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ATTEMPTS TO CONTROL ICHTHYOPHTHIRIASIS IN VEILTAIL AQUARIUM FISH

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SUMMARY

In this study, a controlled experimental infection of Veiltail aquarium fish with I.multifilis has been carried out. Chemical treatment using commercial formalin, methylene blue and sodium chloride at suitable doses and time of exposure, have been used to treat the infected fish. Also the physical treatment through rising of water temperature to 25°C and 30°C with partial of full water change have been tried with great success. The protective immunity as expressed by the parasitic burden on challenged fish and mortality percent was proved in Veiltail fish that were exposed to primary high dose of infection followed by chemical or physical treatments and finally challenged with the same primary infective dose.

INTRODUCTION

Ichthyophthirius multifilis (I.multifilis), the causative agent of white spot disease, is one of the most dangerous ectoparasitic cilliated protozoa of freshwater fishes. The disease is well recognized as a world wide limiting factor in commercial exploitation of feashwater fish including both food and aquarium species (Hines and Spira, 1973; Selosse and Rowlnd, 1990). I. multifilis has a simple , direct life cycle. The trophozoites are located beneath the epidermis of the host, and is visible externally as white spots . On completion of its growth, the mature trophozoite leaves the host and settles onto a solid substrate in water, where it secretes a thin cyst wall, within which it undergoes rapid division and large numbers of infective tomites are released to invade further new hosts (Hoffman, 1978). Ichthyophiriasis is essentially a cool water disease, the causative parasite is active at temperature below 21°C. The time taken for its development on the fish is very temperature-dependent (Richards, 1977), where the optimal duration range of its full development is 4-7 days (Hoffman, 1978). Mortalities resulting from this disease are difficult to be controlled due

to the several developmental stages of the parasite and their temperature-dependence. Most available chemicals have been successful in treating the free-living forms only and do not have to do with the encapsulated trophozoites (Farely and Heckmann, 1980). The application of physcial measures, namely the rise in water temperature up to 28-29°C to kill the infective free tomites have been also used with success (Dickerson and Dawe, 1995). More recently, attention has concentrated around the prospects of preventive immunization as a mean of control, not only to avoid the drastic side effects of chemical therapy, but also due to the fact that the fish may develop some degree of acquired immunity to I.multifilis after exposure and recovery from this parasite, however, the mechanism by which the immunity is elicited after natural infection and recovery remains largely unknown (Dickerson and Dawe, 1995).

As the major problem encountered in the vaccination regime of Ichthyophthiriasis is due to the obligatory pathogenic nature of I.multifilis. Therefore, in this study a controlled laboratory infection was applied in Veilital aquarium fish followed by chemical and physical treatment aiming for monitoring the acquired protection in such experimentally infected and treated fish at challenge with I. multifilis.

MATERIALS AND METHODS

Naive Fish

A total number of 150 apparently healthy, parasites-free Veiltail fish, with 50-60 mm length were obtained alive from aquarium fish breeder at Giza. These fish were bred from brood stock without history of previous parasitic infection. The fish were kept in well aerated, chlorine-free tap water at 20°C.

Ichthyophthirius multifilis

I. multifilis used in this tudy was obtained from heavily infected aquarium fish supplied by some aquarium fish breeders. The parasite was maintained in the laboratory by serial transmission on naive Veiltail fish as described by Dickerson et al., (1989).

The infected fish were placed in a 20 L. aquarium with six naive to I.multifilis at 20°C. When fish become infected, all fish but separate were removed and placed in 10L. aquarium containing aerated water, six naive fish were again added to the aquarium to maintain the infection. Finally, the fish were transferred to new aquarium and the water was gently poured off. The settled trophozoites in the aquarium bottom were rinsed in sterile water, were pipetted into 2 ml wells of tissue culture plate and allowed to divide overnight in the dark at 20°C. The free swimming tomites were collected and their mean of concentration was estimated from the suspension after agitation by direct counting under microscope (Dickerson et al., 1989).

Primary exposur to infection:

All Veiltail fish naive to I.multifilis were exposed to high dose of infective tomites which collected within 4 hours of excystment in dark (app. 1000 tomites/fish) via immersion as described by

Mc Callum (1986). Following exposure for one hour in the dark, the fish were transferred to glass aquarium supplied with aerated, dechlorinated tap water and kept for five days at 20°C. The infected fish were then divided into 9 groups each of 10 fish and the ensuing infection was treated as follows:

a. Chemical treatment:

The infected fish were treated by commercial grade formalin (40% formaldhyde), Methylene blue (medical quality) and Sod.chloride (iodine free). The concentration of these chemicals were calculated on the basis of previous data of effectiveness and toxicity and were applied firstly on small fish samples (Richards, 1977). All chemical treatments were applied at water temperature of 20°C.

b. Physical treatment:

In this type of treatment, there was a half and complete water change at 20°C. The water temperature was then gradually risen to 25°C and 30°C using thermostatically controlled, water heaters.

Control infected fish groups were subjected to similar treatments, but without use of any chemical.

Challenge infection:

All Veiltail fish that treated and survived the infection with I.multifilis and ten naive control fish were immediately challenged through exposure to the same dose of infective tomites as mentioned in the primary infection (app. 1000 tomites/fish). Water temperature was maintained at 20°C. Parasite burden on fish were assessed by direct counting under dissecting microscope daily post-challenge.

RESULTS

In the present investigation, the trails to treat Ichthyophthitiasis in infected Veiltail fish either chemically or physically are met with great success.

Table (1) shows, that, all infected fish groups which were treated with chemicals namely, formalin (25 ppm); Methylene blue (4ppm) and Sod. chloride (30.000 ppm) as short time baths for up to 4 minutes for three times with one day interval, have recovered from infection within 2 weeks post-treatment with complete disappearance of the external clinical signs (white spots). On the other hand, all of the control infected, non treated fish were died.

Table (2) shows that, the infected fish that exposed to physical management in the form of partial and complete water change as well as increase of water temperature to 25°Cand 30°C were recovered from infection within one week post-exposure, however there was some fish loss if compared with the chemical treatment. Also, all fish in the control infected, non-treated group were died.

Concerning the acquired protection in treated fish after challenge infection as measured by the

Table (1):

Efficacy of chemical treatments in *I. multifilis* infected Veiltail fish

Time in weeks	Control		Formalin (25ppm)		Methylene blue (4 ppm)		Sod. Chloride (30.000ppm)	
	degree of Ich.	survived Fish	degree of Ich.	survived Fish	degree of Ich.	survived Fish	degree of Ich.	survived Fish
0	+++*	10	+++	10	+++	10	+++	10
1	+++	8	-	10	-	10	-	10
2	+++	3	-	10	-	10	-	10
3	-**	0	-	10	-	10	-	10
4	-	0	-	10	-	10	-	10

Table (2):

Relation of water temperature and water change on the degree of infection with *I. multifilis* in Veiltail fish

Time in weeks	Water temperature									
	20°C		25°C				30°C			
	Control		Half water change		Full water change		Half water change		Full water change	
	degree of Ich.	survived fish	degree of Ich.	survived Fish	degree of Ich	survived fish	degree of Ich.	survived Fish	degree of Ich	survived fish
0	+++	10	+++	10	+++	10	+++	10	+++	10
1	+++	8	++	10	+	10	++	10	+	10
2	+++	3	-	10	-	9	-	10	-	10
3	-	0	-	10	-	9	-	10	-	9
4	-	0	-	10	-	9	-	10	-	9

N. B. : The number of fish in each group is 10 fish.

* +++ : degree of infection is equal to untreated.

** - : infection with Ich. is negative.

Table (3):

Post-challenge Parasite burden and mortality percent in Veiltail fish

Fish group No.	Mean peak parasite burden/fish	Peak time in days	Duration of infection /days	Mortality No.	Mortality %
(I) Control	40	5	7	9/9	100
(II)					
1. Formalin	15	3	5	0/9	0
2. Methylene blue	14.7	3	5	0/9	0
3. Sod. chloride	0	0	0	0/9	0
(III)					
1. 50%water change at25°C	5	3	5	0/9	0
2. Full water change at 25°C	17	6	7	0/9	0
3. 50%water change at30°C	32	5	7	1/9	11.1
4. Full water change at 30°C	35	5	7	1/9	11.1

* The number of Veiltail fish is 9 in each treatment group

mean peak parasite burden per fish, the day on which the peak parasite burden occurred, the time taken by challenged group to recover and the mortality percentage when compared with the control group, the results as shown in (Table 3) revealed the considerable differences of fish in their ability to acquire resistance to infection. Incomplete resistance to infection was established in fish treated by chemicals (formalin and methylene blue) and physical treatment (partial and complete water change at 30°C and complete water change at 25°C). High degree of sustained immune protection were observed in fish held at 25°C with partial change of water, while full protection was recorded in fish treated with Sod.. chloride at 20°C.

DISCUSSION

The ability of I.multifilis to cause outbreaks in freshwater aquarium and food fish is commonly known . In the literatures, several trails have been undertaken for control such disease problem (Hines and Spira, 1974; Houghton and Matthews, 1990). Acquired protective immunity in fish which survive a primary exposure to I.multifilis have been reported by (Hines and Spira, 1974; Mc Callum, 1986). The assessment of disease resistance in fish is hampered by the inability to expose fish to controlled infection and measure the resulting infection levels on fish (Price, 1985). However, Clayton and Price (1992) have overcome these problems by controlled experimental infections and quantified the resultinf level to the fish.

In this study, a routine laboratory maintenance of the I.multifilis had been used for the controlled infection and challenge. This method was applied as described by Mc Callum, (1986) and Dickerson et al., (1989), who reported that a limited time was available for host location. Ekless and Mathews (1993) attempted to cultivate I.multiiifilis in monophasic media and so could extend the survival time of theronts up to 5 days, 3 days longer than that kept in water.

The results of the treatment experiment indicated that chemical treatments was effective to overcome Ich. disease in two weeks. Also, the rise in water temperature to 25°C and 30°C in combination with half or full water change killed the theronts and could eliminate the infection in one week. These obtained results supported those reported by Farley and Heckmann (1980); Stuart (1983); Griffin (1989) and Selosse and Rowland (1990).

Concerning the challenge infection with I.multiiifilis (Table 3) the results indicated that the parasite didn't follow the same characteristic pattern of infection during challenge as compared with the primary infection. The peak parasite burden was lower and occurred earlier and the infection was of shorter duration in chemically treated group than in the infected, non-treated control group. It was obvious that, infected fish treated with Sod. Chloride proved the absence of parasites post-treatment with a complete acquired resistance to reinfection.

The parasite burden on fish that challenged after the water temperature has been risen to 30°C demonstrated an infection pattern and tomites

growth in a similar manner as of the control infection. Although the parasitic burden on fish challenged at 25°C with full water change were lower than those of challenged fish at 30°C, a significant differences was not detected. Similar results have been reported by Hines and Spira (1974) who immunized mirror carp with sub-lethal doses of tomites and Lom (1969), who proved that immune resistance in carp was a temperature-dependent . Also Rijkers et al., (1980) and Ellis (1988) who recorded that induction of immune memory was absent at temperature below 18°C.

The differences in the degree of fish resistance in these experiments could be attributed to the degenerative changes of the fish skin with the osmoregulatory imbalance in addition to the parasitic stress in the experimental fish. Treatment by chemicals or raising the water temperature with partial or full water change could produce an extra stress on the fish which consequently has an immunosuppressive effect on treated fish. On the other hand, the treatment with Sod. Chloride had the advantage of the availability of Sodium ions in water that help fish to maintain its osmotic balance thus reducing the stress which leads to increase the defense reactions. These results supported those of Hines and Spira (1974).

In this study, an induced protective immunitt in fish against ichthyophthiriasis has been taken in consideration to overcome the problem of lack of any commercial vaccine for I.multifilis. This could be achieved by exposing Veiltail fish to high infective dose of tomites followed by treatment and finally challenge with the infective primary dose. This method of protective immunization should receive further investigations.

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