

Effect of trichlorfon on *Diplectanum aequans* (Monogenea: Diplectanidae) infestations in cultured sea bass, *Dicentrarchus labrax*

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Abstract

The efficacy of oral administration of trichlorfon on cultured sea bass (*Dicentrarchus labrax*) naturally infected with *Diplectanum aequans* has been investigated. Sea bass (weighing 80-100 g, mean weight 92 ± 1 g SD) received a daily dosage of 50 mg kg⁻¹ trichlorfon in feed to for 7 days. On day 0, 7, 14 and 21 of the experiment, fish were randomly sampled and then parasites on the gills were counted. Comparisons were made to untreated control fish. At the beginning of the experiment all examined fish were infected with *D. aequans*. There were no statistical differences ($P > 0.05$) in terms of mean number of *D. aequans* within each group, including the control. During the treatment period, the prevalence decreased in the medicated group by 5 %, 52 %, and 35 % on day 7, 14 and 21, respectively when compared to that of the control group. No mortality was found and adverse drug reactions or palatability problems were not associated with the treatments during the study. The drug may be used during acute outbreaks but due to the risk of anthelmintic resistance this treatment regime cannot be recommended for regular use.

Introduction

Dicentrarchus labrax (L.) is the first most important cultured marine species in Turkey. Its production reached to 49,270 tonnes in 2008 (TUIK, 2009). Production problems have been found associated with heavy parasitic infections caused by the gill monogeneans *Diplectanum aequans* (Wagener, 1857). This parasite may cause gill pathology and predispose the fish to secondary fungal, bacterial and/or viral infections with resulting high mortality (Oliver, 1977; González-Lanza et al., 1991; Tokşen, 1999; Alvarez-Pellitero, 2004; Dezfuli et al., 2007; Yardımcı and Pekmezci, 2012). The infection level may be as

high as thousands of *D. aequans* per fish (Silan and Maillard, 1986). Sexually mature parasites produce high numbers of eggs which increase the parasite population in winter (Silan and Maillard, 1989; Tokşen, 1999) and there is a need for effective control methods. Some successful results have been reported with bath treatments of *D. aequans* (Giavenni, 1983; Cognetti-Varriale et al., 1991; Cognetti-Varriale et al., 1992; Sarti, 1988; Silan et al., 1996; Cruz e Silva et al., 2000), however immersion treatments of large stocks are impractical and stressful to the fish. This advocates for testing drugs administered in feed.

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The purpose of this study was to evaluate the efficacy of orally administered trichlorfon in the control of *D. aequans* infestation in sea bass.

Materials and methods

Trials were conducted in March-May 2006 at a commercial sea bass farm in İzmir, Turkey. Twenty sea bass were sampled before the treatment trials were performed. The gills of fishes were excised and kept at 4 °C as submerged in water before examination under a dissecting microscope. Monogeneans were collected and fixed without coverslip pressure in hot 5% buffered formol saline, stored in fixative 24 hours and then transferred to 70% ethyl alcohol. Specimens were mounted in glycerine jelly or Canada balsam (Gusev, 1983). The monogenean parasites observed were identified following the diagnostic keys outlined by Oliver (1968).

Four net pens, each with a volume of 50 m³ (1 control and 3 treatment groups), were each stocked with 300 sea bass, in total 1200 fish weighing 80-100 g (mean weight: 92 ± 1 g SD). During the study, water temperatures and salinity were recorded daily at 2 m depth.

Sea bass infected with *D. aequans* were exposed to a daily dosage of 50 mg kg⁻¹ trichlorfon for 7 days. Trichlorfon (metrifonate) was top-coated onto commercial sea bass pellet feed with a mechanical mixer and given freshly to the fish. The treatment fish were fed medicated feed for 7 consecutive days (days 0-6) at a rate of 0.8%-1% biomass. The control fish were fed the same but unmedicated feed. On days 7, 14 and 21 of the experiment, 20, 18, 19 and 19, 21, 20 fish from control group and medicated group, respectively, were sacrificed, weighed and parasites on the gills were counted under a stereomicroscope.

The terms prevalence, parasite range and mean abundance follow Bush et al. (1997).

Results

Sea water temperature and salinity in the pens were measured as 16.0-16.5 °C and 35 ppt, respectively. Salinity was assumed to remain stable throughout the trial period. All parasites prepared from the gills of sea bass were identified as *D. aequans*. During the treatments, no abnormal fish behaviour was observed and there was no evidence of reduced appetite or palatability problems associated with administration of medicated feed. When the study began, all of the examined fish were infected with *D. aequans*. There were no statistical differences ($P>0.05$) in terms of mean number of *D. aequans* within each group, including the control. Afterward, trichlorfon (metrifonate) was applied to the groups. Before treatment was initiated 456 parasites were counted on the gills of 20 fish from control group. The parasite range and mean abundance were 5-72 and 22.80, respectively. During the treatment period, the prevalence decreased in the medicated group by 5 %, 52 %, and 35 % on day 7, 14 and 21, respectively when compared to that of the control group. Due to the parasite recruitment, there was an increase in the number of the parasites in the medicated group on day 21. The prevalence and numbers of *D. aequans* are presented in the Table 1.

Discussion

D. aequans is a common parasite of *D. labrax* (Oliver, 1977; González-Lanza et al., 1991; Tokşen, 1999; Sterud, 2002; Cecchini and Cognetti-Varriale, 2003; Colorni and Diamant, 2005; Mladineo, 2005). The infection of the host gills is associated with haemorrhages, disruption and fusion of the secondary lamellae, marked

Table 1: The efficacy of trichlorfon (metrifonate) (50 mg kg⁻¹) against *D. aequans* for 21 days, C: Control group, M: Medicated group.

Time	Dosage (mg kg ⁻¹)	Number of fish	Prevalence	Number of parasites	Parasite range	Mean abundance
Day 0	0 (C)	20	100	456	5-72	22.80
Day 7	0 (C)	20	100	463	7-60	23.15
	50 (M)	19	95	84	0-11	4.42
Day 14	0 (C)	18	100	414	3-54	23.00
	50 (M)	21	48	30	0-8	1.43
Day 21	0 (C)	19	100	470	5-98	24.75
	50 (M)	20	65	43	0-10	2.15

erosion and inflammation of the epithelium of the primary and secondary lamellae histologically (Dezfuli et al., 2007; Yardımcı and Pekmezci, 2012) and may result in mortality (Alvarez-Pellitero, 2004). This accentuates the need for effective control methods. With regard to medical control of *D. aequans*, Giavenni (1983) and Cruz e Silva et al. (2000) could successfully treat infections with juveniles by using 375 ppm of formaldehyde bath treatment, but adult parasites survived even in 1000-1500 ppm concentrations. Even concentrations of 250, 300, 400, 500, 2500 and 5000 ppm of formaldehyde were not found effective against adult *D. aequans* according to Tokşen (1999).

A well-known group of parasiticides used against monogeneans are organophosphates. All nervous and neuromuscular functions stop as a result of the acetylcholinesterase inhibiting action of organophosphates (Buchmann and Møllergaard, 1988; Schmahl, 1991). Schaperclaus (1992) reported that 0.2, 0.4 mg l⁻¹ trichlorfon (metrifonate) for 24 hrs and 6 hrs, respectively, is effective against *Dactylogyrus* and *Gyrodactylus* when used at water temperatures above

10 °C and 5.0 mg l⁻¹ for 30 minutes below 10 °C. Nevertheless, Szekely and Molnar (1987) reported that trichlorfon was not effective against *Pseudodactylogyrus bini* and *P. anguillae* on the gill of eel (*Anguilla anguilla*). According to Cognetti-Varriale et al. (1991) bath treatment with trichlorfon at a 0.15 mg l⁻¹ for 48 hrs was effective against *D. aequans*. In order to decrease the treatment period, they increased the dose to 5 ppm during 24 hrs, but the fish mortality increased. Cruz e Silva et al. (2000) stated that trichlorfon treatment at 5 mg l⁻¹ for 30 minutes was effective against adult *D. aequans* but was toxic to the fish. Likewise, Silva de Assis et al. (1993) and Sturm et al. (1999) stated that trichlorfon causes decreased growth decrease, reduced appetite and loss in motility. But Guimaraes and Calil (2008) stated that trichlorfon bath applications would not cause any significant difference in length or weight growth in *Oreochromis niloticus*. Ruddle and Zhong (1988) and Ludwig (1993) found similar results, suggesting that no adverse effects existed in the use of trichlorfon in aquaculture. The results in this study demonstrated that trichlorfon is an effective drug against infestations of *D. aequans*

at a dose 50 mg kg⁻¹ and no immediate effects on host vitality could be seen.

After the consumption, trichlorfon quickly reduces into dichlorvos which completes its degradation in water after evacuation (Ghittino and Maletto, 1971). Brandal and Egidius (1979) reported that no chemical residue were found in fish tissue 12 days after the treatment. For fish treated with trichlorfon, a 21 withdrawal period is advised, before consumption. The application of such organophosphates must not be used indiscriminately, since other important effects occur, for example the inhibition of the acetylcholinesterase enzyme in the host. In addition, it has been shown several times that repeated use in fish farms with monogenean problems quite easily develop anthelmintic resistance. Thus, both experimentally and in farms anthelmintic resistance in *Pseudodactylogyrus* spp. has been documented to develop after repeated use of anthelmintics (benzimidazoles) (Buchmann et al. 1992; Buchmann et al. 2011) and metrifonate resistance in gyrodactylids (Goven et al., 1980). Therefore, if metrifonate are used against diplectanid infections in sea farms the use should merely be limited for acute cases and not for repeated usage. Alternation between different control methods are needed. Further investigations are also needed to determine the exact amounts, treatment durations, and efficacy of the chemicals used to treat *D. aequans* in sea bass in consideration of EU legislation.

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