotics has shown variable results. *Piscirickettsia salmonis* continues to cause large losses in the Chilean salmon industry, increasing the production cost of the fish as a result of the increased mortalities in some stocks and the necessity of continuous use of antibiotics.

No comparison between isolates from different countries have been reported. Further information regarding horizontal and vertical transmission, pathogenesis, intracellular survival, and immunogenesis is needed in order to establish control strategies for SRS. Identification of risk factors in the production cycle may help to avoid unnecessary management that may induce outbreaks in prevalent populations.

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