

Ichthyophthiriasis: Various Fish Susceptibility or Presence of More than one Strain of the Parasite?

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Abstract: White spot disease is one of the devastating protozoal infections affecting freshwater fish. Commonly known as “Ich”, the Ichthyophthiriasis can infect almost all freshwater fish causing devastating losses in susceptible fish. In the present study, an outbreak of Ichthyophthiriasis erupted in one of the holding tanks of two ornamental fish species, Siamese shark (*Pangasius sutchi*) and goldfish (*Carassius auratus* var. *bicausatus*). Initial observation of the outbreak showed that only *Pangasius sutchi* was affected by typical white spots associated with mortalities. However, *Carassius auratus*, a known susceptible species for *Ichthyophthirius multifiliis* (Ich) in the same aquarium showed only mild erythema that disappeared during the course of infection with no mortalities. To confirm the previous observation, an experimental design was performed in which infection with Ichthyophthirius was induced in *Pangasius sutchi* species alone. Cohabitation was performed between the Ich-induced *Pangasius sutchi* and *Carassius auratus*. Three days after the induction, *Pangasius sutchi* started showing the typical clinical signs. Mortalities associated with severe infection were recorded in *Pangasius sutchi* by 7th day after infection. Associated *Carassius auratus* showed only mild erythema that disappeared by the end of experiment. Histopathological examination of skin from both species in natural and experimental infection was performed to evaluate the severity of infection on the tissue level. Substantial numbers of typical large size trophonts surrounded by layers of fibrous tissue, melanophores and hemorrhages were detected in dermal and epidermal layers. Underlying myodegeneration was also associated the skin lesions in *Pangasius sutchi*. In contrary, pathological changes in the skin of *Carassius auratus* were mild and few numbers of immature trophonts were noticed in the epidermal layers. Possible reasons for such infection discrepancies between the two susceptible species are discussed. [Nature and Science. 2006;4(3):5-13].

Keyword: *Ichthyophthirius multifiliis*, *Pangasius sutchi*, *Carassius auratus*, infection, protozoa, ciliates

Introduction

Commonly known as “Ich”, the white spot disease (Ichthyophthiriasis), can infect almost all freshwater fish (Ventura and Paperna, 1985) and at least one species of amphibian (Gleeson, 1999). The disease is recognized as one of the most pathogenic diseases of fish caused by eukaryote parasites resulting in significant economic losses in the affected cultured fish species (Matthews, 1994). Ich is caused by a hymenostomatid ciliate, *Ichthyophthirius multifiliis* Fouquet, 1876 (*I. Multifiliis*). The parasite is commonly distributed, occurring in tropical, subtropical and temperate regions, and extending north to the Arctic Circle (Matthews, 1994). It causes severe epizootics among different fish species in aquaria, hatcheries, and ponds, as well as in wild fish populations (Ezz El-Dien

et al., 1998; Thilakaratne, *et al.*, 2003; Kim *et al.*, 2002). Naturally occurring outbreaks of Ichthyophthiriasis in wild fish populations can yield devastating effects. For example, natural outbreak of the Ich was blamed for the deaths of 18 millions *Orestias agassi* in Lake Titicaca, Peru (Wurtsbaugh and Tapia, 1988). In intensive aquaculture systems, Ich epizootics are more common (Valtonen and Keränen, 1981) due to the confinement of fish under stressful condition and the exponential increase in parasite numbers (Clark *et al.*, 1995).

The life cycle of *Ichthyophthirius multifiliis* is a direct one and requires no intermediate host (Ewing and Kocan, 1992). Invasion of the infective theront gives rise to the trophont that grows inside the host epithelium to the size of 1 mm in diameter (Lom and Dykova, 1992). The trophont becomes easily visible owing to the

opacity of the cytoplasm in the fish skin (Matthews, 1994) and the formation of somatic cyst by the fish body around the parasite (Price and Bone, 1985). These white spots are easily countable. However, a single white spot does not necessarily represent a single trophont, since aggregations of trophonts can occur in one large white spot as a result of multiple entries at single site (Matthews, 1994). Nevertheless, scoring of individual white spots with appropriate controls provides a direct, quantifiable measure of infection levels on fish (Ventura and Paperna, 1985). The trophonts mature inside the host and develop into tomites, each of which is able to produce up to 3000 tomites which released as theronts. After being released, the free-swimming theronts can infect a new host or re-infect the same host, thus compromising its health status (Lome and Dykova, 1992). Severe damage of the skin epithelium occurs due to the break of the parasites through host skin during infection and their release. This damage might lead to concession of osmoregulatory process and ion regulation and might serve as a portal of entry for secondary invaders, leading eventually to death of fish (Ewing & Kocan, 1987, Ewing *et al.*, 1994 and Tumbol *et al.*, 2001).

Increasing reports are continually published indicating that different fish species and populations have significance difference in their resistance to Ich. These differences in susceptibility were attributed primarily to environmental factors and/or genetic make up of the host (Hines *et al.*, 1974; Clayton and Price, 1992; Clayton and Price, 1994; Price and Clayton, 1999; Gleeson *et al.*, 2000). Another line of reports assumed the presence of more than one strain of *I. Multifiliis* were assumed. This assumption was based primarily on the wide distribution of the parasite, subtle variation in cell morphology and serotypic variations among isolates based on immobilization antigens (Nigrelli *et al.*, 1976, Dickerson *et al.*, 1993 and Leff *et al.*, 1994). Yet, no clinical or field evidences were reported to support this assumption.

Results from the current study report the Ichthyophthiriasis in Siamese shark (*Pangasius sutchi*) for the first time. Initial observations of clinical signs discrepancies of Ichthyophthiriasis in two aquarium fish species under similar conditions were also reported. Investigational approaches might provide a potential clinical clue for the presence of more than one *Ichthyophthirius multifiliis* strain.

Materials and Methods

Natural outbreak

A total of 17 fish; 10 Siamese Shark (*Pangasius sutchi*) and 7 Goldfish (*Carrasius auratus* var. bicausatus) were introduced to one aquarium at the

department of fish disease and management, College of Veterinary Medicine, Cairo University in July of 2001. Two days later, the *Pangasius sutchi* (*P. sutchi*) fish started showing itching behaviors, hemorrhagic patches, fin rot and white spots all over the fish body. Seven of the *Pangasius sutchi* died 10 days after eruption of the clinical signs. The *Carrasius auratus* (*C. auratus*) in the same aquarium showed only mild hemorrhages on the side of fish body by the third day and these signs were completely disappeared by the seventh day. No mortalities or white spots were noticed on goldfish during the course of the outbreak.

Experimental Infection

To confirm the previously mentioned observations, an experiment was designed as in Table 1. A total number of 36 apparently healthy *P. sutchi* (total length 7 ± 2 cm) and 30 fingerlings goldfish (total length 3 ± 1 cm) were used in the experiment. The *P. sutchi* were bought from a commercial dealer while the goldfish were generously donated to the lab by a local ornamental fish breeder with a health history indicating that the fingerlings and their parents had never been previously exposed to ich. The fish were allowed to acclimatize for a period of 4 weeks in glass aquaria and supplied with clean dechlorinated water, aeration and fed on Tetra food fish flakes 2% of the their body weight at water temperature 25°C. After the acclimatization period, the fish (Siamese shark and goldfish) were divided into 4 designated aquaria A, B, C, D and E as shown in (Table 1). The dimension of each aquarium was 60X40X30 cm (Length X Height X Width). At the beginning of experiment, the *P. sutchi* (6 fish) in aquarium (A) were used as the source of infection after induction of Ich. By sudden change in water temperature (5-7°C temperature differences) 3-4 times on 1 hr intervals. Previous work in our lab with ornamental fish indicated that the sudden and repeated water changes of the water temperature induce infection by Ich in the exposed fish within 3-4 days. After induction, the *P. sutchi* fish were split equally between aquarium B and D (3 fish each aquarium). The fish in the 4 aquaria were monitored for a period of two weeks, clinical signs were recorded, and dead fish were removed on a daily basis and kept in 10% formalin. Representative samples (2 fishes) of fish showing clinical signs were also sacrificed and preserved in 10% formalin for histopathological examination.

Histopathological Examination

Tissue specimens from affected fish (Skin, muscles and gills) were collected after clinical and gross examination and immediately fixed in neutral buffered

formalin 10% for 1 week; dehydration was done using ascending grades of ethanol (70, 80, 90, and 100% for 1 hour each). The specimens were then cleared in 2 changes of xylene. After blocking using soft paraffin, serial sections of 4µm thickness were done. The sections were stained using routine hematoxylin and eosin stain (Ezz El-Dien, et al., 1998).

Parasitological Examination

Macroscopic examination of the affected fish was done carefully for detection of visible lesions. Fresh as well as Geimsa stained smears from body surface, fins and gills were microscopically examined according to Pritchard and Kruse (1982). The detected protozoan parasite was photomicrographed and measured using ocular micrometer.

Results

Upon the initial natural outbreaks, typical white spots characteristic of Ich. appeared on *P. sutchi*, while *C. auratus* showed only non specific signs of mild skin irritation. At the beginning of the outbreak “two days after arrival” the *P. sutchi* were restless, swimming erratically and itching against fixed objects. Small white spots appeared on the sides of the fish body, fins and head (Figure 1). In some cases, the small white spots coalesce together forming larger white spots. Hemorrhagic patches appeared on the bases of the fins, fish sides and mouth. Fin rot appeared sometimes on some of the affected fish. Six days later, the fish stopped feeding, appeared lethargic and swam near to the water surface. Seven of the *P. sutchi* died 10 days after arrival. Skin scrapping revealed the presence of different developmental stages of the *I. multifiliis*. The most predominant stage was the mature trophont with the C-shaped macronucleus (Figure 2). During the initial natural outbreaks, the goldfish showed only focal areas of hemorrhages (petichiation), especially at the root of the scales (Figure 3). Sometimes the hemorrhagic spots were enlarged and tinged with mucous. Fish swam erratically during the first 3 days, and then returned to their normal behavioral. Skin scrapping from the goldfish during the clinical signs showed small immature stage of the *I. multifiliis* trophont. It was surprising that no mortalities were

recorded in the goldfish during the initial natural outbreak.

To confirm the previously mentioned observations, the Ich was induced experimentally and allowed to join other non induced fish in aquaria B and D. Control groups (groups C, D and E – Table 1) were not exposed to any handling and were maintained at the same environmental conditions. The fish in the treated and control groups were monitored daily for abnormal signs. Three days later, the Siamese shark started showing erratic swimming behavior, itching, hemorrhagic patches on the sides of the body with few small white spots. The body of the fish was completely covered with white spots by the fifth day. A total of 5 and 7 fish died in tank (B) and (D) respectively by the end of the experiment. Goldfish showed only mild congestion at fin basis and small patches of hemorrhages on their sides and gill covers that disappeared by the fifth day of exposure. No mortalities were recorded in the goldfish during experimental infection exposure. Microscopic examination of the skin scrapping of the affected Siamese shark and goldfish revealed the same picture found in the natural outbreak.

Histological sections of the skin of *P. sutchi* revealed large trophonts of the *I. multifiliis* that were prominently lodged in the epidermal layers. The parasite appeared with large C-shaped macronucleus. Most trophonts observed were adjacent to the basement membrane of the epithelial layer and the surrounding tissue did not show any evidence of damage (Figure 4). In many sections, there were no signs of damage to the epidermal cells surrounding the trophont. In other sections, however, the cells between the parasite and the basement membrane were hydropic, vacuolated and/or necrotic with pyknotic nuclei. In other cases, the macronucleus was not demonstrated in the section either due to the level of the sectioning or the stage of maturation of the trophont. Large aggregations of melanophores were clear around the trophonts and a large number of club cells activation (Figure 5). Epidermal cells around the parasite appeared atrophied. Myodegeneration of the underlying musculature was clearly observed where the muscle fibers were hyalinized with prominent destruction of the nuclei. In goldfish, the detected trophonts in the epidermis were very small in all examined sections. The tissue reaction was less common where the melanophores activation was neglected. Free red blood cells were noticed either between the epidermal cells or in the dermal layers (Figure 6).

Table 1. Design for the experimental infection with *I. Multifiliis*:

Aquarium	Gold fish #	Siamese Shark #	Experimental Group
A	0	6 fish were used for induction then joined later to B& C aquarium	
B	10	10	Treatment group
C	10	10	Control group
D	-	10	Control group
E	10	-	Control group

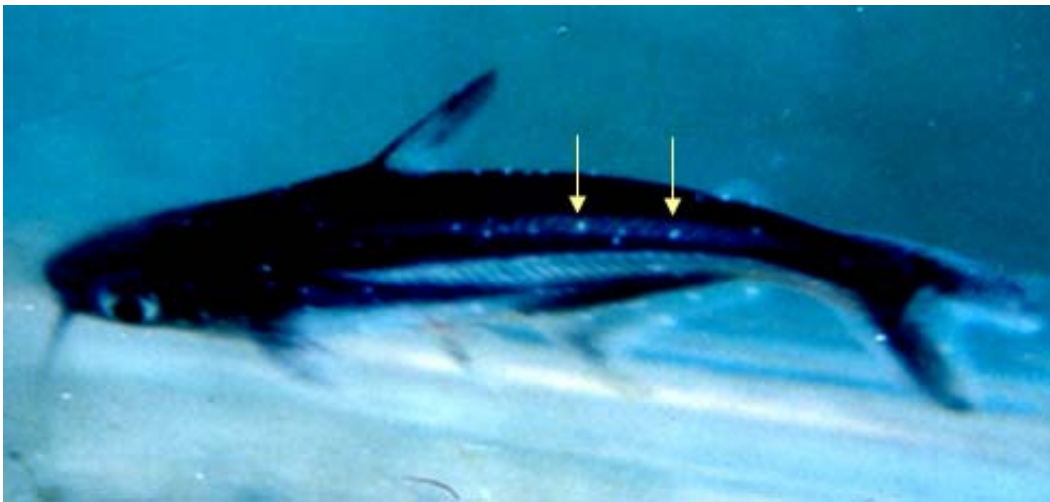


Figure 1. Siamese shark fish infected with Ich. Note the multiple white spots on fish side (arrows)



Figure 2. Wet mount of the Siamese shark skin during infection with Ich. Note the C-shape nucleus of the *I. multifiliis* (bar= 75 μ m).

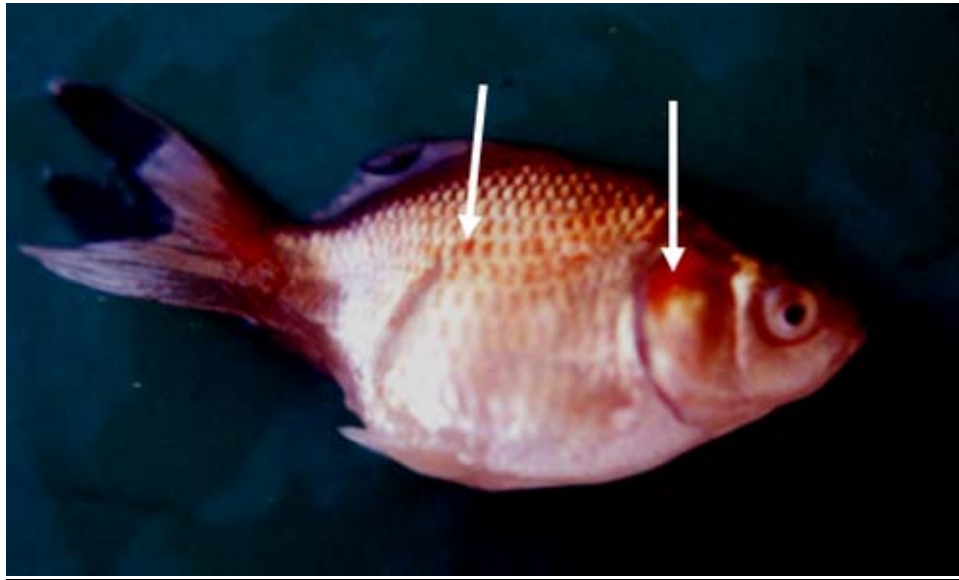


Figure 3. Goldfish showing focal hemorrhages at the base of scales and gill cover (arrows)

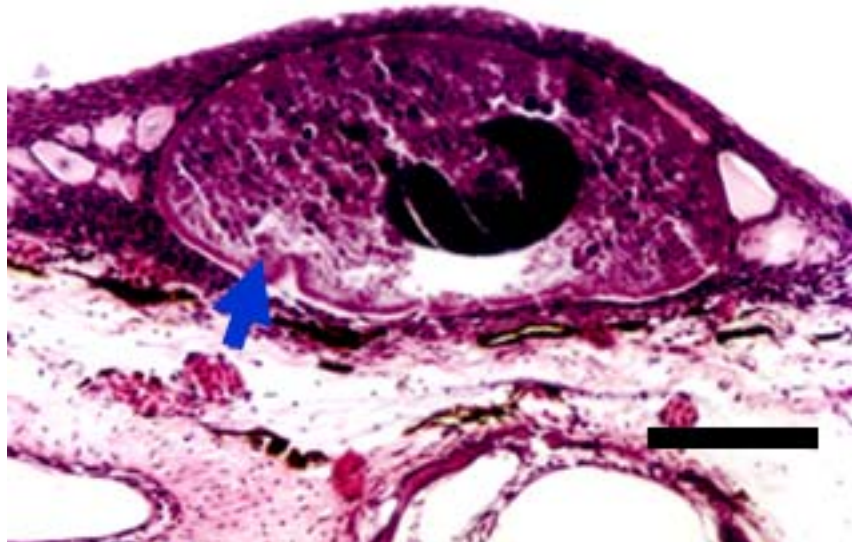


Figure 4. Mature trophont of *Ichthyophthirius multifiliis* in the epidermal layer of Siamese shark (arrow)
(bar = 100 μ m)

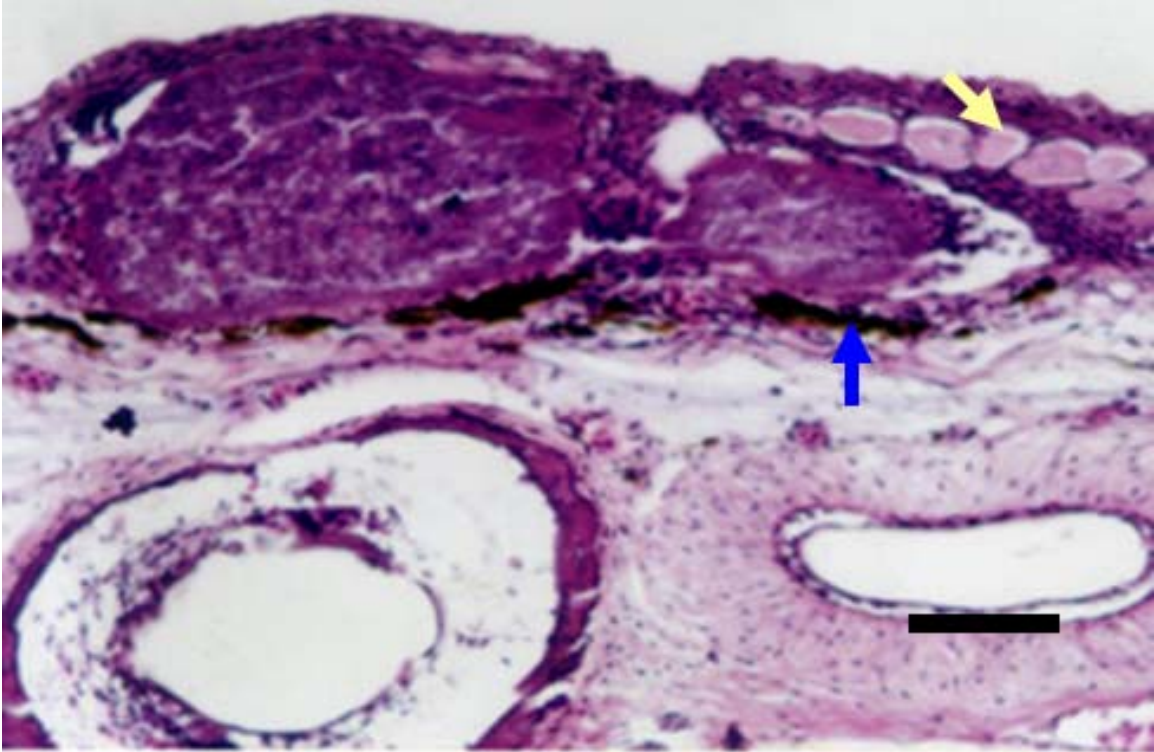


Figure 5. Melanophores accumulation (blue arrow) around the *Ichthyophthirius multifiliis* trophont. Note the activated club cells at the surface of epidermis (yellow arrow) (bar = 100 μ m)

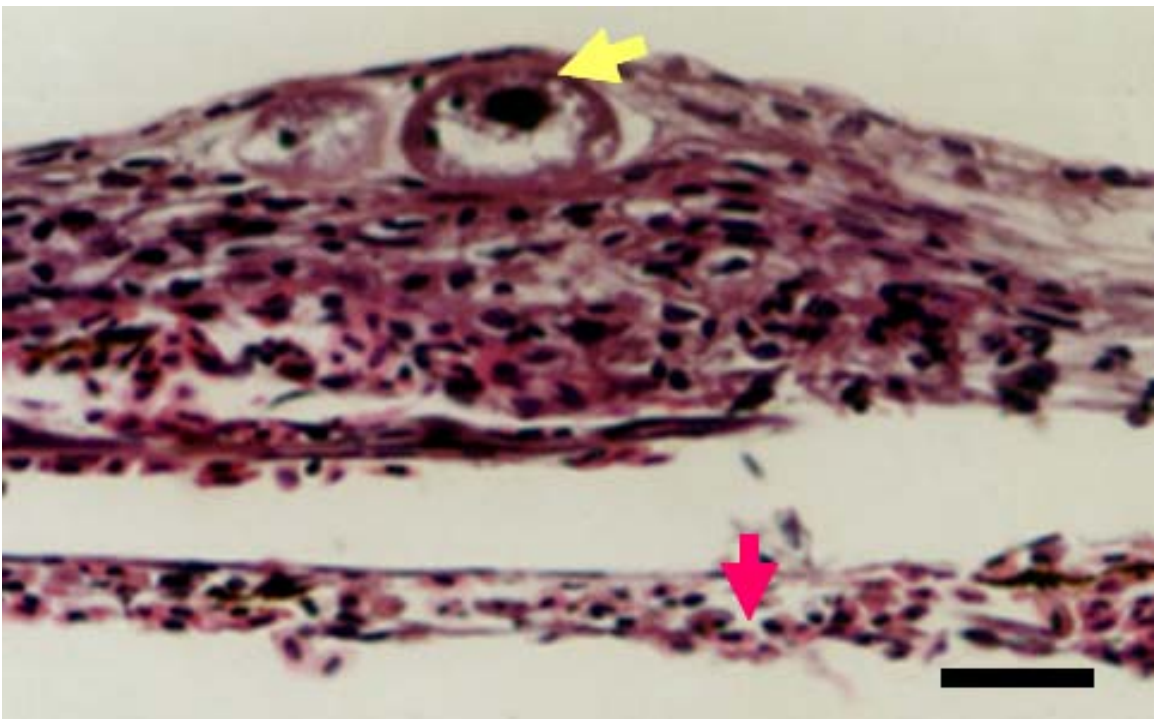


Figure 6. Small immature trophont of *Ichthyophthirius multifiliis* (yellow arrow) in the skin of goldfish. Note the blood cells resulted from the hemorrhages in the dermal layer (red arrow) (bar = 25 μ m)

Discussion

Initial observation of the natural outbreaks indicated that the Siamese sharks showed typical Ichthyophthiriasis signs, while the goldfish showed mild signs of skin irritation. Current stud reports the first record of white spot disease in Siamese shark (*P. suctchi*). However the presence of the atypical mild clinical signs in the goldfish, a known susceptible fish species, was a surprising finding. To confirm these clinical signs, an experiment was designed to mimic the natural outbreak in the Siamese shark. The Ich was induced in *P. suctchi* which were introduced later to aquaria contained both *P. suctchi* and *C. auratus*. The experiment was repeated three times to confirm the clinical signs and ensure consistency of the obtained data. Clinical signs obtained in each time of the experiment were consistent with what was recorded in the natural outbreak.

Discrepancies in the clinical signs between the two exposed fish species were puzzling and could be attributed to fish susceptibility. Earlier reports on ichthyophthiriasis in fish indicated that different fish species show significant differences in their ability to resist disease (Hines et al., 1974). As early as 1947, authors observed that Rainbow trout were more susceptible to infection by *Ichthyophthirius multifiliis* than brown trout (Butcher, 1947). Also, Clayton and Price (1992) demonstrated that susceptibility to Ich varies between different strains of platy, an aquarium fish. However, this assumption contradicted with previous data regarding goldfish. Natural repeated outbreaks of Ich associated with typical clinical signs in goldfish indicated the high susceptibility of this species to *Ichthyophthirius multifiliis* (Ezz Eldin, et al., 1998). A similar report was also published in this regard (Ling et al., 1993). Moreover, goldfish in a controlled experimental infection with Ich usually develop typical clinical signs upon contact with theronts (Ling et al., 1993).

In the current experiment, goldfish developed only atypical mild clinical signs upon contact with the infected Siamese shark fish. This might raise a concern about the variation of exposure of the two species to the infective agent, theront, in the water, infection dose and the method of infection used in the experimental infection. To exclude these assumptions, goldfish and Siamese shark were exposed to the infection through the induced fish at the same time to ensure the exposure of both species to the same infection dose. Moreover the fish during the first 2 days of adding the goldfish were held in a low water column (not exceeding 20 cm) to ensure high concentration of the infective agents.

Previous reports for experimental infection with Ichthyophthiriasis usually used active culture of

Ichthyophthirius multifiliis maintained in the lab for long time (Xu & Klesius 2003 and Sigh & Buchmann, 2000). This active culture usually used the same fish species of origin in the passage or adapted in different species. In either case, the parasite might loss its pathogenicity over long term of passage or become more pathogenic for the new host over long time of passage. In the current study, we used the natural method of inducing infection rather than laboratory culture to investigate the initial observation. Using this method of infection induction mimic the natural infection process and are considered standard to investigate natural infection using controlled experiment. However, a question might rise about the source of theronts in the current study upon sudden temperature change induction. Where is the infective stage come from especially under controlled environmental conditions? Could it be a normal flora of fish skin that flares up upon exposure to stress factors and transform to pathogenic form? Could it be a part of the aquatic system inhabited by any aquatic animal? A trail of questions needed to be thoroughly addressed to clarify the Ich epidemiology completely.

The likelihood of maternal immunity could be raised as another assumption for the mild infection in the goldfish during the natural outbreak. It is well documented that maternal antibodies passed from mothers to their offspring directly via eggs (Mor & Avtalion, 1988 and Sin et al., 1994) or indirectly in mouth-brooding fish via mucus of buccal cavity (Sin et al., 1994). To investigate this possibility, a naïve goldfish from a brood stock that had never been exposed to Ichthyophthiriasis was used in the experimental study. Results excluded the responsibility of maternal immunity for the atypical mild signs in the goldfish during the natural and experimental infection. Meanwhile, the Siamese shark expressed the typical clinical signs for ichthyophthiriasis, associated with mortalities reached to 70% and 50 % in natural infection and experimental infection respectively. This could be an indication of the susceptibility of the fish and/or the host specificity of the parasite. In evaluating discrepancies associated with susceptibility to a disease, there is often a dilemma of having to use a norm such as mortality. There are probable rationales as to why one group of fish differs from another in resistance to a disease (Chevassus & Dorson, 1990). This is rather inadequate since the measurement of resistance (mortality) is rather distant from the authentic infection process. In ichthyophthiriasis, this is not so much a predicament since both exposure to infection and resulting infection levels on the fish can easily be quantified. The presence of white spots along with associated hemorrhages and behavioral changes is

considered the ultimate indication for the presence and susceptibility of any fish species to the infection with ichthyophthiriasis.

Histopathological examination of Siamese shark skin indicated the typical pathological changes induced by *I. multifiliis* infection mentioned in previous reports (Ezz El-dien, et al., 1998). On the other hand, goldfish skin showed only mild inflammatory reaction and trophonts in the epidermis were very small and never reached maturity. This was an indication of the success of the infection process in goldfish, yet it didn't reach the final stages for some reasons. Also, there was no formation of somatic cysts by the fish body around the parasite that typically encountered in typical Ichthyophthiriasis (Price & Bone, 1985). Combine this pathological picture with the previous reports of goldfish susceptibility might indicate that the reason could be related to non specificity of the parasite strain rather than host resistance. Because of the non specificity of this parasite, the host tissue reacted in a way which forced the parasite to leave the body without completing its life cycle. This is consistent with previous studies which suggest that rather than killing the infective stage of the parasite, the host body forces the parasite to exit prematurely in response to immune response (Wahli & Matthews 1999).

Current study assumed the presence of more than one strain of *I. multifiliis* which differ in their pathogenicity to different fish species. Previous record suggested the presence of more than one species of *I. multifiliis* that possess similar morphological criteria (Yunchis 1997). These findings could be describing the atypical mild signs found in the goldfish upon concurrent infection with Siamese shark. However, molecular investigation is needed to prove such assumption. Future research will be focusing on differentiating the *I. multifiliis* isolates from both goldfish and Siamese shark using molecular tools.

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