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SEASONAL INCIDENCE AND HISTOPATHOLOGIC STUDY OF PROTOZOAN INFESTATION IN *Clarias* sp., COLLECTED FROM A LOCAL FISH MARKET OF KOLKATA, WEST BENGAL (INDIA)

Suman Mukherjee*[1] and Lipika Medda[2]

[1]*Parasitology and Immunobiology Laboratory, Post Graduate Department of Zoology, Bidhannagar College, Saltlake City, Kolkata - 700 064, West Bengal (India)*
[2]*Department of Zoology, East Calcutta Girls' College, 237 Lake Town link Road, Kolkata – 700 089, West Bengal (India)*
*e-mail: *biosmukherjee@gmail.com*

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ABSTRACT

Protozoans exhibit highly diverse ecological inter-relationship and most complicated host-relationship. The catfish *Clarias* sp. is important component in human diet in many countries especially those in tropical and subtropical areas where malnutrition is a major problem. *Clarias* sp. (size 20-22 cm, weight 80-90 g) were collected from a local fish market of Kolkata (India) during pre-monsoon, monsoon and post-monsoon seasons of 2017-2019. The blood, gill and skin of catfish were examined in laboratory for protozoan parasites and routine histopathological study of selected tissues was performed. Photo-documentation was done using microscope-fitted with digital camera. The study revealed that the intensity of infection was more in ciliate parasites, and the fish had marked lesions in gills and skin. The highest parasitic infection was observed in post-monsoon season i.e. from November to February, followed by pre-monsoon period; while the lowest infection was noted in monsoon season i.e. from July to October. The study revealed that the environmental factors are responsible for spreading infection.

Keywords: Catfish, gill, protozoa, skin

INTRODUCTION

Parasitic diseases of fishes are common all over the world and these alone or in conjugation with other environmental stress influence the weight or reproduction of host, alter fish population characteristics, or affect their economic value (Rhode, 1993; Ikechukwu *et al.,* 2017). Parasitic infestations adversely influence the fish health by inhibiting the normal growth and inciting the outbreaks with high mortalities (Saha *et al*., 1995). Air breathing *Clarias* sp. (Order - Siluriformis; family - Clariidae) live without water for long time and increasingly use freshwater aquaculture in India due to diverse favourable cultural characteristics such as derelict and swampy waters. *Clarias* sp. is a popular fish having delicious taste and good commercial value in tropical countries (Bush *et al*., 1997). Intensive *Clarias* sp. culture has great potential in livelihood development, employment generation and ensuring nutritional enrichment in regular diet of the people of several Indian states, especially Bengal and Tripura (Debnath, 2011; Omeji *et al.,* 2013). With the worldwide fish production and intensive cultivation system, fish are subjected to a wide spectrum of diseases which lead to great losses in fish production (Adawy and Deeb, 2007). Determination of the incidence of parasitic infections in fish plays an important role in epidemiology of fish parasitic diseases and, in particular, their routes of transmission to other hosts (Masoumian *et al.,* 2005). The parasitic

infections of catfish cause economic losses due to fish mortality as well as by escalating the treatment costs and lessening the growth that reduces the expansion of aquaculture (Hussain, 2010). The present study was aimed to assess the distribution of parasites in blood, gill and skin of edible catfish which hinder the production of *Clarias* sp.

MATERIALS AND METHODS

Study location and sampling

The marketable daily consumable 600 individuals of *Clarias* sp. (size 20-22 cm, weight 80-90 g) were collected from a local fish market of Kolkata, West Bengal (India) and transported to the laboratory in large containers. For seasonal parasitological analysis, the fish were collected in three seasons, *viz*., pre-monsoon (March to June) and monsoon (July to October) and post-monsoon (November to February). The collected fishes were taken immediately to laboratory for examination.

Parasitological studies

Fish samples were taken in live condition at various time intervals, killed by hand and examined immediately for parasitological study. A clean spatula was held to the body of each individual and it was drawn backwards towards the tail in a smooth movement with lifting off a small amount of mucous from different sites of the body for studying the parasites from skin. Later on, for each site the mucous scrapings were placed on clean glass slides and examined under microscope for the presence of parasites. In gill biopsy, a fine pair of scissors was used to cut open the operculum from both sides to reveal the opercula cavity. Gill filaments were taken out by cutting off the two ends of gill arches and kept on Petri-dishes having saline solution (0.007%). For permanent preparations, smears were fixed in Schaudinn's fixative, stained with borax carmine for 30 min and then dehydrated in alcohol graded series of 50, 70, 90 and 100%. The parasites were cleaned with xylene and mounted in Canada balsam. Blood samples from caudal vein were smeared on sterile glass slides, allowed to dry for 15 min followed by fixation with methanol and staining with Giemsa's stain. The stained cells were examined under a light microscope (Olympus BX51, Germany) at different magnifications and photographs taken using digital camera fitted microscope.

Analysis of parasitic infestation

The analyses of parasitic manifestation (abundance) were studied using the modified method of Upadhyay *et al.* (2012).

$$\text{Abundance (\%)} = \frac{\text{Total No. of parasites}}{\text{Total host examined}} \times 100$$

The analyses of parasitic infestation for index were carried out by following formulae of Williams and Jones (1994) as:

$$\text{Index of infection} = \frac{\text{No of host infected x No.of parasites collected}}{\text{Total host examined}}$$

Histopathology

Tissue samples were obtained from *Clarias* sp. and routine histopathological analysis performed as per Butchiram *et al.* (2009).

RESULTS AND DISCUSSION

In present study, the protozoan species *Icthyopthirius* sp. (Fig. 1A), *Trichodina* sp. (Fig. 1B), *Chilodonella* sp. (Fig. 1C) were identified in the skin and gill of *Clarias* sp. *Trypanosoma* sp. (Fig. 1D)

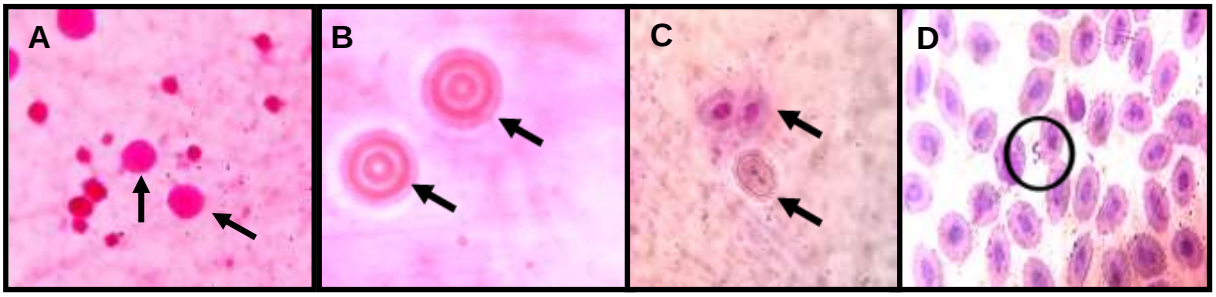

Fig. 1: Protozoan species observed in *Clarias* sp. A) *Ichthyophthirius* sp. (x100), B) *Trichodina* sp. (x1000) and C) *Chilodonella* sp. (x100) from gill and skin lesions, D) *Trypanosoma* sp. (x100) from blood

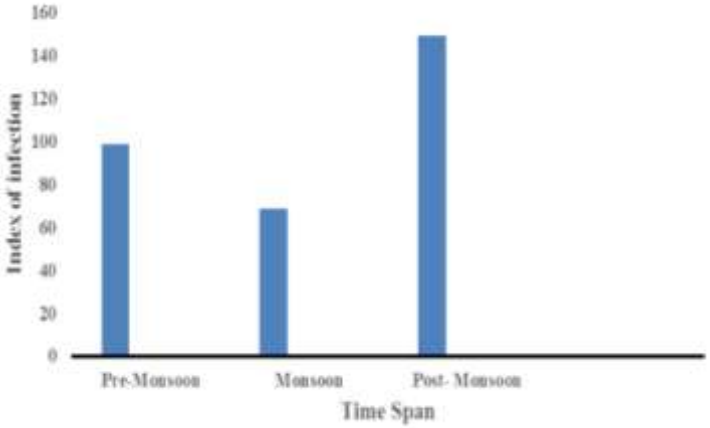

Fig. 2: Parasitic infection index in *Clarias* sp. at varied time span

was prevalent in the blood of catfish which are generally transmitted by cyclops, mollusca and leeches. The analysis for infection index revealed highest index in *Clarias* sp. during post-monsoon season, whereas the lowest index was observed during monsoon season (Fig. 2). The infection index varied in various seasons in fish which may be attributed to the changes in the environmental conditions on definitive host causing physiological changes which may have influenced the occurrence of parasite population. The moderate infection

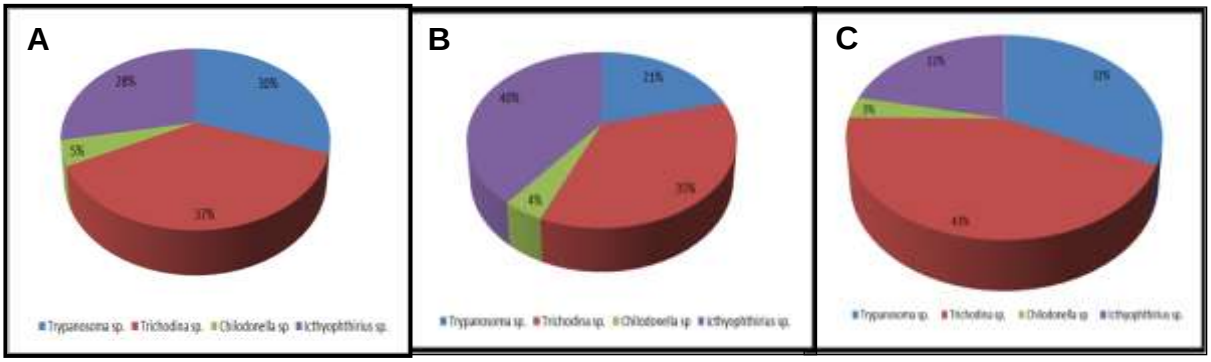

Fig. 3: Parasitic abundance in *Clarias* sp. during various seasons A) pre-monsoon B) monsoon C) post-monsoon; [*Trypanosoma* sp. (blue colour), *Trichodina* sp. (brown colour), *Chilodonella* sp. (green colour) and *Ichthyophthirius* sp. (violet colour)].

index was found during pre-monsoonal season as lower temperature during mid-winter arrested the development of parasites in host and environment. The occurrence of *Trichodina* sp. (43%) and *Trypanosoma* sp. (32%) was maximum in post-monsoon season (Fig. 3C); whereas highest incidence of *Icthyopthirius* sp. (40%) was noticed only in monsoon season (Fig. 3B). Maximum incidence of *Chilodonella* sp. (5%) was noted in pre-monsoon season (Fig. 3A).

Severe damages were caused by parasites on the body parts of sampled fishes (Kuchta *et al.*, 2018). Gills harboured the highest number of protozoan parasites (Fig. 4A), because apparently the

gills are the center of filter feeding and the sites of gaseous exchange. This observation is in line with Emere and Egbe (2006) and Nyaku *et al.* (2007). Parasitic infestation in gills caused severe degeneration, necrosis and consequent degeneration of branchial epithelium and occlusion of capillaries (Fig. 4B). The epithelium degradation of gill was counteracted by extreme process of epithelial hyperplasia (Fig. 4B). The sheer number of parasites covering the gills could cause mechanical hurdle to oxygen exchange mechanism. Fish skin (Fig. 5A) protects against mechanical trauma as well as infections in a constantly changing and challenging environment. The infected fish showed pale skin patches and more slimy skin. Infection with *Trichodina* sp. caused epidermal necrosis and excess**ive** mucus formation **in** skin (Fig. 5B). This resulted in sluggish movement, loss of appetite, emaciation, loss of condition with larger head and darker skin than normal.

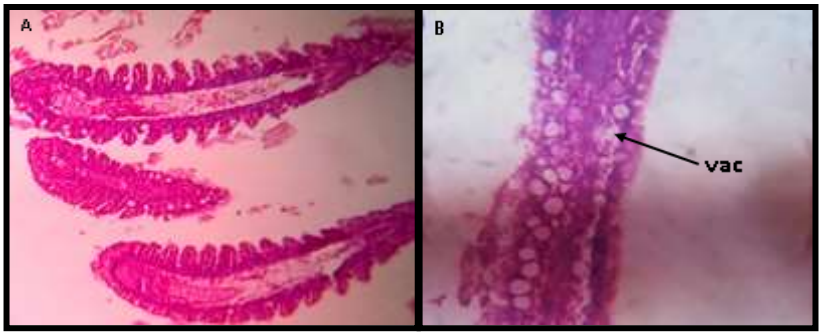

Fig. 4: A section of gill filament of *Clarias* sp. A) Gill lamellae (x100), B) Parasitized gill lamellae with intense vacuolation (vac) and degeneration (x1000)

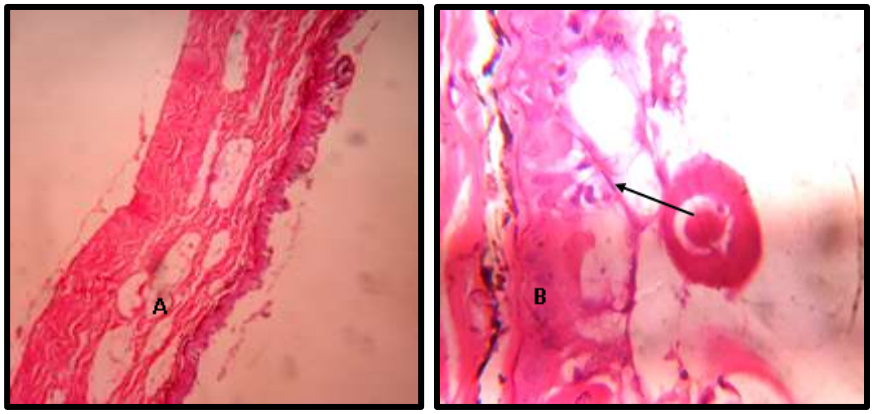

Fig. 5: A section of A) skin of *Clarias* sp. (x1000*);* B) Skin infested with *Trichodina* sp. (x1000)

Fish is important to human populace in trade and economy; and importance in the diet of different countries especially in tropics and subtropics where malnutrition is a major problem (Osuigwe and Obiekezie, 2007). Parasites are most diverse and common pathogens, the aqua- culturist might likely encounter these parasitic diseases in fish all over the world and so their control is of great importance in the tropics (Roberts and Janovy, 2000; Kaur and Ahmad, 2017). Fish parasites inflict huge economic losses and this increases fish mortality and farm inputs expenses via treatment and cause reduction in growth rate and possibly weight loss during and after the period of parasite disease outbreak.

Conclusion The present study reveals that the ciliated and flagellated protozoans in catfish exhibit depilatory effect on its host. Emphasis should be laid on adopting the control measures to interrupt the steps of parasitic transmission of one host to other. Attention demands to control the parasite with a view to increase the protein production together with the rapid growth of the fishes.

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