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EFFICACY OF PRAZIQUANTEL AGAINST EXTERNAL PARASITES INFECTING FRESHWATER FISH

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ABSTRACT

Praziquantel has been used to control external parasites on marine fish but there is little data about its effect on treating external parasites infecting freshwater fish. This study examined whether praziquantel was able to control external parasites on freshwater fish in Vietnam. Four external parasites, monogenean trematode (*Dactylogyrus* sp.), parasitic Crustacea (*Lenear* sp.) and two ciliated Protozoa (*Trichodina* sp. and *Epistylis* sp.) naturally infecting Grass carp (*Ctenopharyngodon idella*), Common carp (*Cyprinus carpio*) and Tilapia (*Oreochromis niloticus*), respectively, were experimentally treated. Infected fish were exposed to praziquantel in baths for periods of 1, 3, 24, 48 and 72 hrs at each of five different concentrations 2.5, 5, 7.5 and 10 mg/l with a control (0 mg/l) treatment. Doses of 7.5-10 mg/l praziquantel administered for 1-3 h for fish infected with two ciliated Protozoa and 24-72h for the other two parasites were effective in eliminating all parasites without killing the fish. Thus, praziquantel of doses at 7.5-10 mg/l immersed within 72h duration is an appropriate therapy for simultaneous treatment of a number of external parasites of freshwater fish. The results have important implications in the management of fish health in current aquaculture systems.

Keywords: External parasites, freshwater fish, praziquantel, treatment.

Hiệu quả điều trị của praziquantel đối với một số ngoại ký sinh trùng ký sinh trên cá nước ngọt

TÓM TẮT

Praziquantel đã được sử dụng phổ biến để điều trị ký sinh trùng trên cá, đặc biệt là các loài cá biển. Tuy nhiên chưa có nhiều nghiên cứu điều trị ký sinh trùng trên cá nước ngọt. Nghiên cứu này được tiến hành trên một số loài cá nước ngọt phổ biến ở Việt Nam. Praziquantel được sử dụng điều trị thử nghiệm 4 loại ngoại ký sinh trùng gồm sán lá đơn chủ (*Dactylogyrus* sp.), trùng mỏ neo (giáp xác ký sinh - *Lenear* sp.) và 2 Protozoa ký sinh gồm trùng bánh xe (*Trichodina* sp.) và trùng loa kèn (*Epistylis* sp.) lần lượt ký sinh trên ba loài cá nước ngọt gồm trắm cỏ (*Ctenopharyngodon idella*), chép (*Cyprinus capio*) và rô phi (*Oreochromis niloticus*). Cá nhiễm bệnh được ngâm praziquantel ở các nồng độ 0; 2,5; 5; 7,5 và 10 mg/l trong khoảng thời gian 1; 3, 24; 48 và 72 giờ. Kết quả nghiên cứu cho thấy, ngâm praziquantel ở nồng độ 7,5-10 mg/l cho cá nhiễm bệnh loại bỏ hoàn toàn 2 Protozoa sau 1-3 giờ và 2 ngoại ký sinh trùng còn lại sau 24-72 giờ và không làm ảnh hưởng đến sức khỏe của cá. Như vậy, ngâm praziquantel nồng độ 7,5-10 mg/l trong vòng 72 giờ có thể dùng như một biện pháp thay thế hữu hiệu và an toàn để điều trị kết hợp nhiều ngoại loài ký sinh trùng trên cá và thay thế cho nhiều loại hóa chất khác. Kết quả nghiên cứu có ý nghĩa rất quan trọng trong việc quản lý sức khỏe các loài cá trong các hệ thống nuôi thủy sản hiện nay.

Từ khóa: Cá nước ngọt, điều trị, ngoại ký sinh trùng, praziquantel

1. INTRODUCTION

In recent years, aquaculture has become a well-established industry in Vietnam. Increased interest in fish culture has also increased awareness of and experience with parasites that affect fish health, growth and survival. Infection of freshwater fish by external parasites has increased in incidence and severity. In general, wild fish are seldom heavily affected by external parasites. In most cases, the outbreaks were caused by common ciliated parasites naturally present on the skin and gills of pond-reared fish. Low intensity of parasitic infection is not harmful, but when fish are crowded or stressed, and water quality deteriorates, parasites multiply rapidly and cause serious damage. Typically, heavily infected fish do not eat well and exhibit low growth rate, discoloration and mucus secretion. Weakened fish become susceptible to opportunistic bacterial pathogens in the water resulting in major stock losses (Kayis et al., 2009; Wang et al., 2008). Parasitic infection is usually controlled by chemicals such as formalin, copper sulphate, potassium permanganate, quinaldine (Crigel et al, 1995), trichlorfon (Thoney, 1990), Aqui-S (Sharp et al., 2004) and toltrazuril (Mehlhorn et al., 1988). Intensive fish farming and the frequent use of chemicals has caused parasites to develop resistance to such treatments and also resulted in damage to the environment. Therefore, the need for alternative chemical treatments and methods of control that are more effective and sustainable has increased considerably in recent years.

Praziquantel has been used to treat various parasites of human, animals and fish (Mitchell, 2004). Several studies demonstrated the effectiveness and safety of this chemical for fish parasites (Chisholm and Whittington, 2002; Janse and Borgsteede, 2003; Katharios et al., 2006; Van et al., 2012). Additionally, praziquantel has been used as a cleaner of residue at the bottom of ponds which can in turn improve water quality. However, most studies on using praziquantel to control parasites have focused on marine fish; few studies have been conducted for freshwater fish although freshwater fish are frequently infected with a multitude of parasite species. Therefore, it is necessary to examine the effect of praziquantel on a wide range of freshwater fish parasites. The aim of this study was to test praziquantel on several common freshwater fish parasitised by four external parasites; *Dactylogyrus* sp., *Lernaea* sp., *Trichodina* sp. and *Epistylis* sp.

2. MATERIALS AND METHODS

2.1. Chemical

Pure praziquantel (Biltricide , Bayer, Germany) was dissolved into ethanol/distilled water 1.75 : 3.25 to obtain the stock solution containing 100 mg/l praziquantel and then this solution was diluted to different concentrations for the final use of the chemical in each treatment.

2.2. Parasites and hosts

Fingerling fish naturally infected with *Dactylogyrus* sp. (Grass carp); *Lernaea* sp. (Common carp); *Trichodina* sp. and *Epistylis* sp. (Tilapia) were obtained from the hatchery in Bac Ninh, Vinh Phuc and Hai Duong provinces, Vietnam from March to August, 2013. The fish were measured for length and weight and the initial infection was examined to confirm prevalence and intensity of the infection prior to the conduct of experiments (Table 1).

Table 1. Information of fish used for the experiment (n =15*)

Fish species	Sample location	Infected Parasites	Length (mm)	Weight (g)	Initial infection	
					Prevalence %	Intensity (parasites/specimen)
Grass carp	Bac Ninh	*Dactylogyrus* sp.	45-67	1.8-3.1	100	> 20
Common carp	Bac Ninh	*Lernaea* sp	87-103	16-19	100	8-15
Tilapia	Hai Duong	*Trichodina* sp.	42-56	1.4-2.6	100	> 20
	Vinh Phuc	*Epistylis* sp.	52-71	2.1-3.2	100	> 50

*Note: *Fish (n=15 per species) were randomly chosen and quickly examined under a microscope to detect parasites before exposed to treatments; Prevelence and intensity of parasites infecting fish were examined following Ky and Te (2007)*

2.3. Experiments

2.3.1. Experimental design

Fish were grown in 2m³ tanks at ambient temperature (23-25°C) and fed daily at 7-10% of fish body weight with commercially pelleted feed supplied by Cargill Company. Treatment of infected fish was conducted in different tanks and at different concentrations. One hundred fingerling fish were incubated in each tank containing 1500l water. Praziquantel dilution was added to each tank to yield final concentrations of 0, 2.5, 5, 7.5 and 10mg /ml. Fish were immersed for 1, 3, 24, 48 and 72h at 23-24°C.

2.3.2. Investigation of the effectiveness of praziquantel treatment

After exposure to Praziquantel treatment, 15 infected fish treated at each of the respective concentrations were randomly chosen and immediately euthenised. Parasites were mounted as specimens on slides in water and examined under a cover slip with a compound microscope at 100 × magnification, except for *Lernaea* sp. which could be counted by visual observation. Mean intensity (mean number of parasites per infected fish) was calculated for each group to investigate the effectiveness of dose and duration treatments.

2.3.3. Data analysis

All data were analysed by using SPSS 16. A One-way ANOVA was used to examine mean parasite intensity over time for treatments and Tukey's HSD test was used for post-hoc analysis.

3. RESULTS

3.1. Behaviour of fish during treatment

Praziquantel treatments with doses from 2.5 to 10 mg/l showed no visible effects within 72h for all fish species during the experiments. Treatments also helped to reduce mortality during the 72h experiments (Table 2). Therefore, praziquantel is safe for fish with current treatment doses and duration.

3.2. Effective treatment of paraziquantel

3.2.1. The efficacy of praziquantel treatments against Dactylogyrus sp.

Grass carp infected with *Dactylogyrus* sp. showed high prevalence (100%) and intensity of infection from 28.96 to 31.26 parasites/fish (Fig. 1, 2) Treatment with praziquantel at a dose rate of 2.5 to 10 mg/l after 3h could remove *Dactylogyrus* sp. from the gills and skin of fish and significantly reduced the intensity of infection compared with control fish ($p<0.05$). However, the infected intensity varied with the concentration of praziquantel and duration of treatment. The effective treatments observed were 7.5 mg/l for 48h and 10 mg/l for 24h treatment which eliminated 100% *Dactylogyrus* sp. from gills and skin of fish (Fig. 1, 3, 4).

Table 2. Comparison of fish mortality between praziquantel treatments and control group during the 72h experiments

Fish species	Treatments		Control	
	Total fish	Mortality (%)	Total fish	Mortality (%)
Grass carp	500	0	100	4
Common carp	500	0	100	2
Tilapia	500	0 8	100	8

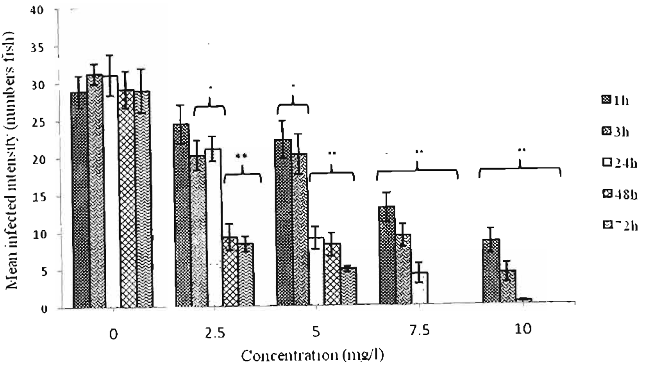

Fig. 1. The relationship between mean intensity of infection and concentration of praziquantel on Grass carp infected with *Dactylogyrus* sp. after different exposure periods to praziquantel

Note: (*) Significantly different with P<0.05; (**) Significantly different with P<0 001

3.2.2. The efficacy of praziquantel treatments against Lernaea sp.

Before treatment, the mean intensity of *Lernaea* sp. on common carp was 10.11 ± 2.31 parasites per fish (Table 2) and the prevalence was 100% (Table 1). This infection level was also found in the control group raised in dechlorinated tapwater following experimental treatments. Praziquantel was not effective in removing the parasitic Crustacea from the skin over a 24h exposure period. After 2 days exposure to treatment, this parasite started to show atrophy and dropped off from the skin. However, examination of the skin of Common carp in experimental tanks showed that praziquantel at concentration of 2.5 to 5 mg/l significantly reduced infection intensity but could not remove all parasites from exposed fish, whereas high doses (7.5 - 10 mg/l) were effective in eradicating all *Lernaea* sp. on the skin of fish within 48-72h (Table 3).

3.2.3. The efficacy of praziquantel treatments against 2 Protozoa (Trichodina sp. and Epistylis sp.)

Tilapia were infected with *Trichodina* sp. and *Epistylis* sp. at the same time with high prevalence and intensity, 100% fish infected with 47.54 ± 2.75 to 56.71 ± 2.12 parasites/fish (*Trichodina* sp.) and 106.52 ± 13.41parasites/fish (*Epistylis* sp.) (Table 4; Fig. 5, 7, 8). After exposure to praziquantel for short duration, all *Epistylis* sp. parasitising the gills of Tilapia were damaged and motionless at a dose of 7.5 and 10 mg/l after 3h immersion (Table 4, Fig. 6). At the lower doses (2.5 and 5 mg/l), a few individual parasites were still attached to the gills after 24 and 48h exposure but following 72h treatment all of them were inactive (data not shown). Therefore, praziquantel is effective in treating *Epistylis* sp. at 2.5 to 10 mg/l for 3-72h duration of immersion. *Trichodina* sp. was less sensitive to this chemical. After incubation in praziquantel at the dose of 2.5 to 5 mg/l for 1h, 64.3 to 75.2 % of parasites demonstrated reduced motility. However, a few parasites were still detected moving on the gills of fish after 72h exposure to chemical. Doses of 7.5 mg/l and 10 mg/l for 1-3h severely damaged parasites and resulted in non-motile parasites; they were then eliminated due to the action of gills during respiration (Table 5; Fig. 9).

Table 3. Prevalence and intensity of parasites of Common carp by parasitic Crustacea *Lenear* sp. after exposure to praziquantel

Praziquantel treatment (mg/l)	48h post expose to treatment		72h post expose to treatment	
	Prevalence (%)	Mean number of parasites per fish ± S.D.	Prevalence (%)	Mean number of parasites per fish ± S.D.
0	93 3	10 11 ± 2.31[a]	86.7	10.42 ± 1.78[a]
2.5	93.3	3 46 ± 0.47[b]	40.0	2.26 ± 0 15[b]
5	43.5	1.24 ± 0 43[c]	26.7	1.07 ± 0.14[c]
7 5	13.3	0.13 ± 0.35[d]	0	0[d]
10	0	0[d]	0	0[d]

Note: The different letter in same column indicated significant difference (P<0 05)

Table 4. The efficacy of praziquantel treatments against *Epistylis* sp. after 3h immersion

Praziquantel treatment (mg/l)	Prevalence (%)	Mean number of parasites actively moving per gill arch ± S.D.
0	100	106.52 ± 13 41[a]
2.5	100	61.43 ± 5.34[b]
5	76	23.45 ± 3.14[c]
7 5	0	0
10	0	0

Note: The different letter in same column indicated significant difference (P<0 05)

Table 5. Intensity of *Trichodina* sp. infecting Tilapia exposed to praziquantel at different doses and duration

Praziquantel treatment (mg/l)	Infected intensity (parasites/fish) over time of treatment (h)				
	1	3	24	48	72
0	48.13 ± 3.31[a]	49 21 ± 2.02[a]	47.54 ± 2.75[a]	54 34 ± 3 29[a]	56.71 ± 2.12[a]
2.5	18 24 ± 2.23[b]	14.12 ± 1.42[b]	12.25 ± 1 87[b]	8.43 ± 1.47[b]	6.36 ± 0.64[b]
5	7.26 ± 1.12[c]	4.34 ± 0 41[c]	4.04 ± 0 17[c]	2 24 ± 0.14[c]	1.03 ± 0 33[c]
7.5	2.32 ± 0.31[d]	0[d]	0[d]	0[d]	0[d]
10	0[e]	0[d]	0[d]	0[d]	0[d]

Note: The different letter in same column indicated the significant difference (P<0.05)

Fig. 2. ***Dactylogyrus*** **sp.**
in gill of Grass carp

Scale = 100μm

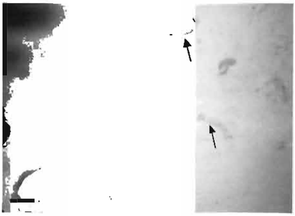

Fig. 3. ***Dactylogyrus*** **sp.**
dropped off and non-motile

Scale = 100μm

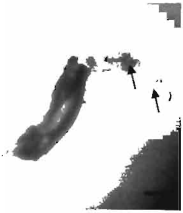

Fig. 4. ***Dactylogyrus*** **sp. damaged after 24h exposed to treatment at 7.5 mg/l**

Scale = 20μm

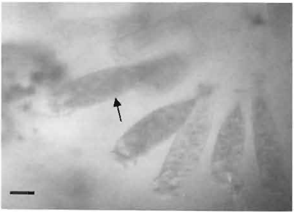

Fig. 5. ***Epistylis*** **sp. before treatment**

Scale = 50μm

Fig. 6. ***Epistylis*** **sp. motionless after 3h immersion in praziquantel at dose of 5 mg/l**

Scale = 100μm

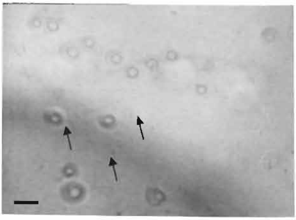

Fig. 7. ***Trichodina*** **sp. moving in control group**

Scale = 50μm

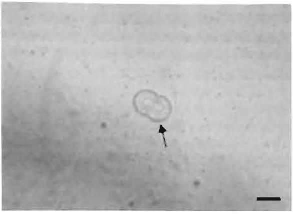

Fig. 8. *Trichodina* sp. reproducing in control group

Scale = 50μm

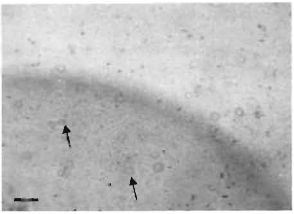

Fig. 9. Non-motile *Trichodina* sp. after 1h treatment at dose of 7.5 mg/l

Scale = 100μm

4. DISCUSSION

Potential treatment methods for fish infected by parasites can be divided into four major groups including mechanical, biological, chemical treatments and egg treatments (Cowell et al., 1993; Hoa and Ut, 2007; Whittington, 2011; Buchmann and Bresciani., 2006) However, chemical treatments are the most widely used in aquaculture. Chemical treatments can only provide short term control as they are only effective on attached parasites stages (Ernst et al., 2005). Numerous chemicals have been trialled to manage monogenean infections with varying success. The most widely used are copper sulphate, formaldehyde, sodium chloride, hydrogen peroxide and oral chemical treatments (Buchmann and Kristensson, 2003; Chisholm and Whittington, 2002; Ellis and Watanabe, 1993; Kim and Choi, 1998; Rach et al., 2000). However, the most important consideration when using these chemicals is the toxicity to the host and the parasite which is dependent on the species as well as biotic and abiotic conditions (Buchmann and Bresciani, 2006). Therefore, extreme caution must be taken and each parasite - host system should be examined specifically prior to using a treatment on a large scale (Whittington and Chisholm, 2008). Praziquantel is the chemical of choice in the control of schistosome and cestode infections in humans and animals. In the 1970s, it was demonstrated to be effective in eradicating various fish parasites. As a result, this chemical has wide application against fish parasites through bathing and oral treatments in marine and freshwater fish culture (Thoney, 1990). Thoney and Hargis (1991) also demonstrated that teleosts and elasmobranchs infected with "skin parasites" bathed with paraziquantel at doses of 10-20mg/l within 1-3h was effectively removed all of skin parasites. Other bath treatments have also been applied to treat *Microcotyle sebastis* infecting rockfish (doses of 100 mg/l within 4min; Kim and Cho, 2000), monogeneans infecting *Rhinobatos typus* (doses of 5mg/l after 40h; Chisholm and Whittington, 2002), *Benedenia seriolae* and *Zeuxapta seriolae* (doses 2.5 mg/l within 24-48h; Sharp et al., 2004) successfully. This chemical was also widely used for oral administration. Praziquantel was investigated as the preferred treatment to treat metacercaria of *Centrocestus formosanus* infecting *Cyprinus carpio* (Van et al., 2012). However, the effectiveness of this method for external parasites varied depending on dose and parasites. For example, oral administration at same dose could reduce *Microcotyle sebastis* infecting Rockfish (Kim and Choi, 1998), but had no effect on *Dendromonocotyle torosa* infecting Spotted eagle ray (Janse and Borgsteede, 2003).

Freshwater fish contribute to a large proportion of aquaculture production in Vietnam. Intensive culture has been developing rapidly creating ideal conditions for disease outbreaks caused by parasites. Therefore, the urgent task is to find chemicals with broad spectrum efficacy in treating various parasites while minimising the potential negative impacts to the cultured fish, environment or humans. The results obtained from the experiments in this study show that praziquantel was successful in treating four external parasites infecting three water fish species including one monogenean (*Dactylogyrus* sp.), two ciliated Protozoa (*Trichodina* sp. and *Epistylis* sp.) and a parasitic crustacea (*Lenear* sp.) within 72h at doses 7.5 to 10 mg/l. This investigation suggests the potential for simultaneous treatment of multiple parasitic infections within a fish pond using only praziquantel. This chemical also demonstrated no negative impacts on fish health or behaviour during treatments. Mortality observed from treatment group was less than that of control group (Table 2) probably due to the adverse effect of praziquantel on parasites and their asexual reproduction, which plays vital role in causing massive reinfection with high intensity and fish mortality. In addition, the advantage of treatment by bathing fish infected with external parasites is that chemical can also treat free living stages of many parasites existing in the water which would otherwise have the potential to reinfect fish if not treated (Hoai et al., 2013) Therefore, it can be concluded that praziquantel meets the requirements for a potential broad spectrum treatment in Vietnamese aquaculture.

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