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PREVALENCE OF TREMATODE PARASITES IN FRESHWATER FISHES IN RIVER KABUL, KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

A total of 135 freshwater fish samples were collected from River Kabul, Khyber Pakhtunkhwa, Pakistan and were examined. Fish samples were subjected to the identification process and out of total 135 fish samples, six different species were identified which included, *Wallago attu, Labeorohita, Cyprinuscarpio, Tor pitutora, Nemacheiluscorica* and *Heteropeneustes fossilis*. Their numbers were 43, 31, 28, 13, 17, and 3, respectively. After identification, all the samples were dissected and their intestines were removed for the trematode parasites determination. After sample analysis, overall 31.85%prevalence of Trematode parasites in the freshwater fishes was recorded. The Trematode species found in the freshwater fishes found were, *Bucephalus polymorphus*, *Proctoeces maculatus*, *Genarchopsis kalriai*, and *Allocreadium danjiangensis* and their prevalence rate was, 8.88%, 11.11%, 7.40%, and 4.44%, respectively. The obtained results showed that *W.attu* was 30.23% infected, *L.rohita* was 51.61%, *C.carpio* was 28.57%, *T. pitutora* was infected 23.07%, *N. corica* was infected 17.64% and *H. fossilis* was infected 0%.

Keywords: Trematode parasites, Prevalence, Freshwater fish, River Kabul.

INTRODUCTION

Fish has an important effect on the lives of the world's population, as it the main source of comparatively low-priced and reasonable essential animal's protein. Fishes link up with the food chain and affect the structure of several water bases **[1]**. Fish fulfill the

protein requirements at a practical price and thus help in reducing the reliance upon the costly protein sources. Moreover, fish oil contains essential fatty acids which help in the proper functioning of the various important organs such as brain, heart as well as the immune system **[2]**. On the other hand, it provides employment to the poor people of the society and thus helps in the socio-economic growth of a country. It also provides chances of refreshment e.g., sport fishing which is a major source of recreation **[3]**.

However, fish all over the world suffers from different kinds of parasitic diseases that cause high mortality in fish either directly or indirectly **[4]**. Especially the helminths parasite of fish that are living in the alimentary tube harm the coating wall of host and some other important organs such as liver and bile duct. The parasitic activities cause injuries to the host tissues which then become infected by the secondary type activity of microorganisms **[5]**. Apart from being harming fishes, some parasites also transmit from fish to human population which has been known since several years ago and thus it is one of the most important difficulties challenging the scientists and fisherman that working with fish **[6]**. Food-borne parasitic contaminations have recently been known as a significant public health problem with widespread viable impact in terms of sickness, loss of profit and healthcare associated costs. In addition to the economic loss to farmers, many of the parasites, particularly trematodes, are also a source of zoonotic importance. Eating raw or improperly cooked or processed fish is the main source of these infections for humans, and this has been reported from various geographical regions **[7]**. The World Health Organization (WHO) has estimated that the number of people currently infected with fish-borne trematodes exceeds 18 million, and many more are at risk **[8]**. Poor cleanness and antediluvian methodologies of food preparation have enhanced the spread of food-borne trematodes infection **[9]**. Fish may work as intermediate or definitive hosts for trematode parasites and are harmful to both human and other animals. Zoonotic infections that result from the consumption of uncooked or under cooked fish include, diphyllobothriasis, opisthor- chiasis, gnathosomiasis, clonorchiasis, and anisakiasis infections **[10]**.

Freshwater fish play a major role as the source of human infections with food-borne trematodes, and these parasites are receiving great attention because of their existence in freshwater bodies and as more information

on their diversity and prevalence appears in some Asian countries **[11, 12]**. Trematode parasites are of two types, those which restricted to the external surface of the body of fishes such as gills, skin, fins and complete their life cycle on a single host, are called the monogenetic trematodes, whereas, those which are confined to the internal organs like stomach, intestine, lever, gall bladder, heart etc., and complete their life cycle on two host or more host are called, the digenetic trematodes. Monogenetic trematodes could be considered as one of the most prevalent parasitic agents that are affecting skin and gills and leading to impairment of breathing as well as economic losses **[13, 14]**.

Infection of fish by encysted metacercariae of digenetic trematode is due to the contamination of water resources with human, animal and birds feces which concealing eggs of these parasites, thus reaching the water stream which contains the specific intermediate snail host completing their life cycle **[15]**. From the public health point of view, the consumption of fishes infected with the larval stages of trematode parasites caught from local water, set up a human threat. Freshwater fishes are considered as one of the important sources of parasitic infection to man and fish eating mammals particularly after the increased pollution of rivers and lakes because several poor regions that heavily depend on freshwater fish as a source of dietary protein suffer with health hazard **[16]**.

On the other hand, unfavorable environmental conditions such as stress, pollution, overfishing, weaken immunity of the fish and open the pathway to these pathogens **[17, 18]**. The clinical image of infected fish discloses no pathogenic anomalies on the external body surface; however, the internal organs become infected and would appear anemic with enlargement and congestion **[19, 20]**.

Keeping in view the importance of the freshwater fishes, its economic losses due to the trematode infections and the as a source of transmitting these parasites to human population and other animals, the present study was thus designed to determine the types of trematode parasites, to document their intensity in freshwater fishes in River Kabul, to spread information amongst the public community to take care while cooking the fish and to take preventive measurements for the stoppage of the trematode parasites infection among the freshwater fishes.

MATERIALS AND METHODS

Study area

The present study was carried out in the Charsadda District, Khyber Pakhtunkhwa and the freshwater fish samples were collected at different sites of River Kabul. River Kabul is located in District Charsadda adjacent to Peshawar, Khyber Pakhtunkhwa Pakistan. It isa plain areaand river Kabul hasfreshwater.

Fish identification

All the fishes which were collected from the River Kabul, District Charsadda were first subjected to the identification process while adopting the methods followed by identified under the standard Taxonomic rules as described by Schmidt **[21]** for the identification of the collected fish samples. Weight and length of each fish was taken and was noted. Also the morphological characteristics were noted shown in **(Table 1).**

Source of collection

The fish net were used for capturing of the fishes at morning time daily. After capturing the fishes, they were transferred to the ice box for preservation and later on transferred to the lab of the department of Zoology, Kohat University of Science and Technology, Kohat for further analysis.

Dissection and extraction of digestive tract

The parasites were identified by the method as described by Souls **[22]**. The sedimentation and floatation techniques were used to isolate the Trematodes parasite. The fish were put ventrally on surface of a table than a small cut were made transversely near the gills and head was separated then, a longitudinal incision were given on the ventral side and the viscera were properly exposed and the guts were removed. The guts were put in sterilized polythene bags and labelled .These was preserved in refrigerator for further processing. The fecal materials of each fish were put in the Petri dishes and labelled than a whole gut was put in petri dish.

Collection of fecal materials

The polythene bags having the guts were opened and fecal materials were squeezed into the Petri dishes then add the concentrated salt solutions and mixed thoroughly.

Then, solution was filtered. Through the cheese cloth and the filtrate were collected and washed and prepared a thin and thick smears and examined the slides under the magnification at 10 x, 40 x, and 100 x microscopically. similarly the filtered solution were centrifuge at 800 x g for 10 minutes and smears were made from the supernatant and also examined under the microscope for eggs and larvae of Trematodes.

Fecal examination

Slides were examined at 10 x, 40 x and 100 x magnification microscope and freeze the photo graph of the images and compare with the positive slides of parasites and eggs/ ova of the fish Trematodes.

Staining

Some slides were not very much vivid under the microscope, so as to get the better results; these slides were given the Giemsa stain and then examined under the microscope.

Prevalence rate

Prevalence rate of the Trematode parasites was determined by the method as described by the souls **[22]**.

Prevalence rate%= No. of fish positive for parasites/Total No. of fish x 100.

Statistical analysis

The obtained results were statistically analyzed by using the Korean Statistic 9 software for significance of the data.

RESULTS

When the fish samples were collected from the different sites of the river Kabul, it was found that the fish samples consist of 6 different fish species and some of that fishes were found infected with several Trematode parasites in the intestine. All of the fishes were dissected and fecal material was removed and examined in which overall 43 (31.85%) fishes were found infected with various Trematode parasites. A statistically significant difference ($P<0.05$) was noted during the data analysis.

Prevalence of Trematodes in freshwater fishes

After the examination of the fecal samples, it was found that *W.attu* showed 30.23% infection rate. Similarly *L.rohita* fishes were infected 51.61%. *T.pitutora* fishes were 23.07% infected. *C.carpio*showed 28.57% infection rate. *N. corica* fishes were infected at a rate of 17.64% while *H.fossilis* showed 0% infection rate. Prevalence rate is shown in **(Table 2).**

Intensity of different Trematode parasite species in the freshwater fishes

Four different types of Trematode species were found in the fecal samples **(Table 2).** These included, *B.polymorphus*, which was found in almost all described fish species and the prevalence intensity was 8.88%. *A. danjiangensis*, species of Trematodes were observed in different described fish species and showed overall intensity of 4.44%. The intensity of *G. kalriai* species of Trematodes was recorded 7.40%. *P. maculates* species was found in almost all fish species. The intensity rate recorded was 11.11% which was highest prevalence rate among the four types of Trematodes.

Table No.1: Morphological characteristic of freshwater fishes of River Kabul, District Charsadda KP Pakistan.

Name of fish Species	No. Of fish Samples	Average length of fish	Average weight of fish	Approximate age of fish	Fish morphology
W. attu	43	51 cm	490 g	mature	Two pairs of barbels, silvery color and sharp teeth on jaws.
L. rohita	31	40 cm	285 g	mature	Barbels two, fins black, abdomen sharp, ventral side lighter.
C. carpio	28	33 cm	320 g	mature	Smooth mouth, dorsal fin long, grayish green color, teeth absent.
T. pitutora	13	26 cm	180 g	mature	Stout and elongated body, large scales, pointed head.
N. corica	17	41cm	370 g	mature	Lateral line complete,strongly curved mouth. Three pairs of long barbels, caudal fin forked.
H. fossilis	03	30 cm	265 g	mature	Villi form teeth and bands on jaws, snout is flat, and mouth is terminal, Four pairs of barbels.

Table 2: Prevalence of Trematodes in freshwater fishes in River Kabul, District Charsadda KP Pakistan

Fish name	Parasite location	Trematode species				Prevalence (%)
		B. polymorphus	*A. danjiangensis*	*G. Kalriai*	*P. maculatus*	
W. attu	Intestine	03	01	04	05	30.23%
L. rohita	Intestine	05	00	03	08	51.61%
C. carpio	Intestine	02	03	03	00	28.57%
T. putitora	Intestine	00	01	00	02	23.07%
N. corica	Intestine	02	01	00	00	17.64%
H. fossilis	Intestine	00	00	00	00	00%
Grand total	----------	8.88%	4.44%	7.40%	11.11%	

DISCUSSION

Trematode parasites are generally found in freshwater fishes throughout the world. The prevalence and intensity of Trematode parasites that has been reported in the present study depends on many factors such as nature of parasite, its life cycle, feeding habits and physical factors of water body where the fish habitat occurs as well as the presence of a transitional host.

It was reported that seven different kinds of larval Trematodes were found in eight freshwater fish species in river Oseep. Of these, two freshwater fish species were infected with the encysted larvae of *E.oviformis* and the remaining freshwater fish species were heavily infected with the cyst larvae of *M.yokogawai*, however the intensity rate of infection with the encysted larvae of *E.oviformis* was very much lower than the intensity rate of infection with the cyst larvae of *M.yokogawai* **[23]**. Also in the present study, six freshwater fish species including, *W. attu, C. Carpio, N. corica*, *T.pitutora, L.rohita and H. fossilis* were examined and it was reported that almost all

fish species except one fish species, *H.fossilis*, were infected with four different Trematode species which included *B.polymorphus*, *P.maculatus*, *A.danjiangensis*, *and G.kalriai*. Of these Trematode species the highest intensity rate of infection was recorded of *P.maculatus*, which was 11.11%while the lowest intensity rate of infection was recorded of *A.danjiangensis* which was 4.44%.

In one of the other previous study the *P.maculatus*, has recovered from a freshwater fish species, *Labrusmarula* of Mediterranian **[24]**. *P.maculatus* also reported from the intestine of a freshwater fish species, *Labeorohita*, from the freshwaters of Kalri Lake, Sindh, Pakistan **[25]**. The same Trematode species was reported from the intestine of several freshwater fish species (*W.attu, L.rohita and T.pitutora*) in river Kabul Pakistan (with highest 11.11% intensity rate of infection).

Similarly, another Trematode species, *G. kalriai* was reported from two freshwater fish species in Kalri Lake, Sindh **[25]**. The intensity rate of the *G.kalriai* Trematode species was 13%. However, in the present study in which a similar Trematode species was found in three freshwater fish species including, *W.attu, L. rohita and C.carpio*in River Kabul, showed 7.40% intensity rate of infection, smaller than the above described value (13%).The fluctuation in intensity rate of the Trematode species in the present study and the previous studies may be due to the kind of freshwater fish species or due to the ecological influences such as temperature and pollution.

As Trematode parasites exist in the freshwater bodies throughout the globe. In Seri Lanka, in a previous research investigation, the prevalence rate of various Trematodes parasite species in freshwater ornamental fishes reported was 0.5% **[26]**. The present study also revealed the existence of Trematode parasites in freshwater fishes and the prevalence rate reported was 31.85%, which is significantly higher and this variations may be due to the different natural habitations, environmental conditions or may be because of the rate of the industrial pollution that goes into the rivers and inconsequent the fishes get various parasitic infections.

In the present study the infection rate in *C.carpio* fish species, reported was 28.57%. A study carried out in Mazreeh district in Khuzestan province of Iran, also reported the same fish species which shown 56% infection rate **[27]**, showing the difference in the quality of freshwater.

The variation in the intensity rate of various Trematode parasites occur because the aquatic environments are affected by various factors such as host habits, climatic changes, environmental and ecological changes or migration of fish from one place to another, its body size or the total density of aquatic population **[28]**.

Ahmad et al. collected 250 fish samples from River Swat, consisted of six different species and observed overall 58% prevalence rate of various parasites including Trematodes which is very high intensity in comparison to the present study**[29]**. Similarly, Ayaz et al. collected 120 fish samples from river Panjkorha, Lower Dir, and observed overall 41.67% of parasitic infection rate**[30]**. In the present study we collected 135 fish samples from river Kabul and observed 31.85% overall Trematode parasites prevalence rate. The difference may be due to the fact that in the previous two studies, the authors have determined various types of parasites and thus shown a total prevalence rate while in the present study we observed and documented only the Trematode parasites.

In the present study, freshwater fish species were collected from many sites of different natural conditions of the River Kabul of Khyber Pakhtunkhwa, Pakistan, however when the prevalence rate was determined in the total fish samples collected from different sites of the river, was found almost correlated.

CONCLUSION

The present research study concluded that different types of Trematode parasites exist in the freshwater bodies of Khyber Pakhtunkhwa province in Pakistan and preventive measures are necessary against these infections to stop their transmission and to make the freshwater environment parasite free. As freshwater fish of the rivers is a good and cheap source to get protein based food and earns income, thus helping the poor people to make their lives better. But the presence of these parasites in the fish bodies causes damage to their health and decreases its market value as well as also deteriorating the freshwater habitat and transmitting parasitic infections to the human population.Thus it has suggested initiating awareness among the people to stop polluting the freshwater bodies and to adopt the recent methods for the production and preservation of the freshwater fauna and flora.

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