



Antiparasitic and immunomodulatory effect of innovative treatments against *Myxobolus* sp. infection in *Diplodus puntazzo*

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Abstract

The potential antiparasitic and immunomodulatory effect of three treatments against myxosporean parasites on the innate immune system of sharpsnout sea bream (*Diplodus puntazzo*) was investigated. Fish naturally infected with *Myxobolus* sp. (Bivalvulida/Platysporina), a histozoic parasite mainly affecting the renal interstitial tissue, were treated by oral administration of a combination of salinomycin with amprolium, *Origanum* essential oil or fumagillin in a small-scale field trial. Various leucocyte functions influenced by myxosporean infection were examined in order to determine treatment effects on leucocyte immunocompetence of treated fish. One month post treatment all drugs caused a significant decrease in prevalence and intensity of infection in comparison to untreated, infected fish. The effect was most prominent in salinomycin with amprolium treated fish, which 1-month post treatment contained either no cysts at all or a few spores free in melanomacrophage centres revealing almost total elimination of the parasite and the antiparasitic action of the treatment. There was no histopathological evidence of drug toxicity. Antiparasitic action was accompanied by a significant enhancement of phagocytic activity demonstrated by ingestion of large numbers of latex beads and the secretion of high levels of reactive nitrogen intermediates by phagocytes in vitro. Complete restoration of the diminished mitogenic responses and serum lysozyme secretion was also detected in salinomycin with amprolium-treated fish compared to untreated, infected fish. These data suggest that salinomycin with amprolium may be a promising treatment for myxosporean infections in intensively cultured warm-water fish, exhibiting action partially via the enhancement of host, innate immune functions and leading to parasite elimination.

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1. Introduction

The probability of disease in Mediterranean aquaculture species is correlated to the intensity of the production system. Under these circumstances, more frequent out-breaks of disease due to myxozoans have been reported. Myxosporeans are the most common parasites affecting intensively reared fish in the Mediterranean basin, inducing a broad spectrum of diseases determined by the parasite species, host sensitivity, environmental and feeding conditions (Sitja-Bobadilla et al., 1992; Alvarez-Pellitero and Sitja-Bobadilla, 1993a,b; Rigos et al., 1997). They are often implicated in episodes of mortality or morbidity in gilthead and sharpsnout sea bream and in sea bass, with serious financial consequences for the fish farming industry (Sitja-Bobadilla and Alvarez-Pellitero, 1993; Diamant et al., 1994; Athanassopoulou et al., 1999; Rigos et al., 1999).

Parasiticides for treatment of myxosporean infections are not commercially available, although successful treatment of various myxosporean infections with fumagillin and its derivatives has been reported in experimental trials in salmonids (Hedrick et al., 1988; Wishkovsky et al., 1990; Kent and Dawe, 1994; Higgins and Kent, 1998). Thus far, efficacy tests of fumagillin have not been followed by studies of the effect of therapeutic doses on the immunity of the treated fish, and toxic side effects ranging from inappetence to mortality (Lauren et al., 1989; Wishkovsky et al., 1990; Sitja-Bobadilla and Alvarez-Pellitero, 1992; Sovenyi, 1992) are major concerns of this drug. Thus, new formulations are being tested to meet the need for novel antimyxosporean medications, especially in sensitive species such as *Diplodus puntazzo* (Le-Breton and Marques, 1995; Athanassopoulou et al., 1999; Rigos et al., 1999; Mladineo, 2003).

Oral administration of several doses and schemes of anticoccidian drugs such as toltrazuril, amprolium, ESB3, salinomycin, *Origanum* essential oils and fumagillin, have previously exhibited differential degrees of protection against myxosporean infections in gilthead and sharpsnout sea bream tested in land-based experimental facilities (Athanassopoulou et al., 2004a,b).

In the present study, three selected treatments, a combination of salinomycin with amprolium, *Origanum* essential oil or fumagillin, were used for the treatment of naturally infected *D. puntazzo* with

Myxobolus sp. (Bivalvulida/Platysporina), a histozoic parasite mainly affecting the renal interstitial tissue, in a small-scale field trial. Along with the estimation of the antiparasitic effect, phagocytic activity, NO induction, lysozyme secretion and T cell lymphoproliferation were examined, in order to determine drug effects on leucocyte immunocompetence of treated fish and to demonstrate their potential immunomodulatory role.

2. Materials and methods

2.1. Experimental fish—small-scale field trial

A total of 1000 fish weighing approximately 1.5 g were transported to commercial cages in a farm in South Greece, which had a history of recurrent myxosporean infections. Fish were monitored for parasite infection and other fish diseases for 10 months. Imprints of gill, skin, gall bladder, liver, spleen, kidney, muscle, brain and gut tissue from freshly killed fish were examined for parasites (Roberts, 1989) and mortalities were recorded weekly. Kidney and spleen tissues were also inoculated onto Tryptone Soy Agar (Oxoid, Hampshire, England) and Thiosulphate Citrate Bile Salt Agar (Oxoid) for bacteriological examination (Roberts and Shepherd, 1997).

In April 2001 incipient *Myxobolus* sp. infection was detected, which reached 15% prevalence early in June of the same year. At this time, 150 fish (~160 g) were put into each of four smaller, experimental cages. Fish were acclimatised for 10 days and starved for another 3 days before feeding three different medicated diets to ensure maximum uptake. Fish in one experimental cage did not receive any medication and they were used as untreated, infected fish. In addition, healthy fish of similar weight and handling were used throughout the study. Healthy fish came from cages of the same farm located 30 km north of the field trial site; these fish had no myxosporean or other infection during the surveillance and trial period. At day 0, 35 and 70 after the commencement of treatment, fish ($n = 15\text{--}40$) from all groups were randomly sampled and tissue samples were prepared for parasitological, histological and immunological investigation. This small-scale field trial was carried out over June, July and August 2001 at a temperature of 20–26 °C, salinity 38‰ and pH 7.0.

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