Pathological, diagnostic, therapeutic and epidemiological aspects of bacterial cold water disease (BCWD) in Chile

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Abstract

The aim of this extended abstract is to compile the current state of knowledge of bacterial cold water disease (BCWD), caused by *Flavobacterium psychrophilum*, in Chile, focusing on important aspects such as pathological, diagnostic, therapeutic and epidemiological aspects. This bacterium was first reported in Chile in 1995 [1], but it is still not included in any of the three lists denoted by the Regulation of Protection, Control and Eradication of High Risk Diseases for Aquaculture species (Chile). According to the National Fishing Service (SERNAPESCA, Spanish acronym) there is no clarity on the economic impact caused by BCWD and therefore difficult to assign a health status. Nowadays *F. psychrophilum* has increased its incidence being isolated from open and closed flow systems as well as from lake fish cultures, affecting rainbow trout (*Oncorhynchus mykiss)*, Coho salmon (*O. kisutch)* and Atlantic salmon (*Salmo salar).* In fact, the cases reported by the diagnostic laboratories from 2005 to 2010 were 1,937 in total, but in 2011 there was an increase of 162% in the number of cases from the 343 reports in 2010 (Fig. 1). An additional 30 BCWD reports have been associated with mixed infections, most of them with the Infectious Necrosis Virus (IPNV) and more exceptionally (in two cases) with internal infection associated with *Piscirickettsia salmonis* (Fig. 2) [2].

The BCWD outbreaks in Chile are chronic because the water temperature is appropriate for the pathogen (8 to 14ºC). In addition, only 7 of the 169 freshwater fish farms perform the full cycle of cultivation and so salmonids are transported once or twice from one fish farm to another in order to fulfill the facilities needed to complete their development, increasing the risks of outbreaks. In fact, fish are moved to the North of Chile in order to reduce culture costs but the susceptibility of fish increases due to stress and in some cases, fish are carriers of *F. psychrophilum*.

In the last years, differences in the clinical signs of BCWD have been observed among the three above mentioned salmonid fish species. In some cases, when rainbow trout are transported to sea cages or lake cultures, skin and systemic infections as well as ulcers, erosions on the head and operculum and skin abscesses with viscous hemorrhagic content can be observed. Internally, splenomegaly and occasionally hemorrhagic petechia in the liver can be noticed. Histologically the clinical signs are characterized by necrotic, subacute to chronic dermatitis/myositis. In Atlantic salmon the signs are characterized by skin and/or gill infections, coincidental to the histological findings and frequently associated to problems with water quality and/or IPNV. Eventually systemic infections can be seen and one of the relevant clinical signs is splenomegaly.

Outbreaks must be informed to the Passive Monitoring Program of SERNAPESCA, but not all fish affected by *F. psychrophilum* outbreaks are taken to a laboratory for diagnosis and in most cases the infection is chronic. In Chile, the presumptive diagnosis of BCWD is currently based on the clinical signs of the affected fish, particularly on gross external lesions (described above) and on microscopic observation of abundant long, thin, rod-shaped bacteria in wet mounts or Gram preparations obtained from gills or skin lesions of symptomatic fish.

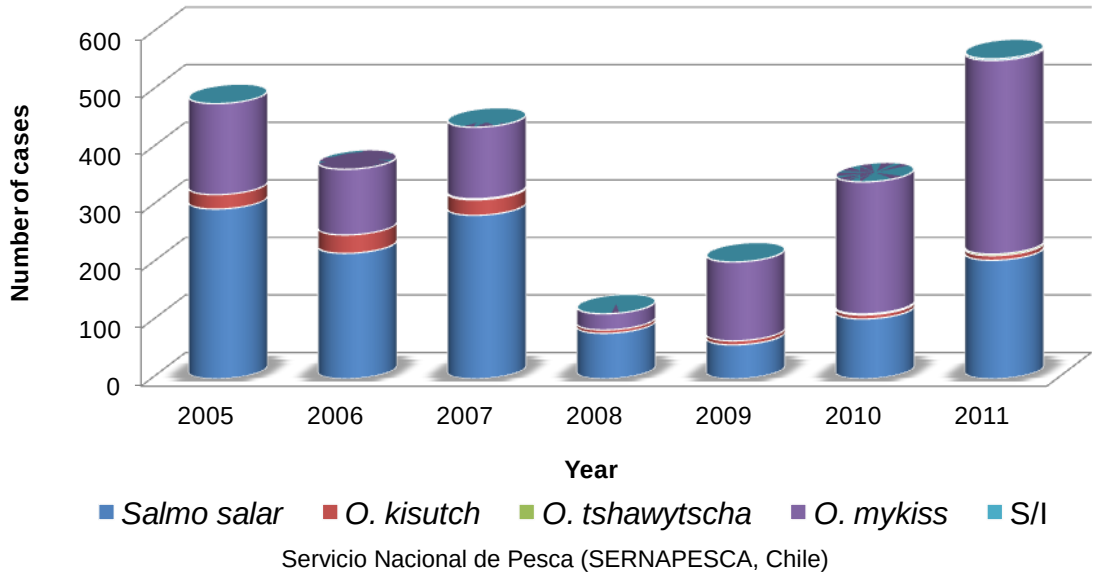

Figure 1. Number of BCWD cases reported by the diagnostic laboratories (2005–2011).

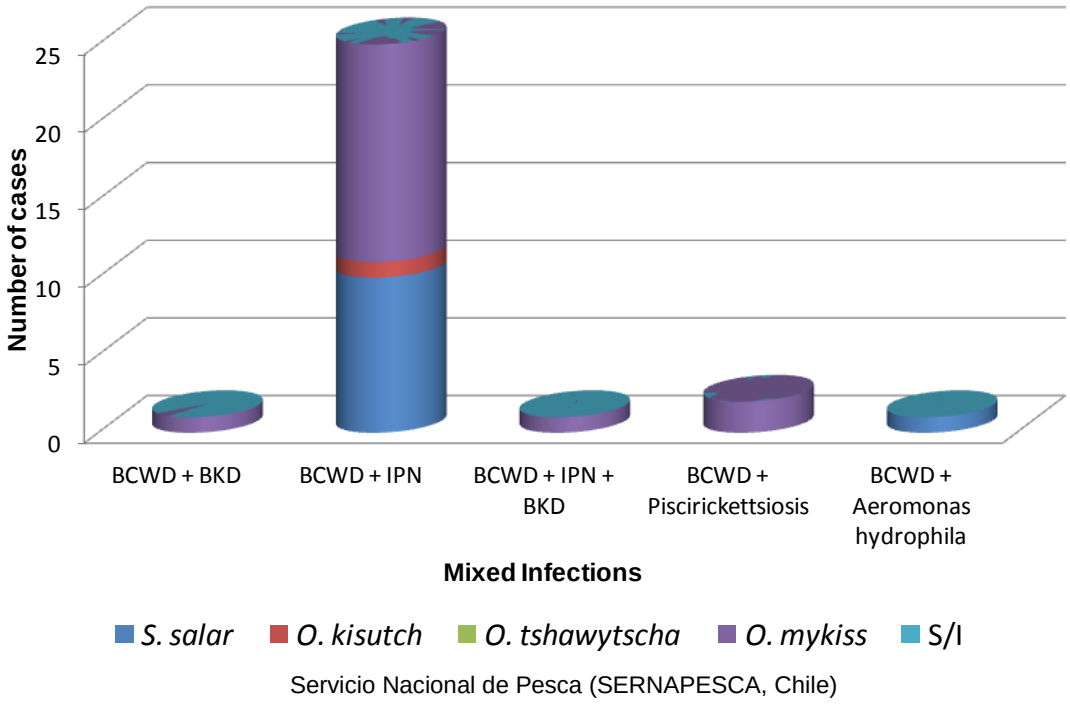

Figure 2. Number of BCWD cases associated to mixed cultures reported by the diagnostic laboratories in 2011 (30 cases).

However, the relative high incidence of secondary bacterial infections, such as those caused by *Chryseobacterium* spp. [3–4], makes visualization difficult and increases the possibility of misdiagnosis. In fact, infections produced by *F. psychrophilum* appear mixed with other phenotypically similar yellow pigmented bacteria, such as *F. araucananum* and *F. chilense*, belonging to the *Flavobacterium* species [5]. Because isolation of the bacterium from diseased fish is not always successful, the definitive diagnosis must be supported by the isolation of colonies of *F. psychrophilum* on appropriate specific media, followed by morphological and biochemical characterization, or by the use of specific molecular DNA-based methods.

Although a tentatively licensed commercial vaccine, containing whole-cells inactivated with formaldehyde, was developed recently, the efficacy of this vaccine was examined using injection-based challenge models that completely bypass the protective function of the skin mucus layer, which could serve as an important infection barrier to the disease in small fry with an immature systemic immune system. Until now, treatment with antimicrobial drugs represents the only recourse to control the disease in farmed fish (Fig. 3). Fish farmers have employed a wide range of antibiotics in the medicated food, including oxytetracycline (59%), florfenicol (34%), amoxicillin (6%), flumequine (<1%) or erythromycin (<1%) with a total volume of 12,778 kg in the year 2010 (Fig. 4). Our country has used for years autochthonous immersion bacterins, made from single farm isolates, against infections with *F. psychrophilum* (issued to prevent this disease [6]), but information about the protection is scarce (e.g. relative percentage survival, RPS). However, a good example of successful results of vaccines used in Chile is the amount of liters sold since 2000. A total volume of 4,640 liters consisting of two autovaccines against *F. psychrophilum* was requested by the first quarter of 2010.

In spite of the auspicious antecedents, up to now antimicrobial chemotherapy with oxytetracycline and florfenicol has often been the method of choice for treating outbreaks. In Chile, treatments are performed according to the outbreak severity. In practice, the treatment commonly used against external disease is bath with oxytetracycline (100–125 ppm for 1 h at closed flow for 3 days); while to internal Flavobacteriosis the most effective compound is oral treatment with florfenicol at 20 mg/kg for 15 days [7]. Recently, we studied the antimicrobial susceptibility pattern and minimum inhibitory concentration (MIC) values of 40 Chilean isolates demonstrating a very high level of resistance in F. psychrophilum which is consistent with the relatively frequent use of antibiotic therapy at the Chilean farms [8]. It is important to note that no correlation was found between the quinolone resistance-determining region (QRDR) and the susceptibility tests; whereas few isolates (n=5) had mutations in the QRDR of a partial region of the A subunit of the DNA gyrase gene (gyrA). Thirty-nine of the 40 isolates possessed a single plasmid (15 isolates with 3.5 kb) or combinations of two plasmids (17 and 7 isolates of sizes 1.5 and 3.5 kb and 2.7 and 3.5 kb, respectively), but a relationship between plasmid carriage and resistance could not be established.

Finally, from a serological and genetic point of view, our studies showed that the Chilean *F. psychrophilum* population is dominated by two antigenic groups and one genetic cluster existing of apparently Chilean-specific sequence-types [9–10].

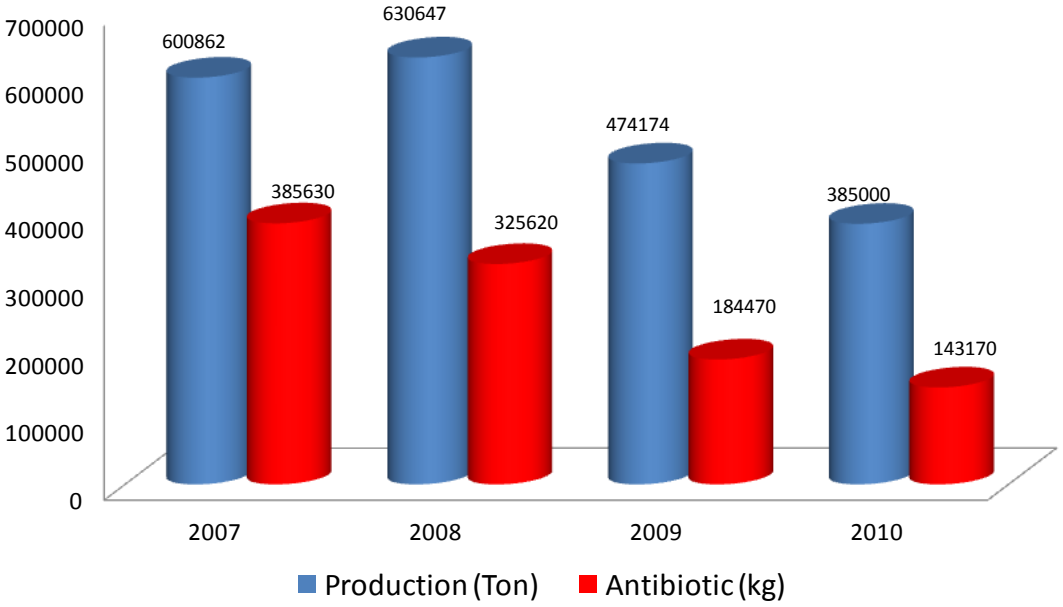

Figure 3. Volume of antibiotics (kg) used in the salmonid culture in Chile.

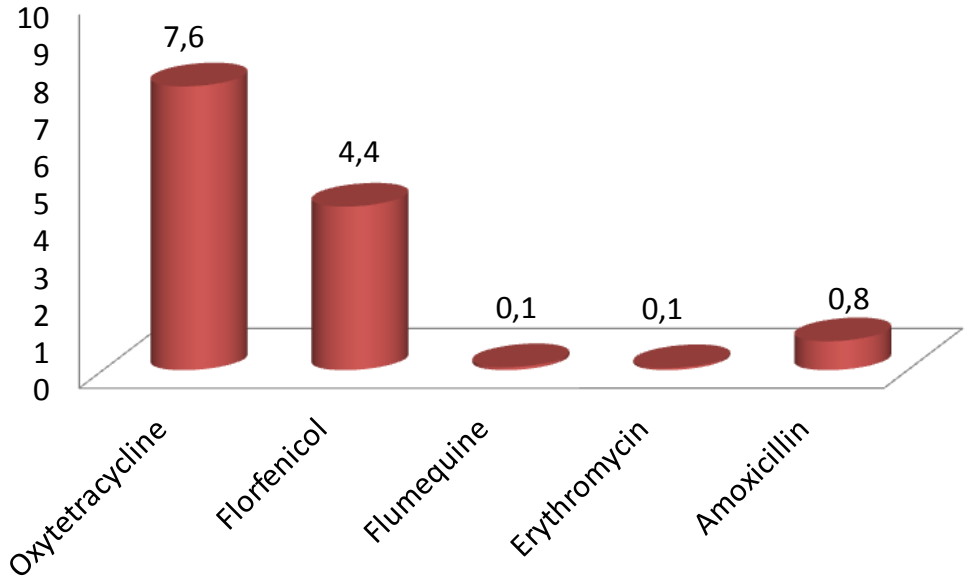

Figure 4. Use of antibiotics (tons) added to fish feed to control *F. psychrophilum*.

Based on our results, BCWD is the main cause of fish mortalities and morbidity in salmonid freshwater aquaculture in Chile and we recommend it to be listed. Since the clinical symptoms are associated to the fish species affected, the use of molecular tools for diagnostics is essential. In addition, the choice and selection of the therapeutic dose to treat the disease must be based on information obtained from antibiograms and/or MIC. Finally, necessary field data must be obtained in order to assess the effectiveness of autovaccines.

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