ACTA ICHTHYOLOGICA ET PISCATORIA (2011) 41 (1): 63–66 DOI: 10.3750/AIP2011.41.1.09

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

Jaafar R.M., Buchmann K. 2011. Toltrazuril (Baycox® vet.) in feed can reduce *Ichthyophthirius multifiliis* invasion of rainbow trout (Salmonidae). Acta Ichthyol. Piscat. 41 (1): 63–66.

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 presently reported 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.

Various compounds are being applied for control of the skin-parasitic ciliate *Ichthyophthirius multifiliis* (commonly known as Ich), which is one of the major pathogen in freshwater fish including rainbow trout (Hines and Spira 1974, Buchmann and Bresciani 1997, Matthews 2005). The inflicted pathology is often referred to as the white spot disease. Although the immune response in fish against these skin parasites offers some protection (Clark et al. 1996, Buchmann et al. 2001, Alishahi and Buchmann 2006, Dickerson 2006, Xu et al. 2006) a number of mechanical (Shinn et al. 2009) or chemical measures must be applied in order reduce morbidity in aquaculture enterprises. Fish farmers are currently using a series of auxiliary substances such as formaldehyde, copper sulphate, and peracetic acid (Straus and Meinelt 2009, Bruzio and Buchmann 2010) or sodium percarbonate (Heinecke and Buchmann 2009) for control of white spot disease through continuous treatment of Ich-infected water. Effective drugs, however, such as malachite green and nitro-imidazoles (Toksen and Nemli 2010) are not allowed for animals intended for consumption. Moreover, formaldehyde and copper sulphate may soon be banned for aquaculture usage due to their adverse environmental and health effects. Therefore farmers need legal and licensed products for control of the disease. In this paper we present a test of a drug which may be used in acute and critical situations against white spot disease. We were able to demonstrate that the invasion of the so-called theronts into trout skin was significantly inhibited after a three day feeding regime involving the feed containing toltrazuril (Baycox® vet.).

The anti-coccidian compound toltrazuril was tested as an in-feed drug for rainbow trout, *Oncorhynchus mykiss*, against both established infections (trophonts in skin) and as a prophylactic treatment against theront invasion of the skin. Two dosages were prepared (5.0 and 2.5 mg $\cdot$ g^{-1} feed with a daily feed administration of 1% of the fish biomass for three successive days at temperature 12–14ºC, 12 h light, 12 h dark).

Fish. Rainbow trout (*Oncorhynchus mykiss*) (body length 17–19 cm, mean body weight 65 ± 2 g) were obtained from the Bornholm salmon hatchery, Nexø,

* Correspondence: Professor Kurt Buchmann, University of Copenhagen, Faculty of Life Sciences, Department of Veterinary Disease Biology, Section of Biomedicine, Stigbøjlen 7, DK-1870 Frederiksberg C., Denmark, phone: +45-35332700, e-mail: *kub@life.ku.dk*.

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 Aquacant, 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 naive rainbow 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.

Counting trophonts in fish skin following parasite exposure. Following challenge the fish in the sample were anaesthetised (on day 6 and 8 post exposure) in 60 mg · L^{-1} MS222 (Sigma-Aldrich, Denmark) and examined under a dissection microscope (7–40× magnification) whereby the total number of visible trophonts were enumerated (fins and body skin). Control fish were examined similarly.

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
Baycox vet.® feed	10	63.1 ± 16.6	25.9 ± 6.7
Control feed	10	247.5 ± 39.5)	154.4 ± 30.6
Statistics		Significantly different $P < 0.05$	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 Weismann (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 theronts. 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-Junghanns et al. 2009), lambs (Gjerde and Helle 1991), cattle (Ghanem et al. 2008), and dogs (Daugschies 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. ii 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.

ACKNOWLEDGEMENTS

This work was supported by the Henrik Henriksen Foundation, Denmark.

REFERENCES

Alishahi M., Buchmann K. 2006. Temperature-dependent protection against *Ichthyophthirius multifiliis* following immunisation of rainbow trout using live theronts. Diseases of Aquatic Organisms **72** (3): 269–273. DOI: 10.3354/dao072269.

Bruzio M., Buchmann K. 2010. The effect of peracetic acid products on parasites causing white spot disease. Fish Farmer **6**: 25–27. www.fishfarmer-magazine.com.

Buchmann K., Bresciani J. 1997. Parasitic infections in pond-reared rainbow trout *Oncorhynchus mykiss* in Denmark. Diseases of Aquatic Animals **28** (2): 125–138.

Buchmann K., Sigh J., Nielsen C.V., Dalgaard M. 2001. Host responses against the fish parasitizing ciliate *Ichthyophthirius multifiliis*. Veterinary Parasitology **100** (1–2): 105–116. DOI: 10.1016/S0304-4017(01)00487-3.

Clark T.G., Lin T.-L., Dickerson H.W. 1996. Surface antigen cross-linking triggers forced exit of a protozoan parasite from its host. Proceedings of the National Academy of Sciences **93** (13): 6825–6829. DOI: 10.1073/pnas.93.13.6825.

Daugschies A., Mundt H.-C., Letkova V. 2000. Toltrazuril treatment of cystoisosporosis in dogs under experimental and field conditions. Parasitology Research **86** (10): 797–799. DOI: 10.1007/s004360000217.

Dickerson H.W. 2006. *Ichthyophthirius multifiliis* and *Cryptocaryon irritans* (Phylum Ciliophora). Pp. 116–153. *In*: Woo P.T.K. (ed.)

Fish diseases and disorders. CAB International, Wallingford, UK. DOI: 10.1079/9780851990156.0116.

Driesen S.J., Fahy V.A. Carland P.G. 2008. The use of toltrazuril for the prevention of coccidiosis in piglets before weaning. Australian Veterinary Journal **72** (4): 139–141. DOI: 10.1111/j.1751-0813.1995.tb15034.x.

EC 2004. EC directive 2004/28/CE, Art. 11, b.ii. Official Journal L136, 30/04/2004 pp. 0058-0084.

Ghanem M.M., Radwaan M.E., Moustafa A.M.M., Ebeid M.H. 2008. Comparative therapeutic effect of toltrazuril, sulphadimidine and amprolium on *Eimeria bovis* and *Eimeria zuernii* given at different times following infection in buffalo calves (*Bubalus bubalis*). Preventive Veterinary Medicine **84** (1–2): 161–170. DOI: 10.1016/j.prevetmed.2007.12.013.

Gjerde B., Helle O. 1991. Chemoprophylaxis of coccidiosis in lambs with a single oral dose of toltrazuril. Veterinary Parasitology **38** (2–3): 97–107. DOI: 10.1016/0304-4017(91)90120-K.

Heinecke R.D., Buchmann K. 2009. Control of *Ichthyophthirius multifiliis* using a combination of water filtration and sodium percarbonate: Dose-response studies. Aquaculture **288** (1–2): 32–35. DOI: 10.1016/j.aquaculture.2008.11.017.

Hines R.S., Spira D.T. 1974. Ichthyophthiriasis in the mirror carp *Cyprinus carpio* (L.). V. Acquired immunity. Journal of Fish Biology **6** (4): 373–378. DOI: 10.1111/j.1095-8649.1974.tb04554.x.

Krautwald-Junghanns M.-E., Zebisch R., Schmidt V. 2009. Relevance and treatment of coccidiosis in domestic pigeons (*Columba livia* forma *domestica*) with particular emphasis on toltrazuril. Journal of Avian Medicine and Surgery **23** (1): 1–5. DOI: 10.1647/2007-049R.1.

Lahnsteiner F., Weismann T. 2007. Treatment of ichthyophthiriasis in rainbow trout and common carp with common and alternative therapeutics. Journal of Aquatic Animal Health **19** (3): 186–194. DOI: 10.1577/H07-002.1.

Matthews R.A. 2005. *Ichthyophthirius multifiliis* Fouquet and ichthyophthiriosis in freshwater teleosts. Advances in Parasitology **59**: 159–241. DOI: 10.1016/S0065-308X(05)59003-1.

Mehlhorn H., Schmahl G., Haberkorn, A. 1988. Toltrazuril effective against a broad spectrum of protozoan parasites. Parasitology Research **75** (1): 64–66. DOI: 10.1007/BF00931192.

Mitchell S.M., Zajac A.M., Davis W.L., Kennedy T.J., Lindsay D. 2005. The effects of ponazuril on development of apicomplexans in vitro. Journal of Eukaryotic Microbiology **52** (3): 231–235. DOI: 10.1111/j.1550-7408.2005.00029.x.

Shinn A.P., Picon-Camacho S.M., Bawden R., Taylor N.G.H. 2009. Mechanical control of *Ichthyophthirius multifiliis* Fouquet, 1876 (Ciliophora) in a rainbow trout hatchery. Aquacultural Engineering **41** (3): 152–157. DOI: 10.1016/j.aquaeng.2009.07.002.

Straus D.L., Meinelt T. 2009. Acute toxicity of peracetic acid (PAA) formulations to *Ichthyophthirius multifiliis* theronts. Parasitology Research **104** (5): 1237–1241. DOI: 10.1007/s00436-009-1361-9.

Toksen E., Nemli E. 2010. Oral treatment trials on telescope fish (*Carassius auratus*) experimentally infected with *Ichthyophthirius multifiliis* (Fouquet, 1876). Bulletin of the European Association for Fish Pathologists **30** (2): 48–54.

Xu D.-H., Klesius P.H., Panangala V.S. 2006. Induced cross-protection in channel catfish, *Ictalurus punctatus* (Rafineque), against different immobilization serotypes of *Ichthyophthirius multifiliis*. Journal of Fish Diseases **29** (3): 131–138. DOI: 10.1111/j.1365-2761.2006.00700.x.

Received: 25 January 2011
Accepted: 15 February 2011
Published electronically: 25 March 2011