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Two year study on the infectivity of *Ichthyophthirius multifiliis* in channel catfish *Ictalurus punctatus*

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ABSTRACT: *Ichthyophthirius multifiliis* Fouquet (Ich) is a fish parasite that causes serious economic loss for aquaculture. A major difficulty in the maintenance of single isolates of Ich for research purposes is the loss of infectivity. After an unknown number of passages or infection cycles the Ich isolate loses its infectivity. This study determined the infectivity of an Ich isolate during 105 infection cycles in channel catfish *Ictalurus punctatus* over a 2 yr period. The mean percentage of fish infected by Ich, the infection levels and the time to trophont emergence were each compared after 4 cyclic periods: 1–25, 26–60, 61–90 and 91–105 Ich cycles. Results of this study demonstrated that Ich was significantly more infective ($p < 0.05$) at 1–25 than 26–105 cycles. Channel catfish were infected at a ratio of 1 infected fish to 8 naïve fish at 1–25 and 26–60 cycles. A higher infection ratio occurred at 61–90 and 91–105 cycles. Trophont emergence was noted to be significantly longer at 91–105 compared to 1–25 cycles, during 7 and 5 d respectively, at 23.4 ± 1.1°C. The results of the present study indicate that the infectivity of *I. multifiliis* started to decrease after 25 infection cycles and was predominant in the single Ich isolate at 61–90 and 91–105 cycles.

KEY WORDS: Senescence · Passage · Infection ratio · *Ichthyophthirius multifiliis* · Channel catfish

INTRODUCTION

Ichthyophthiriasis is a parasitic disease of freshwater fish which leads to high mortality and heavy economic loss for aquaculture (Paperna 1972, Jessop 1995, Traxler et al. 1998). The disease is caused by a ciliated protozoan, *Ichthyophthirius multifiliis* Fouquet, 1876 (Ich), with 3 developmental stages: a reproductive tomont, an infective theront and a parasitic trophont (Hines & Spira 1974, Nigrelli et al. 1976). The trophont is an obligate parasitical stage and requires susceptible fish to propagate. The infection starts when the theront penetrates fish epithelium. The trophont grows there for 5 to 6 d at 20 to 25°C and the adult trophont leaves the host after its completion of normal development.

Long-term maintenance of any Ich isolate is seriously hampered by the loss of infectivity or senescence (Houghton & Matthewes 1986, Burkart et al. 1990). Ekless & Matthews (1993) demonstrated short-term maintenance of Ich in selected monophasic media. However, *in vitro* cultivation in the selected monophasic media was observed not to support growth and development of Ich comparable to host fish. Noe & Dickerson (1995) were able to sustain growth of Ich for 14 serial passages, or infection cycles, by lowering the temperature of the host channel catfish to 9°C. The study demonstrated that viability and surface immobilizing antigen of theronts were not altered at 9°C compared to 25°C. Infectivity, however, was not extended beyond the 14 serial passages in the study. The number of successive passages necessary for an Ich culture to reach senescence is unknown. Houghton & Matthews (1986) noted a loss of viability of Ich culture 9 mo after its isolation. Burkart et al. (1990) maintained an Ich culture at 23 to 25°C for 24 mo by adding new parasite isolates twice from additional infected fish to the Ich culture to increase the parasite virulence. It was not known at which Ich passage the new isolates were added. Presumedly each isolate was kept for 8 to 9 mo and the old Ich isolate was probably replaced after the

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introduction of the new isolate. It is generally accepted that the maintenance of an Ich culture for longer than 1 yr is difficult. However, no data have been found to demonstrate the effect of long-term maintenance on Ich infectivity. The present study evaluated the infectivity of an Ich isolate over 2 yr in channel fish *Ictalurus punctatus* by comparing Ich infection, infection levels and time to trophont emergence after each of 4 Ich infection cycles of 1–25, 26–60, 61–90 and 91–105.

MATERIALS AND METHODS

Fish and parasite cultures. Juvenile channel catfish (13.3 ± 3.7 cm in length and 27.1 ± 15.2 g in weight, mean ± SD) were used as host fish and reared from eggs at the US Department of Agriculture, Aquatic Animal Health Research Laboratory, Auburn, Alabama. Fish were treated with formalin at a concentration of 100 mg l^{-1} (equivalent to 37 mg l^{-1} formaldehyde) for 1 h to eliminate external parasites and kept in tanks with flowing water for 1 wk before used for the maintenance of Ich culture. The *Ichthyophthirius multifiliis* isolate was obtained initially from an infected goldfish *Carassius auratus* L. purchased at a pet shop. This single isolate was designated as ARS-2.

Ich infectivity. A total of 105 serial infectivity experiments of a single Ich isolate were performed using 2056 channel catfish from April 2001 to March 2003. Fish were kept in tanks supplied with flowing dechlorinated water, undergravel filter, and aeration at an average temperature of 23.4 ± 1.1 or 25.5 ± 0.6°C. The results of these serial infectivity experiments were averaged into 4 periods with Ich infection cycles of 1–25, 26–60, 61–90 and 91–105. For Ich passages from 1–60, 8 Ich naïve catfish were stocked into a 50 l aquarium filled with dechlorinated water. A single Ich infected fish was killed and put in the tank with Ich naïve fish as described by Beckert (1967). The dead fish were removed after 24 h and flowing water was provided at approximately 0.3 to 0.4 l min^{-1}. The Ich naïve fish were observed daily for mortality, infection, and time to trophont emergence. The infection levels were determined by the number of visible trophonts on each fish. The levels, including non infection, <50, 50 to 100, >100 trophonts per fish and killed by Ich, were scored as 0, 1, 2, 3, and 4, respectively. Non-infected fish were those without visible trophonts 7 to 8 d post-infection. Since the infectivity of Ich was reduced after serial passages, the ratio of infected fish to naïve fish was periodically increased in order to maintain the infection. The ratios of Ich-infected fish to naïve fish were 1:4 at 61–90 cycles and 1:3 at 91–105 cycles. The resulting infection was considered ideal when fish showed heavy infection (>100 trophonts per fish) but not killed by Ich. Since the ratio of Ich-infected fish to naïve fish influenced the infectivity, an adjusted infection level was used for this study. Adjusted infection level = infection percentage × infection level/infection ratio.

Statistics. The data for fish mortality, adjusted infection levels and time to trophont emergence were compared for 4 passage periods with Duncan's multiple range test. The relationship between the number of Ich passages and infection ratio was evaluated with Spearman correlation (SAS Institute 1989). Probabilities of 0.05 or less were considered statistically significant.

RESULTS

Ich infectivity after different numbers of cycles

Fish killed by Ich were noted to vary from 24.3–17.0% in the 1–25 and 26–60 cycles. However, the fish mortality was significantly reduced to 8.0–11.3% in the other infection cycles (Table 1). The adjusted infection level was higher ($p < 0.05$) at 1–25 cycles than all further cycles. The Ich infectivity declined gradually with the increments of Ich infection cycles.

Time to trophont emergence

The time to emergence of trophonts was noted to significantly increase up to 7 d post-infection at 91–105 cycles at 23.4 ± 1.1°C (Table 2). Five days was the mean time required for trophont emergence at 1–25 and 26–60 cycles. No significant difference was noted for the emergence time of trophonts after different infection cycles between 23.4 ± 1.1 and 25.5 ± 0.6°C (Table 2).

Table 1. *Ichthyophthirius multifiliis* infectivity to *Ictalurus punctatus*. Mean mortality of channel catfish killed by *I. multifiliis* (Ich), % of infected fish, and adjusted infection level of fish infected by Ich after different numbers of passages. Values are means ± SD. Within a column, means followed by the same lower-case letter are not significantly different ($p > 0.05$)

Ich cycles	Fish (N)	Mean % of mortality	Mean % of infected fish	Mean Ich infected level	Mean adjusted infected level
1–25	369	24.3 ± 7.2^a	90.7 ± 4.5^a	2.58 ± 0.18^a	2003.1 ± 156.4^a
26–60	781	17.0 ± 5.2^b	92.5 ± 3.7^a	1.91 ± 0.19^b	1650.0 ± 158.5^b
61–90	462	11.3 ± 4.4^c	91.4 ± 6.3^a	1.81 ± 0.20^b	788.7 ± 104.5^c
91–105	444	8.0 ± 4.0^c	88.5 ± 4.0^a	2.31 ± 0.16^c	627.1 ± 60.5^d

Table 2. *Ichthyophthirius multifiliis* infectivity to *Ictalurus punctatus*. Effect of number of *I. multifiliis* (Ich) passages and water temperature on trophont emergence on channel catfish after Ich challenge infection. Within a column, means followed by the same lower-case letter are not significantly different ($p > 0.05$). Within a row for time of trophont emergence on fish at different Ich passage, means followed by the same upper-case letter are not significantly different. Fish uninfected or killed by Ich over the infection period were excluded from this analysis

Ich cycles	23.4 ± 1.1°C		25.5 ± 0.6°C	
	Fish (N)	Mean time to trophont emergence (d)	Fish (N)	Mean time to trophont emergence (d)
1–25	23	$5.3 \pm 0.3^{a,A}$	232	$5.3 \pm 0.2^{a,A}$
26–60	390	$5.6 \pm 0.1^{a,A}$	134	$5.5 \pm 0.4^{a,A}$
61–90	204	$6.0 \pm 0.3^{b,A}$	52	$5.9 \pm 0.3^{b,A}$
91–105	131	$7.1 \pm 0.3^{c,A}$	223	$6.6 \pm 0.2^{c,A}$

Correlation between Ich passages and the infection ratio

There was a positive correlation between the number of Ich passages and the infection ratio (coefficient 0.728, $p < 0.01$) for 2056 fish. Naïve catfish were infected at the ratio of 0.125 (1 infected fish:8 naïve fish) at 1–60 cycles. However, the infection ratio had to be increased to 0.25 for 61–90 cycles and 0.33 for 91–105 cycles in order to maintain an ideal infection of fish by the parasite.

DISCUSSION

No data has been found to assess the Ich infectivity after continuous passage of a single isolate. In the present study, Ich infectivity was evaluated for 105 serial passages at room temperature for 2 yr. The adjusted infection level was significantly lower at 26–60 than 1–25 cycles, indicating the start of Ich senescence. Infectivity of the Ich isolate gradually decreased with the increment of Ich passages. After 60 successive passages (approximately 1 yr), higher ratios of infected fish to naïve fish were needed for maintenance of Ich culture. The loss of infectivity of *Ichthyophthirius multifiliis* was predominant in the single Ich isolate at 61–90 and 91–105 cycles.

The percentage of infected fish and infection level could not be compared for different Ich passages if the ratios of infected to naïve fish were varied. The Ich with weak infectivity could cause heavy infection if more trophonts were introduced into a tank by using more infected fish. The problem was solved in this study by using the adjusted infection level. The adjusted infection level evaluated the infectivity of Ich by considering simultaneously (1) percentage of fish infected, (2) infection level, and (3) infection ratio. The adjusted infection level was higher when fish in a tank showed a high infection percentage and level when using a low infection ratio to infect fish.

The occurrence of senescence has been found in other ciliates or protozoa. *Tetrahymena thermophila* maintained in the laboratory for a prolonged period became senescent and gradually lost the ability to produce viable progeny at conjugation (Nanney 1980). In a study on the virulence of *Trapanosoma cruzi*, Basombrío et al. (2000) noted that successive *in vitro* culture of the parasite resulted in an attenuated stock of parasites with low infectivity to invade mammal hosts.

Some factors have an influence on Ich infectivity, such as temperature, water quality, host species, and immune response of the host (Clayton & Price 1992, Dickerson & Dawe 1995). In this study, these factors were not major concerns since we used channel catfish which had not been previously exposed to Ich, and maintained similar water quality and laboratory conditions for every passage of Ich. Two factors may be considered when addressing the senescence of the parasite: population selection and conjugation. Unlike fish in the wild or in ponds, fish in the laboratory are usually kept in a small volume of water and limited in swimming speed. Ich theronts are purposely allowed to reach a high concentration in tanks in order to achieve a good infection of fish. These theronts have a high chance of contacting the host fish, including theronts with weak virulence. Prolonged maintenance of Ich stock in the laboratory results in the selection of Ich populations with low infectivity. Conversely, only Ich theronts with strong infectivity have the chance to infect fish in the wild or in ponds since the fish actively swim in large volumes of water. Successive infection cycles of the parasite select Ich populations with high virulence to invade fish. *Tetrahymena pyriformis*, a ciliate similar to Ich theronts, usually maintains its numbers by asexual reproduction. Under certain conditions, such as lack of food, *T. pyriformis* undergoes a sexual phase involving conjugation to exchange micronuclei material (Elliott 1973). No conjugation has been observed for Ich maintained in our laboratory. However, the possibility that Ich undergoes conjugation in the wild to keep the parasite from loss of virulence cannot be excluded.

In summary, the results of this study demonstrate that Ich was significantly more infective at 1–25 than 26–105 cycles. The loss of infectivity of *Ichthyophthirius multifiliis* started after 25 serial passages and was predominant in the single Ich isolate at 61–90 and 91–105 cycles. Maintenance of the Ich isolate at room temperature required an increased ratio of infected fish to naïve fish when Ich became senescent.

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