TOLTRAZURIL (BAYCOX® VET.) IN FEED CAN REDUCE

ICHTHYOPHTHIRIUS MULTIFILIIS INVASION OF RAINBOW TROUT (SALMONIDAE)

Rzgar M. JAAFAR and Kurt BUCHMANN*

Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen,
Frederiksberg C, Denmark

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Abstract. Various compounds have been applied for control of Ichthyophthirius multifiliis (commonly known as Ich) which is the parasitic pathogen, responsible for the white spot disease, in freshwater aquaculture worldwide. Available methods are based on disinfection of fish tank water, targeting infective free-swimming theronts and escaped tomonts. No legal drug is available and licensed for treatment or prevention of the disease. The present study was performed to test the potential of toltrazuril (Baycox® vet.)—a drug licensed for treatment of coccidial infections in other animal species—to reduce Ich infections when administered orally. Commercial pelleted feed containing 5.0 or 2.5 mg toltrazuril per 1 g of feed was offered to rainbow trout, Oncorhynchus mykiss Walbaum, 1792 (Actinopterygii: Salmoniformes: Salmonidae), during a three day feeding regime (1% of biomass offered per day). Two trials were performed: one with feeding before exposure to parasites and one where already infected fish were treated. Before the treatment it was tested if fish would eat feed containing different drug concentrations and it was found that feed with the high drug concentration was not eaten by the fish whereas all feed containing 2.5 mg per 1 g feed was eaten. Following the exposure to infective I. multifiliis theronts it was found that the fish treated with toltrazuril before challenge obtained a significantly lower parasite burden (number of trophonts in the skin) compared to untreated control. On the other hand, toltrazuril proved to be ineffective when administered to the fish which were already infected before the treatment.

Keywords: Rainbow trout, white spot disease, Ich, Ichthyophthirius multifiliis, toltrazuril, Baycox® vet.
Bornholm where they had been hatched and reared under pathogen-free conditions at 13°C in municipal tap water. They were then brought to the experimental facilities at the University of Copenhagen. Prior to the experimental feeding fish were acclimated in two recirculated 200-L tanks at 12–14°C for 4 weeks under a constant 12:12 h light–dark cycle. The fish were, before the experimental start, fed with control pelleted trout feed (Biomar, Denmark) at the rate of 1% of the biomass per day. Water was replenished daily and concentrations of nitrite, nitrate, ammonia, and pH-levels were measured on a regular basis (Merck Aquaquant, Germany).

**Experimental fish feed.** Control feed was 1.5-mm pelleted dry feed based on fish-meal and fish oil (protein 46%, fat 28%, carbohydrate 16%, and ash 7%) and the experimental feed was similarly composed but enriched with toltrazuril (Baycox® vet.) (Bayer Health Care, Leverkusen, Germany) to concentrations 5 mg · g⁻¹ or 2.5 mg · g⁻¹ feed. The product was a suspension for oral use (50 mg active drug mL⁻¹ suspension) licensed for piglets and the drug was added by spraying the solution onto the pelleted feed.

**Parasite culture.** A laboratory culture of *I. multifiliis* obtained from the Pøle River (Zealand, Denmark) was established. The parasite population was maintained in laboratory aquaria through serial passage to naïve rain-bow trout for two years before experimental start.

Challenge infection. Highly infected fish were kept in a small fish tank of 250 L volume in order to produce a high infection pressure. Trophonts escaped the fish epidermis and subsequently transformed into tomocysts releasing theronts. The number of viable theronts in the tank water was determined with a 1-mL Sedgewick–Rafter counting chamber. Viability of theronts was assessed by their movements. Fish were then challenged by introducing them into the tank. They were tagged by a minor incision into the tail fin which allowed untreated fish (cut in upper part of the tail fin) to be differentiated from treated fish (cut in the lower part of the caudal fin).

**Parasite challenge. Trial 1.** Ten fish from each of the two groups were exposed to 50 000 theronts in the above mentioned 250 L fish tank (2500 theronts fish⁻¹) immediately after end of the 3 day experimental feeding. **Trial 2.** Fish (ten in each group) were exposed to 250 000 theronts in the 250-L fish tank (12 500 theronts fish⁻¹). These fish developed white spots on day 3 and were subsequently treated by a similar three-day feeding scheme.

**Statistical analysis.** The mean number of parasites on fish in different control and experimental groups were compared using the Mann–Whitney U-test. A 5% probability level was applied in all tests.

**Ethics.** The experiments were performed under the approval of the committee for animal experimentation, The Danish Ministry of Justice, Copenhagen, Denmark.

**Response of fish to feed.** Trout refused to eat feed with the highest drug concentration (5.0 mg · g⁻¹) whereas no effect on feed intake was registered when using 2.5 mg · g⁻¹. Feeding was observed following food administration confirming that all feed pellets were eaten in all groups.

### Table 1

Effect of prophylactic treatment of rainbow trout, *Oncorhynchus mykiss* (infected by *Ichthyophthirius multifiliis*) with toltrazuril (2.5 mg g⁻¹ feed) (offered for three days as 1% of the fish biomass per day); Invasion and development of Ich parasites in fish skin and fins following exposure was significantly reduced

| No. of trout | No. of parasites* Mean ± SD | No. of parasites** Mean ± SD | Statistics |
|--------------|-----------------------------|------------------------------|------------|
| Baycox vet.® feed | 10 63.1 ± 16.6 | 25.9 ± 6.7 | Significantly different, P < 0.05 |
| Control feed | 10 247.5 ± 39.5 | 154.4 ± 30.6 | Significantly different, P < 0.05 |

* per fish at day 6 after challenge;  
** per fish at day 8 after challenge.

### Table 2

Effect of toltrazuril treatment (three days with 2.5 mg toltrazuril g⁻¹ feed offered as 1% of the fish biomass per day) on an already established Ich infection in rainbow trout, *Oncorhynchus mykiss*. No significant decrease was seen

| No. of trout | No. of parasites* Mean ± SD | No. of parasites** Mean ± SD | Statistics |
|--------------|-----------------------------|------------------------------|------------|
| Baycox vet.® feed | 10 1097.0 ± 180.3 | 947.8 ± 145.9 | No difference, P > 0.05 |
| Control feed | 10 1056.0 ± 174.3 | 707.5 ± 222.3 | No difference, P > 0.05 |

* per fish before treatment was initiated;  
** per fish two days after treatment (three days feeding).
Fish mortality. During the entire experiment one fish died in trial 1 (following challenge and during counting) and 4 fish (infected and treated) died in trial 2 during the counting procedure.

Trophonts. Fish fed the toltrazuril feed for three days and subsequently challenged became infected with significantly fewer detectable trophonts than those fed the standard feed. This was seen when fish were examined both 6 and 8 days post-exposure where treated fish had obtained a significantly (Mann–Whitney U-test, \( P < 0.05 \)) weaker infection (means in the two groups 63 and 25 parasites per fish, respectively) compared to the infection of non-medicated fish (means in the groups 247 and 154 parasites per fish, respectively) (Table 1).

Using the same drug concentration in feed for treatment of infected fish carrying established and visible trophonts in the skin no significant effect of treatment was recorded (Table 2). This was seen in a trial in which groups of 10 rainbow trout infected by 1097 and 1056 trophonts per fish were treated with medicated or control feed, respectively. No significant decrease was seen in parasite count, neither in medicated fish (947 parasites per fish) or control fish (708 parasites per fish) when fish were examined on day 2 post-treatment. Further it was observed that the parasites in medicated fish were alive (cilia movements observed) and able to exit the host skin and swim freely in water.

The compound toltrazuril was recommended for bath treatments against the trophont stage of Ich by Mehlhorn et al. (1988) studying drug effects at the ultrastructural level but effects on infection intensities in fish by this bath treatment could not be confirmed by Lahnsteiner and Weissmann (2007). However, inclusion of the drug in feed was in our study found to reduce infection success significantly when fish were exposed to infection after being fed the drug. Thus, it was found that toltrazuril administered with a concentration of 2.5 mg · g\(^{-1}\) feed for three days reduced the infection obtained following exposure to trophonts. Due to rejection of higher concentrations of drugs by trout the low concentration must be applied. The fact that trout refused to eat feed with the high drug concentration indicates that the toltrazuril molecule may be sensed by olfactory and/or other sensory cells in the fish but it is unknown if parasites also are able to sense the drug. This compound and its metabolite ponazuril interfere with cytokinesis and elicit vacuolization in coccidia (Mitchell et al. 2005) but the mode of action of this drug against Ich is unknown. No immediate killing of parasites has been seen but it cannot be excluded that invading theronts evade skin of medicated fish due to a repelling action of toltrazuril. Fish did not like the taste of feed with a drug concentration of 5 mg · g\(^{-1}\) feed and theronts may also be suggested to avoid fish tissue with a content of toltrazuril. The feeding of already infected fish with this compound did not reduce infection and live trophonts were able to escape to water when examined. However it cannot be excluded that treatment may produce alterations in the trophonts which may affect later stages of the life cycle. This possibility was not assessed in the present study but must be investigated in future programmes. Likewise, further studies should explore if increased daily feed administration (e.g., 2%–3% of the biomass) with the low concentration feed (2.5 mg · g\(^{-1}\)) will increase the preventive or parasiticidal effect.

The rationale for using oral treatment and not bath treatments is the efficacy of the former method and the potential environmental and economical losses by using the latter method. Bath treatments are not effective against theronts (Mehlhorn et al. 1988) and have an unsatisfactory effect on trophonts (Lahnsteiner and Weissmann 2007). Further, bath treatments use higher amounts of drug which eventually may be lost to the environment.

The drug toltrazuril used is licensed for use against protozoan parasites (coccidians) in various animals such as pigs (Driesen et al. 2008), poultry (Krautwald-Junghans et al. 2009), lambs (Gjerde and Helle 1991), cattle (Ghamem et al. 2008), and dogs (Daugschiehs et al. 2000) in a range of countries. It is not specifically licensed for use in fish but in the European community antiparasitic drugs can be used for fish if the so-called “cascade principle” (EC 2004) is taken into account. This rule is as defined in EC Directive 2004/28/CE, Article 11. b. i may be applied for use of a drug licensed for another host animal if no drug is available for treatment of a disease in a specific species. Therefore toltrazuril may be considered to have a future as a legal drug against Ich within the EC.

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