

OCCURRENCE OF BLACK SPOT DISEASE IN *LABEO ROHITA* (HAMILTON) FRY IN CARP FISH HATCHERY LAHORE, PAKISTAN

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ABSTRACT

Two hundred fry of *Labeo rohita* were obtained from Central Fish Seed Hatchery Lahore. The mean total length of fry was 20.47 mm. The fry were examined to be infected with metacercaria of a digenetic fluke *Posthodiplostomum cuticola*. The infection resulted in the formation of black spots on the skin of fry. This infection is commonly called as a Black spot disease or Posthodiplostomiasis in fish. The prevalence of *P. cuticola* was 100% and mean intensity of infection was 4.495. The size of metacercaria varied from 0.70 - 0.98 mm. A moderate significant relationship existed between the total length of the fry and number of black spots ($r^2 = 0.15$). The black spots were randomly distributed on the body of fry. The ventral side of the fry (Section-I); was having a significantly higher number of the black spots than the dorsal side (Section II) ($\chi^2 = 3.75$; $P = 0.05$). No skeletal deformation was observed in the infected fry. The black spot disease in *L. rohita* fry, its relationship to pond management and control is discussed.

Keywords: *Posthodiplostomum cuticola*, black spot disease, *Labeo rohita*, fry, control.

INTRODUCTION

Posthodiplostomum cuticola is a digenetic fluke. Adult *P. cuticola* lives in the intestine of piscivorous birds of family Ardeidae, such as common heron, *Ardea cinerea* L. purple heron *Ardea purpurea* L. squacco heron *Ardeola ralloides* (Mierzejewska *et al.*, 2004). The eggs of *P. Cuticola* are released into the water with bird's feces and hatch into free living larvae, miracidia. The miracidia enters into aquatic snail *Planorbis planorbis* and *Planorbis carinatus* the first intermediate host of this fluke. In the snail miracidia produce numerous sporocysts, which develop into furcocercaria. Furcocercaria leaves the snail and burrows into the skin and fins of the fish, the second intermediate host. The infection in fish by metacercaria results in the formation of black spots on the skin and fins of fry. Hence, this infection is commonly called as a Black spot disease or Posthodiplostomiasis in fish. It is common disease in earthen bottom ponds and lakes. Once the infected fish is eaten by bird, the metacercariae develops into an adult fluke in the intestine of the bird (Anon, 2010).

The black spot or the cyst accumulates black pigment cells (Mierzejewska *et al.*, 2004). Juvenile and young fish are more susceptible to infection. The black spot disease is not deadly to fish in general. However, the disease easily spread throughout the pond quickly. The *P. cuticola* infection is common in many species of fish,

primarily cyprinids (Bauer *et al.*, 1973; Mierzejewska *et al.*, 2004; Rolbiecki, 2004; Zrnici *et al.*, 2009). Infected fish show symptoms like; weight reduction, pathological changes in the blood, backbone and musculature deformation, kidney and liver dystrophy, appetite of fish is poor and it often die (Williams and Jones, 1994; Rolbiecki, 2004). The aim of this study was to investigate *P. cuticola* infection in *L. rohita* fry and to evaluate parasitological indices such as prevalence and mean intensity of infection and suggest control measures for this infection.

MATERIALS AND METHODS

The infected *L. rohita* fry were collected from a fry rearing cemented tank (earthen bottom) located at Central Fish Seed Hatchery, Lahore, during October 2001. The fry were brought live in polyethylene bag in pond water to the Fish Pathology Laboratory, Fisheries Research and Training Institute, Lahore (FR&TI) and kept in 40 liters glass aquaria in aerated water. Two water samples were taken and analyzed at Water quality Laboratory at FR&TI, according to APHA (1992) protocol. The snails were also collected from the pond bottom and identified according to Huet (1974). The water plants present in the pond were also collected for identification. Total length of fry ranged from 15 to 27mm, mean 20.47mm.

In order to better visualize the parasite distribution and to

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improve statistical treatment the body of fry was divided into two sections. Section-I covered the area from the middle of the fry upwards. Section-II covered area from middle of the fry downwards (Fig. 1). The number of black spots was counted from Section-I and Section-II of the fry on both left and right side.

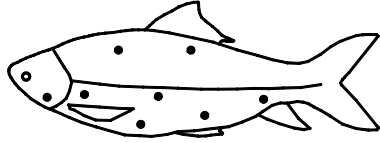


Fig. 1. Location of black spots on *Labeo rohita*.

The cysts were removed from the fish with the help of scalpel and opened with needles to expose the metacercaria. The metacercaria were fixed in 4% hot formalin, and preserved in 70% ethanol and stained with iron acetocarmine dehydrated in an alcohol series, cleared in eugenol and mounted in Canada balsam (Bauer *et al.*, 1973; Moravec *et al.*, 1991). The mounted worm were measured with the help of eye piece graticule fitted in the microscope and were photographed. Two cattle egret (*Bubulcus ibis* L.), from the fish hatchery complex were obtained and dissected. One mature *P. cuticola* worm was recorded from the intestine of one bird. The prevalence and mean intensity of infection was calculated according to Margolis *et al.* (1982). The differences between numbers of black spots in Section-I and Section-II of the body of fry were tested for statistical significance with the Chi square test. The relationship between the number of black spot/cyst and total length of fry was calculated by regression analysis.

RESULTS

1. Management of Infected pond

The infected fry sampling tank measured 62 x 31 x 1.40m. The pond has earthen bottom and cemented vertical walls. Organic manure (cow dung) and inorganic

fertilizer (Urea) are added to the pond to maintain productivity in the pond before and after stocking of fry. The pond was supplied with ground water through electric tube-well. The water quality parameters of the pond water (water temperature, dissolved oxygen, pH, total hardness and total alkalinity) are given in table 1. The pond had thick vegetation of aquatic plants; *Hydrilla* sp. and *Vallisneria* sp. spread throughout the pond. The visibility through water was 30.5-35.5cm in various parts of the pond. The snails, *Planorbis* sp. and *Lymnaea* sp. were also obtained from the pond. However, the snail population could not be estimated due to sampling difficulties at the pond bottom.

Infection of *Labeo rohita* with *Posthodiplostomum cuticola*

In total 200 fry were examined and all were infected. The prevalence was 100% mean total length of fry was 20.47mm. The total length of the fry ranged from 15 to 27mm. The fry with total length 17, 18, 23, 24 and 25mm were not present in the sample (Table 2). A total of 899 black spots were recorded on 200 fry. The mean number of black spots per fry was 4.49 ± 1.53 . The mean number of black spots increased with the rise in the total length of the fry (Table 2). The fry were divided into three groups with different length range, to observe the infection pattern of *P. cuticola* (Table 3). Second length group (19-22mm) was the most prominent as it comprised 60% of all the fry examined (Table 2). There was a statistically significant difference in the mean intensity with the existing length groups of the fry. A positive correlation between number of black spots and total length of the fry existed. The regression equation can be expressed as under;

$$\text{No. of black spots} = 1.18143 + 0.161874 \text{ Length } (r^2 = 0.15)$$

The length of metacercaria ranged from 0.70 – 0.98mm (Fig. 2) and diameter of the black spot/cyst was 0.79-1.01mm. The mature worm obtained from egret was 3.50mm in length (Fig. 3). No abnormality in the body shape of any fry was observed. However, the fry with

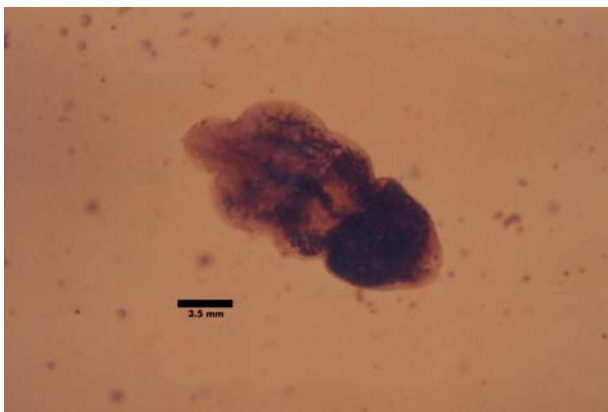


Fig. 2. A metacercaria of *Posthodiplostomum cuticola* from *Labeo rohita* fry.



Fig. 3. A mature *Posthodiplostomum cuticola* from *Bubulcus ibis*.

Table 1. Water quality parameters of infected fry rearing pond.

Transparency of water (cm)	Temp. °C	Dissolved oxygen mg/l	pH	Alkalinity mg/l	Total hardness mg/l
30.5-35.5	21.5	6.95	8.60	287	1560

Table 2. Occurrence of black spots in *Labeo rohita* fry.

S. No.	Total Length (mm)	No. of Fry	Total no. of black spots	Mean No. Black spots/fry	Black spots on Section-I	Black spots on Section-II
1	15	30	97	3.23±0.858	30	67
2	16	10	37	3.70±0.483	13	24
3	19	40	172	4.30±1.264	49	123
4	20	40	190	4.75±1.970	58	132
5	21	12	58	4.83±1.585	15	43
6	22	28	138	4.93±1.864	47	91
7	26	24	122	5.08±0.829	37	85
8	27	16	85	5.31±0.793	27	58
Total		200	899	4.495±1.533	276(30.70%)	623(69.30%)

Table 3. Mean intensity of *P. cuticola* in three length groups of *L. rohita* fry.

Length Group	No. of fry	Mean total length (mm)	No. and range of black spots	Mean Intensity	Black spots on Section-I	Black spots on Section-II
1(15-16mm)	40	15.25±0.438	134(1-4)	3.350	43	91
2(19-22mm)	120	20.23±1.150	558(2-8)	4.650	69	389
3(26-27mm)	40	26.40±0.496	207(4-6)	5.175	64	143
Total	200	20.47±3.668	899(1-8)	4.495	276	623

higher number of black spots on the body were more conspicuous and showed slow swimming movement and looked lethargic. The black spots were present randomly on both left and right side of the body of the fry. Section-I and Section-II of the body of the fry had 30.70% and 69.30% black spots respectively and it was significantly different ($\chi^2=3.75$; $P = 0.05$) (Table 3).

DISCUSSION

Black spot disease was investigated in *L. rohita* fry. The causative agent of this disease is *P. cuticola*, which is commonly found in many freshwater fish families like, Cyprinidae, Percidae, Ecocidae, Acipenseridae, Siluridae (Bykhovskaya-Pavlovskaya *et al.*, 1964). *Posthodiplostomum cuticola* is widely distributed in Europe and over 50 fish species are thought to be potential hosts (Hoole *et al.*, 2001). The infection level was 100% and mean intensity 4.495. Whereas, Shukerova (2005) reported low prevalence and mean intensity (8.33%, 0.39) of *P. cuticola* in *Carassius gibelio*. Shukerova *et al.* (2010) observed low prevalence (0.6%) of *P. cuticola* in perch, *Perca fluviatilis*. In another study Shukerova and Kirin (2008) reported high prevalence and mean intensity (92.22%, 8.58 ±7.80) of *P. cuticola* from

rudd, *Scardinius erythrophthalmus*. Our results are comparable to Zrncic *et al.* (2009) who reported 47.83-100% infection of *P. cuticola* in some freshwater fishes.

The present study is first documented report of *P. cuticola* infection in *L. rohita* from Pakistan. Shukerova (2005) reported *P. cuticola* in prusian carp, *C. gibelio* for the first time in Bulgaria. *Posthodiplostomum cuticola* infection in eight freshwater fishes was reported by Zrncic *et al.* (2009). Infection of *Abramis brama*, *Rutilus rutilus* and *Cyprinus capio* fry with *P. cuticola* in rearing ponds has also been reported. However, the susceptibility of *P. cuticola* infection decreases with age of the fish (Bauer *et al.*, 1973). Infection of *P. cuticola* in commercial fishes like *Catla catla* (Ganapati and Rao, 1962) silver carp, *Hypophthalmichthys molitrix*, grass carp *Ctenopharyngodon idella* (Bauer *et al.*, 1973) and now in *L. rohita* can cause losses in fish farms. *Labeo rohita* is a popular food fish in Pakistan. This fish has high fecundity and Gondosometric index (Iqbal and Batool, 2013) and grow fast in polyculture system in ponds. Being a highly commercial fish, its health status is of great concern to the fish farmers and hatchery managers. Khan *et al.* (2011) and Iqbal *et al.* (2013) has reported that some commercially important fishes like *L. rohita* are

declining in natural waters of the Province of Punjab, Pakistan; due to high population of invasive exotic species and other factors. The infection such as lernaesis, abdominal dropsy and aspergillomycosis has been reported in *L. rohita* from fish ponds (Iqbal *et al.*, 2001; Minhas *et al.*, 2001; Iqbal *et al.*, 2012).

No skeletal abnormality was observed in the infected fry, which indicates that, the infection was recent. However, the black spots can be quite pathogenic if they are located in sensitive areas like gills. Gill infection leads to respiratory problems (Hoole *et al.*, 2001) and the fish with eye infection may be blinded. Heavily infected fry experience retarded growth, physiological stress and even death. The disturbed coordination of infected fry reduces its mobility and makes it easy prey for the birds the final host of *P. cuticola* (Bauer *et al.*, 1973). This mechanism, hence facilitate transmission of parasite from fish to bird. This view has also been discussed by Ondrackova *et al.* (2006) and Zrncic *et al.* (2009).

The pond water was alkaline and within the normal range required for carp culture (Boyd and Tucker, 1998; Ali *et al.*, 2001). High prevalence of *P. cuticola* in *L. rohita* fry may be associated to the snails and egrets populations in and around the pond. The rich organic sediment of pond, abundance of decomposers and aquatic plants probably made the pond environment suitable for snail to multiply and facilitated the completion of digeneans life cycle (Garg *et al.*, 2009). Habitat degradation increases the incidence of black spot disease (Steedman, 1991) and high prevalence and abundance of *P. cuticola* infection in fish in water bodies is associated with thick vegetation (Ondrackova *et al.*, 1999). Hence, the environmental conditions observed in infected pond were important factors which supported high parasitic infection in fry. The control of snails and fish eating birds is essential to minimize black spot disease. The eradication of snails is difficult because they can escape themselves from chemicals by burrowing into substrate (Brown, 1991). However, birds can be driven off. These steps can prevent further infection, but it takes years for black spot to eliminate from the fish (Lane and Morris, 2000). It may be concluded that the infection of *P. cuticola* in *L. rohita* fry may be linked to the organically rich pond bottom, decomposers and aquatic plants, snail and egrets populations in and around the pond.

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