Incidence of White Spot Disease in Freshwater Ornamental Fishes imported to Pakistan

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ABSTRACT

The aim of this study was to investigate incidence of white spot disease in four types of tropical freshwater ornamental fishes imported to Pakistan. One hundred and twenty ornamental fishes (goldfish, comet, shubunkin, oranda, \(n=30\) each) were studied for *Ichthyophthirius multifiliis* infection. Goldfish showed highest prevalence (26.6%) and mean intensity 5.25. The prevalence was 20% in shubunkin but it had highest mean intensity (9.16). Whereas, comet and oranda showed low prevalence (13.13% and 10%) but mean intensity was 4.0 and 6.66 respectively. The maximum white spots in a fish were seen in goldfish (16) and minimum (7) in comet. Only gills were infected in goldfish and shubunkin. The fins were the only infected sites in oranda. But, in comet 50% infection was on gills and other 50% infection was on fins of the host. Gills seemed to be more infected as compared to fins. Both immature and mature trophonts were observed on fins, which indicate either infection is a continuous process or different growth rate of parasite in the host. There was little inflammatory response by the fin tissue around the parasite. However, affected gill filament appeared degenerated. The variable infection rate and mean intensity in these four fish types indicates that, there is probably more than one strain of *I. multifiliis* which differs in its pathogenicity and virulence to different varieties of goldfish.

Key Words: *Ichthyophthirius multifiliis*, white spot disease, ornamental fishes, goldfish

INTRODUCTION

White Spot disease (*Ichthyophthiriasis*) is commonly called as “itch”, and is caused by a ciliate parasite *Ichthyophthirius multifiliis* Fouquet, 1876. *Ichthyophthirius multifiliis* is a three stage parasite with direct life cycle (Ewing and Kocan, 1992). When the adult parasite leaves the infected fish, it is called tomites. Tomont is attached to a suitable substrate in water and form a thin walled cyst. Within the cyst tomites divide many times and form small 2000-3000 tomites (Durborrow et al., 1998; Lom & Dykova, 1992). The tomites when expelled from the cyst is elongated in shape (20-60mm) and become theront. The theront swim to fish host and penetrate into the epithelium using a penetrating gland and the strong swimming action of their cilia (Durborrow et al., 1998). After penetration into the fish skin they are called trophont, which grow inside fish epithelium and become 1mm in diameter (Lom & Dykova, 1992). The fish body form a somatic cyst around the trophont (Price & Bone, 1985) and cysts appear as white spot, which are easily visible and countable. All life stages are extremely temperature dependents which ranges from 18-25⁰C (Osman et al., 2009).

*Ichthyophthirius multifiliis* is one of the most important protozoan pathogen of almost all freshwater fishes worldwide (El-Dien & Abdel-Gaber, 2009) and is distributed in tropical, subtropical, temperate regions and even extends up to Arctic Circle (Matthews, 1994, Ventura & Paperna, 1985). White Spot disease is considered as the most pathogenic diseases of fish, which cause significant economic losses of affected cultured fish. The parasite is capable of killing large number of fish in short period of time (Durborrow, et al, 1998). This parasite can cause serious epizootic in different species of fish in aquarium, ponds and hatcheries and even in wild fish populations (Ezz El-Dien et al, 1998; Kim et al, 2002, Thilkaratne et al, 2003). White spot disease is more common in intensive fish farming system due to the confinement of fish under stressful conditions. The aim of this study was to investigate incidence of white spot disease in some tropical freshwater ornamental fishes imported to Pakistan.

MATERIALS AND METHODS

One hundred and twenty freshwater ornamental; goldfish \(n=30\) and three of its varieties (shubunkin, oranda and comet, 30 each) were examined for *Ichthyophthirius multifiliis* infection from Feb. to Oct. 2012. The fishes were obtained from local pet shops in Lahore and brought to laboratory in sterilized polyethylene bags containing aerated water and maintained in glass aquarium separately. Total length and body weight of each specimen was recorded. The fish skin and fins were
examined for the ulcer and lesions and presence of white spots and symptoms of the disease. Gills were removed and placed into petri dish containing distilled water and examined for the presence of *I. multifiliis*. Parasites quantification was performed directly on wet mount of fins and gills under microscope. The identification of parasites was made following Kabata (1985), Durborow et al., (1998); Elsayed et al., (2006) and Osman et al., (2009). Mean intensity and prevalence of parasites were determined according to Margolis, et al., (1981) and compared in all four types of fishes. The photographs were taken with Olympus camera fitted on microscope.

**RESULTS**

The morphometric data total length, standard length, body width and body weight of the experimental fishes is given in Table 1.

**Table 1: Morphometric observations of four types of experimental fish**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Fish</th>
<th>No. of Fish exam</th>
<th>Total length range (cm)</th>
<th>Standard length range (cm)</th>
<th>Body width range (cm)</th>
<th>Body weight range (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Goldfish</td>
<td>30</td>
<td>5.6-9.5</td>
<td>3.5-5.6</td>
<td>1.9-3.9</td>
<td>4.3-7.9</td>
</tr>
<tr>
<td>2</td>
<td>Comet</td>
<td>30</td>
<td>6.5-11.4</td>
<td>4.1-6.9</td>
<td>1.7-3.0</td>
<td>5.3-11.3</td>
</tr>
<tr>
<td>3</td>
<td>Shubunkin*</td>
<td>30</td>
<td>3.3-11.6</td>
<td>2.4-3.7</td>
<td>1.1-3.7</td>
<td>1.2-11.8</td>
</tr>
<tr>
<td>4</td>
<td>Oranda</td>
<td>30</td>
<td>5.8-8.6</td>
<td>3.9-4.2</td>
<td>1.9-3.6</td>
<td>4.3-11.3</td>
</tr>
</tbody>
</table>

(*From Iqbal & Hussain 2013)*

**Infection of fish with *Ichthyophthirius multifiliis***

The gills and fins were two sites of infection in these fishes. In goldfish, shubunkin and oranda, 100% infection was on one sites either gills or fins. The prevalence in these fishes was 26.6%, 20.0% and 10.0%; mean intensity was 5.25, 9.16 and 6.6, respectively (Table 2).

**Table 2: Occurrence of *Ichthyophthirius multifiliis* in four types of ornamental fishes**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Fish</th>
<th>No. of Fish exam</th>
<th>Fish infect</th>
<th>Prevalence (%)</th>
<th>Maxl. Parasites</th>
<th>Total Parasites</th>
<th>Mean Intensity</th>
<th>Parasites No and Site of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Goldfish</td>
<td>30</td>
<td>8</td>
<td>26.66</td>
<td>16</td>
<td>42</td>
<td>5.25</td>
<td>42 -</td>
</tr>
<tr>
<td>2</td>
<td>Shubunkin*</td>
<td>30</td>
<td>6</td>
<td>20.0</td>
<td>13</td>
<td>55</td>
<td>9.16</td>
<td>55 -</td>
</tr>
<tr>
<td>3</td>
<td>Comet</td>
<td>30</td>
<td>4</td>
<td>13.33</td>
<td>07</td>
<td>32</td>
<td>4.0</td>
<td>09 23</td>
</tr>
<tr>
<td>4</td>
<td>Oranda</td>
<td>30</td>
<td>3</td>
<td>10.0</td>
<td>09</td>
<td>20</td>
<td>6.66</td>
<td>- 20</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>120</td>
<td>21</td>
<td>17.50</td>
<td>16</td>
<td>149</td>
<td>7.09</td>
<td>106 43</td>
</tr>
</tbody>
</table>

(*From Iqbal & Hussain 2013)*

In comet both gills and caudal fins were infected in the same host at the same time in all infected fishes. The prevalence was 13.33%. However, mean intensity of infection on gills was 2.25 and that of fins was 5.75. The detail of gills infection by *I. multifiliis* and *Dactylogyrus* sp. in shubunkin is discussed elsewhere (Iqbal & Hussain, 2013). Infected fish showed typical but mild clinical signs and symptoms. Mucus secretion from gills and skin was clear which was sloughing off. Shubunkin showed aggressive flashing behavior in the aquarium. The other three fishes showed less aggressive behavior than shubunkin. The white spots on the fins were very clear. Both mature and immature stages of parasite, (the trophont) were located under mucus and layers of cells (epithelium) on gills and between fin rays. The wet mount preparation of the parasites from the gills and fins showed, oval to round shape parasite (trophont) with characteristic C-shaped or horseshoe shaped macronucleus (Fig.2). In the immature stages of parasites, the macronucleus was not prominent (Fig. 1). Not much pathological changes due to parasitic infection on fins were observed. The tissue surrounding the trophont did not show any damage. There seems to be little inflammatory response by the fin tissue to the parasite. *Ichthyophthirius multifiliis* were more dominant on gills than on fins. The infected gill filament looked degenerated and desquamated. The gill surface became thick and
showed edema at the point of attachment of the parasite (Fig. 3).

**DISCUSSION**

The prevalence and mean intensity in these fishes varied from 10.0% to 26.6% and 4.0 to 9.16 respectively. Thilakaratne et al., (2003) reported low prevalence of *I. multifiliis* from five ornamental fishes. Pizza et al., (2006) found variable infection of *I. multifiliis* from platy, *Xiphophorus maculates*, sword tail *Xiphophorus helleri* and molly *Poecilia sphenops*. Tavares-Dias et al., (2010) also reported variable mean intensity of *I. multifiliis* from six ornamental fishes, *Carnegiella strigata*, *Carnegiella martae*, *Hyphessobrycon copelandi*, *Nannostomus eques*, *Nannostomus unifasciatus*, and *Pterophyllum scalare* in Brazil.

Goldfish shows high susceptibility to *I. multifiliis* as exhibited by natural repeated outbreak of Itch (Ezz Eldin et al., 1998). The moderate prevalence and mean intensity and maximum number of cysts in one goldfish in present study support this point that goldfish is more susceptible to Itch, than other three fishes, shubunkin, oranda and comet. Shubunkin with highest mean intensity and maximum parasites (13) in a fish seems having fair susceptibility for Itch. Comet and oranda with low prevalence and mean intensity can still can be placed as less susceptible to Itch compared to goldfish. The variations in infection level and mean intensity among four varieties of goldfish probably point the variable susceptibility of these fishes to *I. multifiliis* attack. This explanation may be compared with care to Hines et al., (1974) who indicated that different fish species show significant difference in their ability to resist diseases.

There are reports indicating that different fish species have significant difference in their resistance to Itch. These differences in susceptibility have been associated primarily to environmental factors or genetic make up of the host (Hines et al., 1974; Clayton & Price, 1992, 1994; Price & Clayton, 1999, Gleeson et al., 2000). Butcher (1947) reported that rainbow trout *Oncorhynchus mykiss* were more susceptible to infection by *I. multifiliis* than brown trout *Salmo trutta*. Clayton & Price, (1992) stated that susceptibility to Itch varies between different strains of platy. Our results show slightly higher infection in goldfish and shubunkin compared to other two varieties. Variation in infection of these four types of fishes with *I. multifiliis* may be associated with one assumption that there is probably more than one strain of *I. multifiliis*. This view is strengthened by the fact that the source of ornamental fish was different in each sample. This point has already been highlighted by Leff et al., (1994). The mild infection in comet and oranda may be explained by already reported fact that maternal
antibodies pass from mothers to their offspring directly via egg (Mor & Avtalion, 1988, Sin et al., 1994). The presence of atypical signs such as poor inflammatory response on fins in our fishes such as comet and oranda is probably an indication of the success of the infection process, but at the same time points to non specificity of the parasite strain for the host rather than host resistance. Molnar et al., (2012) stated that the protozoan parasites of fish, such as; Eimeria anguilae, E. daviesae, E. praecae, E. Variabilis, E. rutili and E. nemethi are related but distinct from those that infect terrestrial vertebrates, such as Eimeria bovis, E. zuernii, E. cylindrica, E. subsperica reported from cattle (Qamar et al., 2011).

Probably goldfish and shubunkin show similar interaction with I. multifiliis as both fishes had infection on gills. Comet showed different pattern by having infection on gills and fins at the same time and oranda showed entire different pattern of infection by having infection just on fins. Eissa (2004) reported that sublethal infection provides the fish with a protection against re-infection. Mild infection observed in present study may also be explained by one point that infection with monogenean gill parasites such as Dactylogyrus sp. seems to protect the fish partly against Itch. This is just one explanation of low I. multifiliis infection, as observed in case of shubunkin, where Dactylogyrus was attached to gill along with I. multifiliis. But in contrast to this Buchmann et al., (1999) found that the activation of the response against other parasites did not induce any protection against Itch. The four types of fishes investigated in this study basically belonged to one species Carassius auratus. Hence, the understanding of their susceptibility to Itch, host response, success of establishment of parasite in host and finally its maturity and completion of life cycle needs a very detailed in vitro investigation.

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punctatus (Rafinesque), against two immobilization serotypes of *Ichthyophthirius multifiliis* (Fouquet). *J. Fish Dis.*, **17**: 49-432.


