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Studies on Infestations of Monogenean Ectoparasites on Indian Major Carps of District Sultanpur, Uttar Pradesh, India

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ABSTRACT

Fishes are the good source of aquatic food that provides nutrients and gives nourishment to the human`s body and promotes growth. This study was carried out to determine the prevalence of monogenean ectoparasites on fresh water Indian major carps inhabited in the cultured ponds and natural ponds lakes and river of district Sultanpur, Uttar Pradesh, India, in a bid to suggesting ways of curbing them. Total 360 fishes were examined from cultured fish ponds consisting of 120 Bhakur (*Catla catla*), 120 Rohu (*Labeo rohita*) and 120 Mrigal (*Cirrhinus mrigala*) and 360 fishes from natural water bodies such as ponds, pools, lakes and river also consisting of 120 Bhakur (*Catla catla*), 120 Rohu (*Labeo rohita*) and 120 Mrigal (*Cirrhinus mrigala*). Higher infestations were recorded in cultured fish farm in rainy season whereas, in natural water bodies in summer season. The higher monogenean infestation was recorded in Indian major carp Bhakur (*Catla catla*) in both natural as well as cultured fish farm. The captive fishes are more infested in rainy season due to high pollution load but the fishes inhabited in natural water bodies are highly infested in summer season, when water level falls and fishes come in contact with each other. Prevention of monogenean ectoparasites infestation by given appropriate quarantine is preferable to treatment of the parasites after they have become established in a system. Administration of praziquantel and hydrogen peroxide should be used for the removal of monogenean ectoparasites and prevents the Indian major carps from infestation of monogenean ectoparasites. In present study, the larger fishes recorded higher infestation than smaller ones. Therefore, routine checkup of physico-chemical parameters, water level, fish density and monitoring of parasitic status of fishes would reduce the mortality and loss in fish production.

KEYWORDS: *Infestations, Indian major carps, Monogenean-ectoparasites, Sultanpur*

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1. INTRODUCTION

Monogenea are small parasitic flatworms mainly found on skin or gills of fish. They are rarely longer than about 2 cm. Monogeneans are mainly ectoparasites of fishes, occasionally they are found endoparasitic **(Mishra, 2007)**[18]. Among parasites infecting fishes, the monogeneans constitute a group, which plays an important role as pathogens of severe diseases **(Tripathi, 1955)**[32]. Most monogeneans are browsers that move about freely on the fish`s body surface feeding on mucus and epithelial cells of the skin and gills; however, a few adult monogeneans will remain attached permanently to a single site on the host **(Mishra, 2014a)**[20]. Some monogenean species invade the rectal cavity, ureter, body cavity and even the blood vascular system. The monogeneans affecting internal organs are presently insufficiently known and role of such species in the pathological process has not been adequately studied **(Mishra and Pande 2008)**[19]. Approximately 4,000 to 5,000 species of monogeneans have been described. Monogeneans are small to medium sized trematodes which complete their life cycle on single host **(Mishra, 2014b)**[21].

Morbidity and mortality epidemics caused by excessive parasite loads are not uncommon in captive fishes and have also occurred in wild fishes. Captive fishes are usually held in more crowded conditions than fishes in the natural environment. This practice allows hatching monogeneans to easily find host fish. The monogeneans are commonly found on wild fish, they seldom cause disease or death in free-ranging populations because under natural conditions they are usually not present in high numbers on individual fish **(Barai and Chandra, 2005)**[2]. However, any change that results in crowding of wild fish, which could include drought and diversion of water, may increase the density of parasites on wild fish, and consequently result in disease. In addition, the release of monogenean-infected fishes to the natural environment can have potentially devastating effects **(Onyedineke, et. al., 2008)**[29].

In majority of cases, monogeneans cause dual mode of injury to their hosts **(Mishra, 2014c)**[22]. Monogeneans have a specialized holdfast organ or haptor that has hooks or clamps that enable them to attach to their host. The haptor is usually shaped like a disc. One to three pairs of hook-like structures called anchors or hamuli are commonly located in the centre of the haptor **(Chandra and Jannat, 2002)**[3]. In many monogenean species, the anchors are used to attach to the host by penetration of the host`s skin. These organs of attachment break the continuity at the site of attachment and result in the localized hemorrhages. Suction created using the haptor itself is another way some monogeneans stick to their host **(Mishra, 2015)**[23]. Monogeneans parasitizes especially on gills but some on the skin and fins and cause damage of host tissue by their haptor, anchors and hooks during feeding **(Gussev, 1976)**[9]. Monogeneans have a direct life cycle, which means they go directly from host to host (fish to fish). There are three species of Indian major carps i.e., Rohu (*Labeo rohita*), Bhakur (*Catla catla*) and Mrigal (*Cirrhinus mrigala*) are the principal fishes cultured throughout India particularly eastern Uttar Pradesh. Mortalities of these fishes are accompanied by *Dactylogyrus* and *Gyrodactylus* infestations of the gills and skin. The monogeneans are cosmopolitan parasites occurring highly in larval and adult stages produce a wide variety of effects on fishes.

Fishes infested with monogeneans may become lethargic and end up swimming towards the surface of the water. They may be seen rubbing the bottom or sides of their skin where the parasite is located. Infected skin where the parasite is attached may show areas of scale loss and may produce a pinkish fluid. The skin may vary in color where the parasites have fed. Heavy gill infestations result in respiratory diseases **(Mishra, 2020a)**[24]. Gills that are infected may appear swollen and pale, respiration rate may be increased, and fish will be less tolerant of low oxygen conditions. "Pipping" which is gulping for air at the water surface, could indicate severe respiratory distress. Large numbers of monogeneans on either the skin or gills may result in significant damage and mortality **(Mishra, 2020b)**[25]. Secondary infections with bacteria and water molds are common on tissue that has been damaged by monogeneans.

Prevalence of fish disease has negative economic impact on fish culture. Studies of fish diseases in India especially in eastern Uttar Pradesh are limited to diagnosis, characterization and control of pathogens involved **(Hossain, et. al., 2007)**[11]. Some studies have been undertaken only on socio-economic aspects of developing pond fish culture. These studies did not assess the loss of fish diversity due to disease **(Hoffman, 1967)**[10]. There is a need to understand not only the prevalence of various diseases and pathogens but also the need to understand the economic losses resulting from disease outbreak. Production-loss assessment, assessment of economic impact of disease on production and optional investment for fish disease control are essential. Field survey is the most practical way in collecting such information directly from a large number of retailers, fishermen and fish-farmers.

The present work, on monogenean infestation on fish culture is one of the significant and priority areas. The above background keeping in mind, the present research programme is considered to be undertaken with the study of seasonal infestations of monogeneans in different carp species and general pattern of infestation of monogeneans in different seasons on cultured and natural Indian major carps of district Sultanpur, Uttar Pradesh, India.

2. MATERIAL AND METHODS

For the investigation of monogenean ectoparasites, the samples were collectedfrom various fish farms and natural water bodies situated in district Sultanpur, Uttar Pradesh, India. To observe the seasonal variation three distinct seasons were used as summer season (February - May), winter season (October - January) and rainy season (June - September) in the experimental period. The Indian major carps Rohu (*Labeo rohita*), Bhakur (*Catla catla*) and Mrigal (*Cirrhinus mrigala*) were selected as experimental fishes and sampled regularly from all sampling stations. Experimental fishes were collected for a period of 12 months from February 2020 to January 2021. Samples were collected from each sampling station at monthly intervals with the help of

fisherman. Per month 10 samples of each fish were collected from cultured ponds and 10natural water bodies. The samples were transported to the laboratory of department of Zoology, Ganpat Sahai P.G. College Sultanpur with plastic container and bags for further investigation.

2.1. Collection of Parasites

The monogeneans were collected especially from gill region by using **Mizelle`s (1936[26] and 1938[27])** freezing techniques. The external examination were made by scrapping the skin and examining smear using a magnifying glass or by under microscope. Cut both the opercula of fish and remove gills. The dissected gills were placed in petri dish containing clean water and kept in refrigerator for 8-48 hours. The low temperature not only relaxes the parasites but also help in automatic removal of mucous in which these flukes were entangled **(Hossain, et. al., 2007)**[12]. Subsequently, the gills were removed, placed in separate tubes, half filled with water and sake vigorously. This solution now poured in clean petridishes, diluted with water and examined under binocular microscope. The live monogeneans were gently rubbed to dislodge from the gill filaments with the help of a triangular pointed needle and forceps as suggested by **Malmberg, (1957)**[15]. The monogeneans were removed and picked out using a finepipette with a small drop of water on a clean slide and covered with a cover slip (**Das, et. al., 2016**)[7].

2.2. Fixation of Parasites

Monogeneans were fixed with a drop of ammonium picrate or Malmberg fixative introduced beneath the cover slip to fix and clean the parasite. Sometimes monogeneans were immediately fixed in 70% ethyl alcohol or 10% neutral formalin for further processing. The fixed monogeneans were studied under microscope and their size, shape and chitinoid structure was noted. Then the slides were marked by a permanent marker pen according to probable monogenetic trematode. The identification of the parasites was done following **Yamaguti, (1953)**[32]and **Chandra, (2008)**[5]**.**

2.3. Quantification of Parasites

Prevalence, mean intensity and abundance are suitable descriptors to quantify parasites in hosts. The prevalence, mean intensity of infestation and abundance (relative density of parasites) were used after **Margolis, et. al., (1982)**[16].

Prevalence is the number of disease cases present in a particular population at a given time, whereas incidence is the number of new cases that develop during a specified time period. Prevalence is calculated as-

$$Prevalence = \frac{Number\ of\ Host\ Infested}{Number\ of\ Host\ Examined} \times 100$$

Mean intensity is the arithematic mean of the number of individuals of a particular parasite species per infected host in a sample. It is calculated as-

$$Prevalence = \frac{Number\ of\ Parasite\ observed}{Number\ of\ Host\ Infested}$$

Abundance is the arithematic mean of the number of individuals of a particular parasite species per host examined. It is calculated as-

$$Prevalence = \frac{Number\ of\ Parasite\ observed}{Number\ of\ Host\ Examined}$$

3. OBSERVATION

The skin mucous and gills of Indian major carps was taken by using scrapping put on object glass, added one drop of aquadest and covered with cover glass. Observation was done by binocular microscope with 400X to identify monogenean ectoparasites. The prevalence, mean intensity and abundance of monogenean ectoparasites were found fluctuated in irregular pattern over the study period in natural as well as cultured fish farm of district Sultanpur, Uttar Pradesh, India.

Table 1: Seasonal Changes in Infestations of Monogenean Ectoparasites of fresh-water Indian Major Carps in Cultured Fish Farms:

Season	Name of Fish	No. of Host Fish		Total No. of Parasites	Prevalence (%)	Mean-Intensity	Abundance
		Examined	Infested				
SUMMER	Bhakur	40	23	161	57.50	7.00	4.03
	Rohu	40	19	131	47.50	6.89	3.28
	Mrigal	40	17	115	42.50	6.76	2.88
	Total	**120**	**59**	**407**	**49.17**	**6.90**	**3.39**
RAINY	Bhakur	40	25	189	62.50	7.56	4.73
	Rohu	40	22	161	55.00	7.32	4.03
	Mrigal	40	18	127	45.00	7.06	3.18
	Total	**120**	**65**	**477**	**54.17**	**7.34**	**3.96**

WINTER	Bhakur	40	22	129	55.00	5.86	3.23
	Rohu	40	17	105	42.50	6.18	2.63
	Mrigal	40	16	86	40.00	5.38	2.15
	Total	**120**	**55**	**320**	**45.83**	**5.82**	**2.67**
Grand	**Total**	**360**	**179**	**1204**	**49.72**	**6.73**	**3.44**

In cultured fish farm of Indian major carps shows highest prevalence (62.50%), mean intensity (7.56) and abundance (4.73) of monogenean ectoparasites found in **rainy season** in Indian major carp Bhakur (*Catla catla*) and lowest prevalence (40.00%), mean intensity (5.38) and abundance (2.15) of monogenean ectoparasites found in **winter season** in Indian major carp Mrigal (*Cirrhinus mrigala*) **Table 1.**

Table 2: Seasonal Changes in Infestations of Monogenean Ectoparasites of fresh-water Indian Major Carps in Natural Habitats of Fishes:

Season	Name of Fish	No. of Host Fish		Total No. of Parasites	Prevalence (%)	Mean-Intensity	Abundance
		Examined	Infested				
SUMMER	Bhakur	40	24	181	60.00	7.54	4.53
	Rohu	40	22	152	55.00	6.91	3.80
	Mrigal	40	21	132	52.50	6.29	3.30
	Total	**120**	**67**	**465**	**55.83**	**6.94**	**3.86**
RAINY	Bhakur	40	19	104	47.50	5.47	2.60
	Rohu	40	16	85	40.00	5.31	2.13
	Mrigal	40	14	68	35.00	4.86	1.70
	Total	**120**	**49**	**257**	**40.83**	**5.24**	**2.14**
WINTER	Bhakur	40	21	129	52.50	6.14	3.23
	Rohu	40	19	114	47.50	6.00	2.85
	Mrigal	40	17	105	42.50	6.18	2.63
	Total	**120**	**57**	**348**	**39.17**	**6.11**	**2.90**
Grand	**Total**	**360**	**173**	**1070**	**48.06**	**6.19**	**2.97**

In natural habitats of Indian major carps shows highest prevalence (60.00%), mean intensity (7.54) and abundance (4.53) of monogenean ectoparasites found in **summer season** in Indian major carp Bhakur (*Catla catla*) and lowest prevalence (35.00%), mean intensity (4.86) and abundance (1.70) of monogenean ectoparasites found in **rainy season** in Indian major carp Mrigal (*Cirrhinus mrigala*) **Table 2.**

4. MANAGEMENT

The best way to manage monogeneans is to avoid introducing parasites to a new system. This can be done by following a quarantine protocol. If quarantine is not possible, a simple way to minimize the introduction of monogeneans is to dip fish in fresh or salt water, depending on the fish species. Dipping freshwater fish in salt water will reduce the number of parasites. Regardless of the salt concentration used, the minimum contact time is 10 minutes (15 minutes for some monogeneans see hyper salinity in the section on treatment). However, if the fish roll over before 10 minutes has lapsed, they should be immediately removed from the dip. Dipping fish will not completely eliminate the risk of introducing parasites to an established tank or system, but it may help minimize the number brought in **(Johnsen and Jensen, 1986)**[13].

A number of chemicals have been used to control monogenean infestations of fish. However, treatment of monogeneans must be accompanied by identification and reduction of environmental or husbandry-related stressors, if they are present. Stressful conditions can not only inhibit the immune system oh the fish, but also result in release of cortisol (a stress hormone). Take steps to prevent monogenean infestation whenever new fish are to be introduced to a system, even when the new fish appear healthy. Some fishes are capable of mounting an immune response that will prevent monogeneans from reaching lethal numbers. Such fishes may appear normal, but can serve as a reservoir of monogeneans that can infest nave fishes **(Mashego, 2001)**[17].

Monogeneans don't live after a fish dies, so examination of a live or fresh dead fish is best. They may be diagnosed by performing biopsies of fin gill and skin mucus and examining these tissues with a light microscope. Preservation of monogeneans for identification and future study should both permanent mounts and 95% ethanol-fixed specimens.

5. PREVENTION AND TREATMENT

For the prevention of monogenean infestation, fish should be quarantined for at least 04 weeks before they are placed in to a new system. The design of a quarantine system should be very simple so that fish are readily accessible for observation and handling and so that water can be easily changed and treatments easily administered. A number of chemicals have been used to control monogenean infestations of fish **(Obiekezie and Osuigwe, 2007)**[28]. Treatment of monogeneans must be accompanied by reduction of environmental or husbandry related stressors, if they are present. Stressful conditions can not only inhibit the immune system of the fish, but also result in release of a stress hormone, cortisol that induced monogenean reproduction. Formalin, when administered at 30 mg/L can also be used; however, vigorous aeration is required to maintain an acceptable level of dissolved oxygen. Potassium permanganate is also moderately effective against monogeneans; it can be administered as a prolonged bath at a concentration of 2 mg/L or as a short-term bath (10-30 minutes) at a concentration of 10 mg/L.The treatment of choice for monogeneans for fresh water Indian major carp is praziquantel; however, praziquantel is not approved by the US Food and Drug Administration (FDA) for use in fishes. If praziquantel is used to treat monogenean ectoparasites, its use should be restricted to enclosed tank and any effluent from the treated tank should be run through an activated carbon filter before it is discharged. A common treatment method is to use praziquantel at 25 mg/L in a prolonged bath for 23 weeks. Infested fish should be re-examined 12 weeks post-treatment to determine if treatment should be repeated **(Paperna, 1975)**[30]. Praziquantel is the most effective drug for controlling and possibly eliminating monogenean ectoparasites and it is safer for the host. Praziquantel may also be given orally by mixing it in to feed at the rate of 40 mg/kg of body weight per day; praziquantel- medicated feed should be administered for 11 days. High doses (300560 mg/L) of hydrogen peroxide for 10 minutes have also been used successfully for the removal of monogenean ectoparasites. A final method of control is to empty, dry and disinfect tanks or ponds before restocking them. Except for this method, it is very difficult to completely eliminate monogeneans once they are introduced.

6. DISCUSSION

Infestations of monogenean ectoparasites are very common in fresh water Indian major carps. During the investigation, prevalence of monogenean ectoparasites in Indian major carps {Bhakur (*Catla catla*), Rohu (*Labeo rohita*) and Mrigal (*Cirrhinus mrigala*)} was quite high and fishes of larger size groups were more susceptible. However, several authors have noted only the correlation between outbreak of monogenean ectoparasites and stocking densities. It was also observed in our study that infestations of monogenean ectoparasites were higher in those fishes which are present in highly condensed form or in fishes inhabited with highly polluted water **(FAO, 1999)**[8]. The infestation of monogenean ectoparasites are more in captive fishes than fishes inhabited in natural water bodies. The captive fishes are more infested in rainy season due to high pollution load but the fishes inhabited in natural water bodies are highly infested in summer season, when water level falls and fishes come in contact with each other **(Majumdar and Agarwal, 1988)**[14].

The prevalence of monogenean ectoparasites was higher in captive Indian major carps in rainy season than summer and winter seasons whereas, the prevalence of monogenean ectoparasites on fishes inhabited in natural water bodies was higher in summer season than rainy and winter seasons **(Chandra, 2006)**[4]. In both of these habitats captive as well as natural, the infestation was higher in Indian major carp Bhakur (*Catla catla*) than Rohu (*Labeo rohita*) and Mrigal (*Cirrhinus mrigala*). The Indian major carp Bhakur (*Catla catla*) was more susceptible for infestation of monogenean ectoparasites than Rohu (Labeo rohita) and Mrigal (*Cirrhinus mrigala*). The infestation of monogenean ectoparasites was highest in rainy season and lowest in summer season in captive Indian major carps whereas, the infestation was higher in summer season and lower in rainy season in fishes inhabited in natural water bodies because of the highest stocking density. This result agrees with **Das, (2003)**[6] **and Banerjee and Bandyopadhyay, (2010)**[1]. According to **Barai and Chandra, (2005)**[2] the highest monogenean ectoparasites infestation were found in fry and fingerlings of Indian major carps during rainy season (June to September) because of highest stocking density and unfavorable environmental condition in captive fish farms. In natural water bodies especially in river (running water) the infestation of monogenean ectoparasites were found highest in summer season due to fall of water level that causes environmental or behavioral stressors and increases the infestation of monogenean ectoparasites on Indian major carps.

7. CONCLUSION

The present study highlighted the infestation of monogenean ectoparasites and disease problems in Indian major carps at district Sultanpur, Uttar Pradesh, India. Monogenean ectoparasites were found

mainly on gills but sometimes occur on skin and fins of fishes. Infestation begins at the early stage of fish life and persists up to the adult stage. The captive fishes are more infested in rainy season due to high pollution load but the fishes inhabited in natural water bodies are highly infested in summer season, when water level falls and fishes come in contact with each other. Monogenean ectoparasites infestation also provides platforms for secondary infections of protozoans, bacteria, viruses and crustaceans. Monogenean ectoparasites were a common problem for fresh water Indian major carps of India especially in eastern Uttar Pradesh. Prevention of monogenean ectoparasites infestation by given appropriate quarantine is preferable to treatment of the parasites after they have become established in a system. Administration of praziquantel and hydrogen peroxide should be used for the removal of monogenean ectoparasites and prevents the Indian major carps from infestation of monogenean ectoparasites.

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