Veterinary Parasitology 168 (2010) 212–216

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Prevention of *Ichthyophthirius multifiliis* infestation in goldfish (*Carassius auratus*) by potassium ferrate(VI) treatment

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ARTICLE INFO

Article history:
Received 19 July 2009
Received in revised form 3 November 2009
Accepted 11 November 2009

Keywords:
Ichthyophthirius multifiliis
Potassium ferrate(VI)
Goldfish
Infestation

ABSTRACT

Ichthyophthirius multifiliis is an important freshwater teleost pathogen that often leads to significant economic losses to the aquaculture industry. The purpose of this study was to assess the acute toxicity of potassium ferrate(VI) to *I. multifiliis* theront and the concentration needed to prevent *I. multifiliis* infestation in goldfish, *Carassius auratus*. Five hundred theronts were exposed to concentrations of potassium ferrate(VI) in each well of a 96-well microtiter plate and observed for 4 h to determine the acute toxicity. Results showed that the exposure of *I. multifiliis* theronts to potassium ferrate(VI) at concentrations of 4.80 mg/L or more resulted in 100% mortality by 4 h; the LC_{50} value was estimated to be 1.71 mg/L. Aqueous static renewal 96-h bioassays were carried out to determine the acute toxicity of potassium ferrate(VI) to goldfish. The LC_{50} value for potassium ferrate(VI) in goldfish was 42.51 mg/L. Goldfish were exposed to 4000 theronts/fish in aerated tap water (a dose previously shown to result in consistent infestation) and treated with a single dose of potassium ferrate(VI) after 30 min contact with theronts. Infection level and prevalence were recorded everyday after exposure. The results revealed that potassium ferrate(VI) at the 4.80 mg/L or more concentrations can significantly reduce not only the number of trophonts on the fin of goldfish on day 3 ($P < 0.05$), but also the prevalence of ichthyophthiriasis ($P < 0.05$). Potassium ferrate(VI) at a concentration of 4.80 mg/L was considered to be the lowest effective dose to prevent infestation of *I. multifiliis* in goldfish.

1. Introduction

The ciliate, *Ichthyophthirius multifiliis*, is the main parasitic threat to freshwater fish in large parts of the world (Buchmann et al., 2001). The disease ichthyophthiriasis, commonly known as white spot disease, can result in considerable economic losses to the aquaculture industry, including the freshwater ornamental fish trade. The ciliate life cycle, which consists of three stages: an infective theront, a parasitic trophont and a reproductive tomont, is well documented (Nigrelli et al., 1976; Noe and Dickerson, 1995; Swennes et al., 2006). Free-swimming theronts enter into the epidermis of fish to feed on mucus and tissue and rapidly differentiate into trophonts, and following a period of growth and development, the trophonts leave the host actively and transform to encysted tomonts. The tomonts undergo mitosis in the cyst and release theronts, the stage infective to the fish host.

In terms of current strategies for controlling ichthyophthiriasis in aquaculture, chemical agents aimed at interrupting the life cycle by killing the free-living stages of the parasite play the major role, although in some situations water management and vaccine can also be effective (Matthews, 2005). However, the application of

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0304-4017/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.vetpar.2009.11.009

chemical treatments in aquatic systems has to face two significant problems: toxicity to fish and safety to the environment. Additionally, many effective and widely used chemotherapeutants (e.g. malachite green) against *I. multifiliis* are no longer permitted to be used by some government agencies, such as the Food and Drug Administration (FDA) of the USA. So, the search for an effective drug for ichthyophthiriasis, which is not only safe for fish and the environment, but also permitted by legislation in some areas, becomes stringent.

Potassium ferrate(VI) is an environmental friendly strong oxidant in the entire pH range, which is from −2.2 V in acid to −0.7 V in base (Wood, 1958; Ma and Liu, 2002). Because potassium ferrate(VI) combines disinfectant and coagulative functions, it can potentially be used as a dual-function chemical reagent for water and wastewater treatment (Jiang and Lloyd, 2002). Recently, some researchers have proven that potassium permanganate can be used to control ichthyophthiriasis (Straus and Griffin, 2001, 2002). Potassium ferrate(VI), is not only a stronger oxidant, but also less toxic to animals, and safer for the environment and humans. Potassium ferrate(VI)'s potential as a therapeutic agent for external protozoan parasite infections is worthy of investigation.

The purpose of this study was to assess the acute toxicity of potassium ferrate(VI) to free-living *I. multifiliis* theronts and to examine the efficacy of this chemical against *I. multifiliis* infestations in goldfish in aerated tap water. The present study also evaluated the acute toxicity of potassium ferrate(VI) to goldfish. Such information will be useful in formulating safe treatment rates for ornamental and food fish.

2. Materials and methods

2.1. Fish

Goldfish (*Carassius auratus*), weighting 4.27 ± 0.72 g, were utilized throughout the study. All fish, referred to as "naïve fish", were kept in several 300 L opaque tanks and supplied with a constant flow of aerated tap water (flow rate 1.0–1.5 L min^{-1}), at 22.0 ± 2 °C, pH 7.0 ± 0.3, with dissolved oxygen 6.0–7.8 mg/L, ammonia content (total nitrogen) 0.5–2.0 mg/L and total hardness ($CaCO_3$) 85.0–104.5 mg/L. They were fed once at 1% body weight daily with commercial fish pellet feed, produced by the Institute of Hydrobiology, Chinese Academy of Sciences.

2.2. Parasite

A local strain of *I. multifiliis* was isolated from goldfish, obtained from a pet shop and its passage was as Ling et al. (2009) described. The fish were held at 22 ± 2 °C in a static 40 L aquarium equipped with an outside biological filter and air stones to maintain enough dissolved oxygen (greater than 5 mg/L). *I. multifiliis* was collected using a method described by Clayton and Price (1988). Several heavily infected fish were placed into 300 mL of filtered aquarium water for 30 min. Mature trophonts were allowed to dislodge from the host by body movements of the fish while in close proximity. The cysts thus obtained were incubated at 23.5 ± 0.5 °C for 18–20 h, and theronts were allowed to emerge naturally. The infectious theronts were used to determine the acute toxicity of potassium ferrate(VI) to *I. multifiliis* and to challenge fish during experiments. Theront concentrations were estimated by pipetting 2-μL droplets of the theront suspension onto a glass slide and counting the organisms (40× magnification). The final concentration was extrapolated using a mean of 10 droplets from the theront suspension (Schlenk et al., 1998; Straus and Griffin, 2001).

2.3. Potassium ferrate(VI)

The potassium ferrate(VI) used in this study was supplied by the Xi'an Tian Shun Fine Chemical Plant, and the preparation of this reagent was followed by the wet oxidation method described by Jiang and Lloyd (2002). In order to obtain an accurate dosage for treatment, the concentration of potassium ferrate(VI) was measured using both chromate titration and spectroscopy methods (Jia et al., 1999; Jiang and Lloyd, 2002). This reagent containing 96% of potassium ferrate(VI) was used throughout this study.

2.4. Acute toxicity of potassium ferrate(VI) to I. multifiliis theronts

An in vitro study was designed to determine the acute toxicity of potassium ferrate(VI) to *I. multifiliis* theronts according to an immobilization method (Sin et al., 1991; Ling et al., 1993; Straus and Griffin, 2001; Buchmann et al., 2003). The theronts were placed into 96-well microtiter plates at a final concentration of 500 theronts per well with 100 μL of solution and exposed to concentrations of potassium ferrate(VI) at 0, 0.096, 0.96, 1.92, 4.80, 9.60, 14.40, 19.20, 24.00, and 48.00 mg/L, respectively. Acute toxicity was assessed directly by dissection microscopic examination (16–40× magnification) of each well at various intervals up to 4 h after treatment. The theront cells with the absence of motility and abnormal morphology were considered dead. The experiment was conducted at 23.5 ± 0.5 °C, and replicated three times using separate populations of theronts for each potassium ferrate(VI) concentration.

2.5. Determination of infective dosage

In order to achieve consistent infestation of goldfish, an experiment was conducted to determine the appropriate number of infective theronts. Sixty healthy goldfish were divided into six groups ($N = 10$), and exposed in opaque beakers to 0, 1000, 2000, 4000, 8000, and 16,000 theronts per fish, respectively. The infection protocol was referred to Ling et al. (2009). For each group, theronts were placed into an opaque 2 L beaker prior to infection and the goldfish were transferred into the beaker at a density of one fish per 100 mL of aerated tap water. After 30 min, during which time infection occurred (McCallum, 1982), all the contents of each beaker were respectively placed into six static 20 L aquaria, equipped with air stones and in which the fish had been previously acclimated for at least 1 week. The half of each aquarium water was renewed on alternate days with aerated tap water. The experiment was