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Fish Parasites prevailing in the fishes of Indus River at D.I. Khan Khyber Pakhtunkhwa, Pakistan

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Abstract
The present study was conducted to investigate the parasitic profile and their level of infestation in freshwater fishes of river Indus, D.I. KHAN, Khyber Pakhtunkhwa, Pakistan. A total of 109 fishes belonging to seven different species were identified and examined for infection. Five species of different parasites, including *Saprolegnia, microsporadia, Lernaea cyprinacea, Spherospora auretus, Carpora athritus*, were found in fishes. Among parasites *saprolignia* was most frequent prevailed. The Infections were found on the whole body, including fins, skin, head, gills and internal system. Intense infection was present in some fishes as their whole internal body system was damaged and present in the form of liquid and spores. The frequency and number of parasites were more pronounced than the diversity of parasites in this area of river Indus, D.I. Khan.

Keywords: Fresh water, fish, river Indus, parasites, infection, D.I. Khan

1. Introduction
Fishes are one of the most important group of vertebrates which give benefits to human beings in several ways. These are more common and widely distributed in almost all parts of the world. The quality and quantity may vary, but they are used by the man everywhere. Three - fourth of the earth's surface is covered by sea. Nearly all fish freshwater and marine are edible and have been an important sources of protein food. Fresh fish flesh provides an excellent source of protein. This protein is relatively of high digestibility, biological and growth promoting value for human consumption. Nutritional studies have proved that fish proteins rank in the same class as chicken protein and superior to milk, beef protein and egg albumen [1]. The indigenous fish fauna of river Indus are one of the earliest local fish faunas known to Science. Mainly they can be divided in two groups scaly and scaleless fishes. Scaly mostly includes carp fishes and some others and non-scaly include catfish and others. High prevalence of parasites is mostly susceptible in scaly fishes, including caps and some non-carps. The expected diseases may be fungal, bacterial, viral, or other parasitic diseases [2]. Every fresh water fish is exposed to at least one species of fungus during its lifetime [3]. Lernaea species, commonly known as “anchor worms,” are crustacean, copepod parasites that can infect and cause disease and mortality in many types of freshwater fishes, especially wild-caught and pond-raised species. Infestations with *Lernaea* are most prevalent in the summer months and occur more commonly in stagnant or slow-moving water bodies. Approximately 110 species of lernaeids (Lernaea and Lernaea-like parasites) have been described. *Lernaea cyprinacea*, one of the more common species, is found worldwide. It is most common in Cyprinids, including Koi, common carp, grass carp and goldfish; however, it can infect other species of fish and has caused major kills in Arkansas catfish in ponds that were also stocked with bighead carp. Because *Lernaea* is a copepod and crustacean, it is related to crabs, lobsters, and shrimp, all of which have a multistage life cycle. Lernaea is unusual, however, as most common copepod species are free-living and do not cause disease. Although organisms belonging to this group have complex life cycles, they do not need to pass through an intermediate host; rather they can spread directly from fish to fish. This direct life cycle can take from 18 to 25 days to complete, and only a fish (or an amphibian) is necessary for the organism to develop from egg to mature adult [4].

Renal dropsy in fishes is caused by the parasite, *Sphaerospora auratus*. There is no treatment for this kidney disorder and it usually causes death of the infected fish. Similarly swim bladder inflammation is also caused *common carp* and its parasite is unknown [5]. A xenoma (also known as a 'xenoparasitic complex') is a growth caused by various protists and fungi, most notably *microsporidia*. It can occur in numerous organisms; however, is predominantly found in fish. In most cases the host cell and nuclei suffers from hypertrophy resulting in a change in the organization of the cell and its structure and can result in polyploid nuclei. This outcome is due to the *microsporidian* parasite proliferating inside the host cell. This results in a 'symbiotic co-existence' between the parasite and the host cell [6]. The fresh water, edible fish found susceptible to fungal infection *Aspergillus fumigatus* and *Aspergillus niger* is a pathogenic group of fungi causing infection in freshwater fishes. The pathogenesis of *Aspergillus fumigatus* and *Aspergillus niger* has been reported in freshwater fishes [7-8] The aim of the research work was to find out the fish parasite prevalence in the Indus river of D. I. Khan.

2.1 Materials and Methods

Study Area

The area of study is river Indus, district D. I. Khan, KPK, Pakistan. The Indus River has fish fauna of great variety and is a major southerner-flowing river in South Asia.

Fish Sample collection and identification

Fish sampling was done by using fish gears to catch the fishes from river Indus. The samples were collected from March to May of 2016. Fish were identified through a key and brought to lab for experimental work by Jayaram [9].

Experimental analysis

Total length (TL) and weight of fish were recorded. For the isolation of ecto-parasites like crustaceans, protozoans and other gill parasites, external surface and gills were carefully examined with the help of a magnifying glass. Smears were prepared from the gills and skin of fish to locate the protozoans. Smears were allowed to air dry and fixed in absolute methanol for 20 minutes, stained (Giemsa) for 10 minutes an de-stained with distilled water and then microscopy was done. To locate other parasites, the gills were scraped with fine forceps and were examined like others. All smears were examined with the help of digital microscope. geimsa Stain was prepared by dissolve 3.8g of Giemsa powder into 250ml of methanol and heated the solution to 60ºC. Slowly add in 250ml of glycerin to the solution filter the solution. Fresh smear was prepared from gills, intestine, liver, stomach on the slide and allow the smear to air dry at room temperature then fixed with absolute methanol for twenty minutes. Put the drops of Geimsa stain on the smear and leave for ten minutes, then wash the slide with distilled water, then allowed to dry. Parasites were examined by using digital, dissecting and compound microscope on 10x and 40x. Stained slides were easily visible due to its different colors on 40x with bright light while transparent slides were visible at 10x with the dim light of the microscope. Large no of body spores of fishes were studied under a digital microscope [10].

Results

During the research period of March 2016 to May 2016 a total 109 fish of 07 different species were examined from the river Indus, D I KHAN. Total percentage of infection was 22.93% (50 fishes). The highest prevalence of parasites was in *L. rohita* as 34.21%, while low prevalence was in W. attu as 12.5%, respectively. The highest prevailing parasites were *Saprolegnia* and *microsporadia.* The rate of infection was increased with increase in temperature from March to May. In the month of March total 38 fishes were examined and among them 07 fishes were infected by different parasites like *lernea, microsporadia*, and *Saprolegnia* spp. The rate of infection in that month was 18.42%. Most prevalent fish to infection was *L. rohita* while *C. carpio* was normal during examination of prevalence *L. rohita* had the highest prevalence of infection with the percentage of 34.21%. The infection was same in *L. rohita* and *C. calbasu* which was found on the whole body, including skin and fins (caudal) in the form of white small spots clearly showed fungal infection. Internal and external hemorrhage were present on the head, eyes and skin. The organs of fish were fully converted with spores and differentiation of any organ wasn't possible, which was due to *Saprolegnia* specie. Parts covered with fungus spores were not transparent and light couldn't pass from them (**Figure-I).**

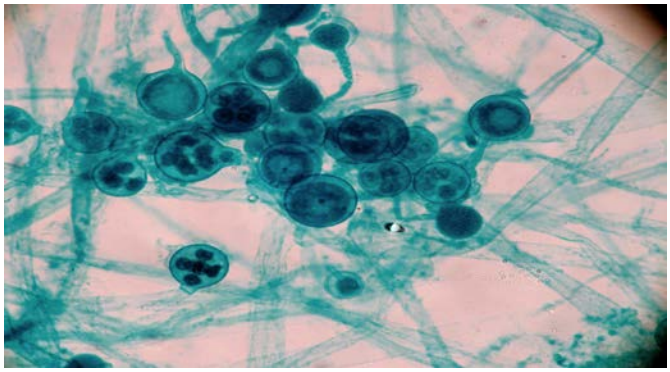

Fig I: Microscopy showed *Saprolegnia* parasite in fish

W. attu suffered from xenoma disease caused by fungus *microsporadia* in which hypertrophy of cells takes place (Figure-II).

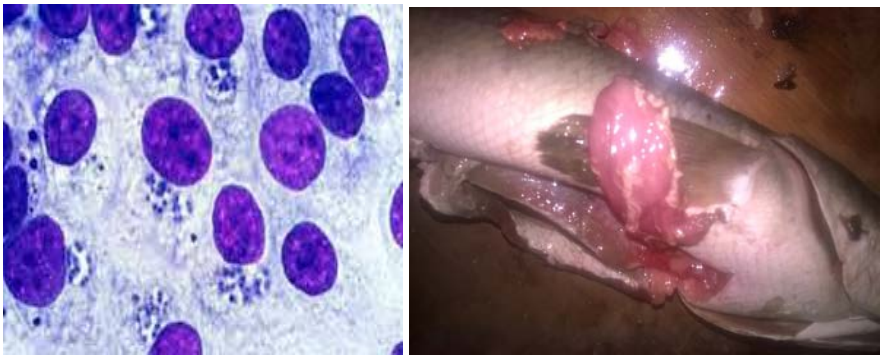

Fig II: *W.attu* and Microsporadia showing xenoma to naked eye and Microscopy

C. idella was found to be suffering from mature *lernea cyprinacea*, and severe fungal infection. Whole body, including fins and skin contained white spots. The gills were compacted in fish. Slight hemorrhage was also seen in the eye region. The infection in fish can be seen by naked eyes (Figure-III) (Table-I).

Fig III: *C. idella* containing white spots on the whole body, including fins

Table I: Prevalence of parasites in different fishes of river Indus in month of March

No	Fish name	No, of Fishes Examined	Fishes infected	Infection %	Parasites found	Infected organ
1	*C. idella*	12	3	25	*Lernea cyprinacea*	Whole skin, including fins
2	*C.carpio*	10	0	00	Nil	Nil
3	*L.rohita*	08	3	37.5	*Saprolegnia*	Whole internal system
4	*Wallago attu*	08	1	12.5	*Microsporadia*	Intestine, gills
Total		38	7	18.42		

Total 27 fishes were examined in the month of April, of which 06 were infected and rate of infection was 22.2%. Most infected fish was again *C. carpio* with 27.27%, while *N. Notopterus* was least infected with 16.6% (**Table-II)**.

Table II: Prevalence of parasites in different fishes of river Indus in month of April

No	Fish specie	No of fish examined	No of infected fishes	Percentage of infection %	Parasites found	Site of infection
1	*C.carpio*	11	3	27.27	*Spherospora auretus*	Kidney, swim bladder
2	*L.rohita*	10	2	20	*Saprolegnia*	Whole internal system
3	*Notopterousnotopterous*	6	1	16.6	*Corpora arthritus*	Eggs of fish
	Total	27	6	22%		

In *N. notopterous* size of the egg is large and is visible to naked eyes. The disease caused by corporal arthritis abnormally enlarged the size of eggs that covered almost all places over the swim bladder and system was poorly visible (Figure-IV).

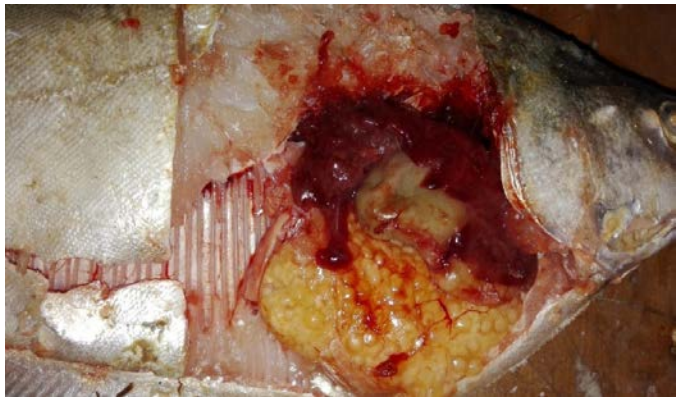

Fig IV: *N.notopterus* infected by Corporate arthritis causing enlargement of eggs

As far as month of May is concerned total 44 fishes of three different species were examined of which 13 got infected. The rate of infection was 29.54%. Most prevalent species were again *L. rohita* with 40% infection rate while low prevalent specie was *C. reba* with 18.18%. During this month both *C. reba* and *C. calbasu* was infected with fungus as primary infection while bacterial as a secondary infection. Evidences showed it, but no proper parasites were recovered to recognize the proper disease (Table-III).

Table III: Prevalence of parasites in different fishes of river Indus in month of May

No	Fish specie	No of fish examined	No of fish infected	Percentage of infections	Parasites found	Site of infection
1	*L.rohita*	20	8	40	*Saprolegnia*	Whole internal system
2	*C. reba*	11	2	18.18	Primary fungal and secondary bacterial infection	Whole internal system including skin
3	*L.calbasu*	13	3	23	Primary fungal and secondary bacterial infection	Whole internal system except gills
	Total	44	13	29.54%		

Percentage of infections increased with respect to each month. Infection percentage was 18.42%, 22% and 29.54% in the month of March, April and May respectively (Figure-V).

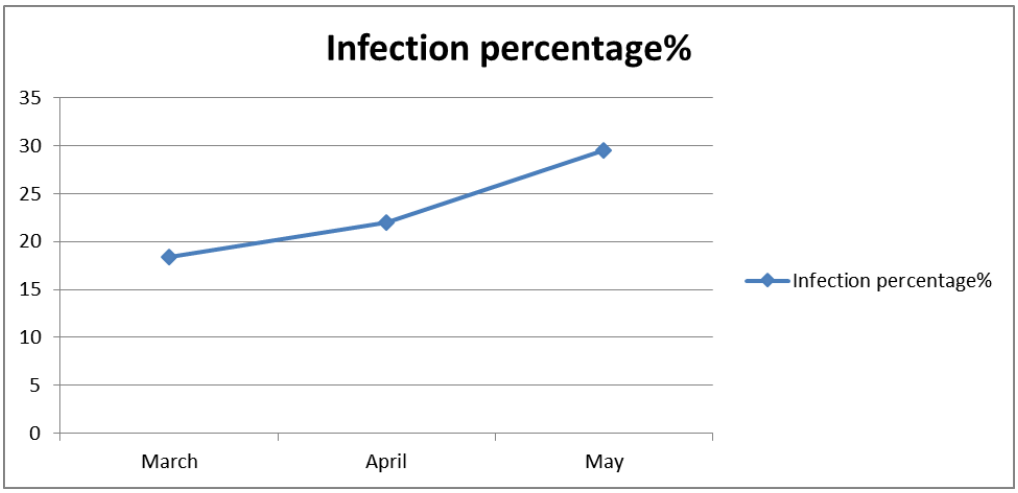

Fig V: Infection percentage in fishes shown in a line graph.

Most infected species in the whole research period was *L. rohita* with 34.57%, while low prevalent was *W. attu* with 12.5% (Table-IV). In infection of *L. rohita* was characterized by a damaged and dissolved thin layer of tissues inside the body, attached to muscles.

Table IV: Prevalence of parasites in all fish species during research period.

Fish specie	Examined fishes	Infected fishes	Prevalence of parasites %
C.idella	12	3	25
C.carpio	21	3	14.28
L.rohita	38	13	34.21
c.reba	11	2	18.18
C.calbesu	13	3	23.07
N.notopterus	6	1	16.6
W.attu	8	1	12.5

C. carpio whose overall rates of infection was found up to14.28% in the study. The parasites were not properly separated but the result was based on evidences which indicated the presence of these parasites. The fish was dissected and showed swim bladder inflammation (Figure-VI)

In case of *C. reba* the skin of fish contained white patches due to which skin was fully damaged and scales became milky white. The system, especially intestine was slightly floating outside the body (Figure-VII).

Fig VII: *C. reba* white patches externally and damage internal system.

3. Discussion

Parasites are generally found in all freshwater fishes. The parasite prevalence and intensity depend on many factors like its life cycle, host and its feeding habits and the physical factors of water body where the fish inhabit. It also depends upon the presence of intermediate host [11]. Different parasites were found in fresh water fishes of river Indus, D.I. Khan. A total of 109 fishes of 7 different species in which 38 fishes of 4 species in March, 27 fishes of 03 species in April and 44 fishes of 03 species in the month of May were examined. Total 25 fishes got infected of which 7 in March, 6 in April and 13 in May were found infected. Total percentage of infection was 22.93% in which 18.42% were in March, 22% in April and 29.54% in the month of May. The prevalence of parasites in fishes depends on location of sample collection, study area, season, climatic condition, volume of sample; size of population, etc., due to the above factors transmission of parasites in fishes is different in emerging countries then that of advanced countries. With the passage of time, in each month the rate of infection increased due to temperature. Previous studies have shown that the parasitic infestation is more common in fishes of warm water at higher temperatures [12], which is the agreement of the present findings. As most infected fish was *L. rohita*. Total 38 fishes were examined in which 08 in March, 10 in April and 20 in the month of May for parasitic infection, of which 13 were infected in which 03 in March, 02 in April and 08 in May got infected. The overall percentage of infections of *L. rohita* were 34.57% in which 37.5% were in March, 20% in April and 40% in May respectively. Overall 13 samples were shown positive with parasitic infection out of total 38 samples. *L. rohita* was infected by *Saprolegnia* spp. While in previous studies *Aspergillus* was also isolated from *L rohita* and mixed infection also identified. This species presumably causes infection via entry into the fish through contaminated feed [13].The whole body of the fish was converted into spores. Almost all infection causing agents were fungi because fungi can also cause disease at very low and slightly high temperature as well. Ecological differences play an important role in species diversity of fungi that develop on both fish and eggs [14]. Interaction of physiochemical factors generally has influence on the diversity of water molds [15].

4. Conclusion

From the above results it is clear that the highest prevalence studied during the present endeavor of infection occurred in summer and lowest in winter in all fish species. The rainy season which starts in the spring and continued to early summer, makes the environmental conditions more favorable for the development and survival of the pre-parasitic stages of all parasites and leads to an increased availability of infective stages in rainy and post-rainy seasons. This results in higher prevalence and level of parasitism in summer and post-summer months; therefore, parasite burden also reaches maximum levels in summer and autumn. In other words the fluctuation of temperature also increases during summer season therefore fishes falls in stress and shock and it leads to easy and effective parasitic attack.

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