Detection and Prevalence of *Capillaria Pterophylli*, an Endoparasitic Nematode, in *Labeo Rohita* (Rohu), *Cyprinus Carpio* (Common Carp), and *Oreochromis Niloticus* (Nile Tilapia) From the River Indus, Dera Ismail Khan

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DOI: https://doi.org/10.51584/IJRIAS.2025.10040091

Received: 18 April 2025; Accepted: 22 April 2025; Published: 22 May 2025

ABSTRACT

Fish are solely aquatic and cold-blooded vertebrates having a streamlined body with lateral line sense organs. Keeping in view the importance of fish endoparasites, the present study was designed to assess the prevalence of endoparasites Capillaria pterophylli in freshwater fishes of the Indus River at D.I. Khan. Capillaria pterophylli are smooth, cylindrical, large, relatively long roundworms that appear rather frequently in the gut of fish. The samples of fish were collected from December 2023 to April 2024 from the Indus River. In total, 360 fishes were examined for parasites, of which 23 fishes (6.3 % of the total sample) were infected with parasites. Among the fish species, Oreochromis niloticus showed the maximum prevalence of infection, with 7.5%, and Cyprinus carpio with 6.6%. Labeor rohita showed a minimum prevalence of 5%. Among all fishes, the infection of nematode was highest with a percentage of 6.3%. It is found that freshwater fish are highly diverse in terms of endoparasites. In this context, prevention is always the best option for the nematode that infects the gastrointestinal tracts of fish. Since the use of fish as a ready source of food is increasing, the overall health status of fish in any system is becoming more valuable.

Key words: Endoparasites, freshwater fish, Labeo rohita, Oreochromis niloticus, Cyprinus carpio, prevalence

INTRODUCTION

Fish are purely aquatic and cold-blooded vertebrates with streamlined bodies and lateral line sense organs [1]. Fish is a substantial source of vitamins A and D and other vitamins of B-group [2]. There is the presence of at least 193 fish species representing the freshwater fish fauna of Pakistan. These species belong to class Actinopterygii, sub-class Teleostei, 3 cohorts, 6 superorder's, 13 orders, 30 families and 86 genera [3,4].Labeo rohita belongs to the family Cyprinidae, order Cypriniforms, and is commonly known as rui, rohit, rohu. There are 12 species of Labeo, among which only Labeo boggut does not occur in Bangladesh. [5]. Rohu is commonly found in freshwater ponds, lakes, rivers, and streams. Rohu is an important major carp in our aquaculture and a vital source of protein food supply for the people.[6].

The Nile tilapia Oreochromis niloticus reduces local biodiversity by competing with other aquatic species for a vailable food. No hunting and changing environmental conditions affect the fish composition of Nile tilapia [7,8]. Fish are constantly exposed to a wide range of harmful parasites, including protozoans and metazoans. Their physiological characteristics make them perfect hosts for a variety of parasites [9]. Fish are the most important hosts for parasites, particularly helminths among animals. The majority of fishes contain parasites, and they not only serve as hosts for various parasites, but they also act as carriers for a variety of larval parasitic forms that grow and can cause significant diseases in many vertebrates, including humans[10].Nematodes are among the most typical and important infectious parasites of fish found worldwide in freshwater, brackish water, and marine habitats [11].Aquatic parasites nematodes are abundant and diverse in both freshwater and marine

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue IV April 2025

habitats [12].However, their biodiversity is still largely unexplored [13].Nematodes are found all over the world, particularly in species that are using fish as intermediate or temporary hosts. They can infect all organs of their hosts, with predatory fishes experiencing more severe infections.[14]. Capillaria pterophylli is distinguished by smooth, cylindrical, big, and somewhat long roundworms typically seen in fish guts, which are often identified by their double operculated eggs in the female worms. Capillaris pterophylli falls under the following taxonomy: Kingdom: Eukaryota; Phylum: Nematoda; Class: Adenophorea; Subclass: Enoplia; Order: Enoplida; Suborder: Trichinellina; Family: Trichuridae; Genus: Capillaria; Species: Capillaria pterophylli .Capillaria species are relatively translucent, and if only young or male worms are present, they can be readily missed during necropsy. When females with the distinctive barrel-shaped eggs with a polar plug on either end (opercula) or eggs alone are detected in the intestinal tract, capillarid infection can be easily diagnosed. Capillaria species have direct life cycles and can be transmitted from one fish to another through consumption of infected larvae. Capillaria eggs can take up to three weeks at 68-73°F (less time at warmer temperatures) to form embryos that are infectious when consumed by a fish. At these temperatures, it takes around three months from infection until mature adult parasites produce eggs or larvae. Even though Capillaria species have direct life cycles, a tubifex worm can serve as a paratenic (alternative) host, "carrying" infective Capillaria stages to the fish that consumes them. Capillaria species are relatively translucent, and if only juvenile or male nematodes are present, they can easily be overlooked during necropsy. However, Capillarid infection is rather simple to diagnose when females with the unique barrel-shaped eggs with a polar plug on either end (opercula) or eggs alone are observed in the digestive tract.The study aims to identify endoparasites Caprillari pterophylli in the guts of freshwater fish such as Common Carp, Rohu, and Nile Tilpia. Endoparasites Caprillari pterophylli are prevalent in the guts of freshwater fish, including Common Carp, Rohu, and Nile Tilpia. In the Indus River, D I Khan.

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.

Sampling Sites

Fish sampling was carried out in river Indus district. Fish sampling was carried out from December 2023 to April 2024 from River Indus, District D.I Khan, KPK. The fish specimen was collected randomly from the study area with the help of different fish nets such as gill nets, cast nets, drag nets, and hand nets two times a month for four months. Fish were identified through a key and brought to lab for experimental work by Jayaram [15].

A)

Figure 1: Sample Of Fish from River Indus of Nile Tilapia, Common Carp and Rohu are shown in A).

Sample Processing

Total length (TL) and weight of fish were recorded of all species of fish. Small samples were stored directly in 10% formalin, and large samples were injected intraperitoneally and pres erved in 10% formalin and transported to the Fisheries Research Laboratory of the Department of Zoology. Sa

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue IV April 2025

mples taken from each location are packaged in separate plastic boxes according to date, location, time and loc ation. Fish samples were identified with the help of the following publications [16,17,18,15].

Dissection for Parasites

The fish were transferred onto a dissecting board for dissection. To pick up each of the fish in the collection of endoparasites using a single disposable hand glove, they were individually picked up, properly inspected for the presence of any anomaly on its body and then laid down on the dissecting board. The body cavity was slit from the hind region up to the throat with the help of sharp scissors. Great care was taken to separate the guts. The gut was then gently coaxed out into a large Petri dish. Longitudinal openings of the separated sections were done using a scalpel blade to expose the inner surface which was then washed into test tubes containing normal saline. Place a drop of the stain (Giemsa) on the slide and examine under x 10 and x 40 objectives of the light microscope as described [19]. The identification of parasites was performed using standard keys and catalogues [20,21,22,23,24]. Ecological studies have been performed according to Bush et al [25].

Figure 2: Dissection Of Gut of Fish for Parasite

Fecal Material Form Fishes Gut

After the body cavity was opened, the gut was placed in a large Petri dish and got fecal material form gut of fish intestines (common carp, rohu, and Nile tilapia). The fecal material was solved with distilled water for some time until large particles took away easily. Whole feces were collected and mixed thoroughly before analysis. Sampled fecal material was stored at 4°C, within 6 h following collection. The sample analyses were never later than 3 days after the sampling. This fecal solution was then transferred into a breaker for easy handling at a later stage.

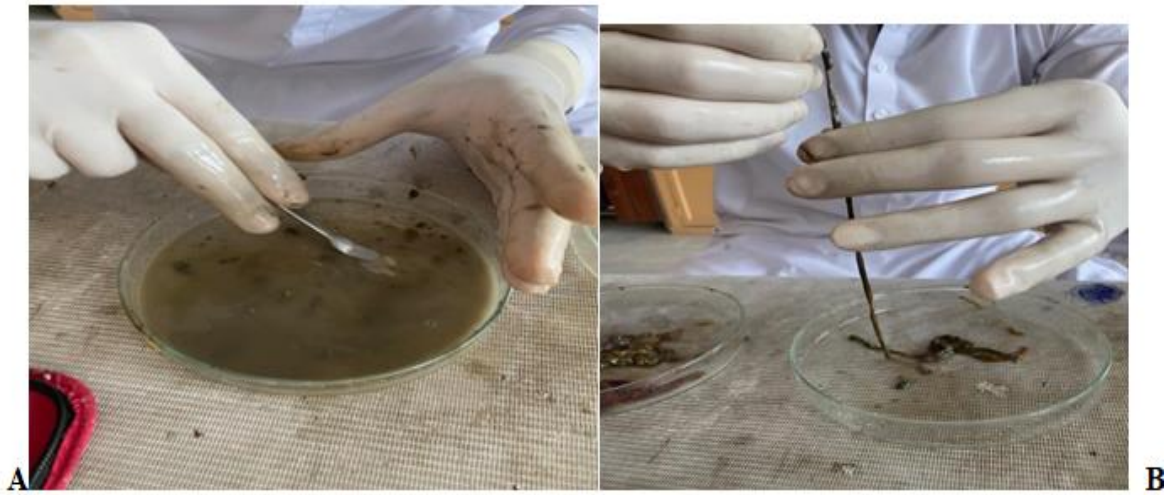

Figure 3: Obtained Fecal Material Form Fishes (A) and Mixing in Distilled Water for Filtration

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Filtration

Using Whatman filter paper, we carried out the filter process by putting the fecal solution with the help of a beaker into a funnel on which a filter paper was adjected. Following some time usually 15 to 25 minutes for a filtration process to be complete, we washed the filter paper to get a sample for centrifugation in 5 percent formalin solution in the bottle.

Centrifugation

The samples were placed in centrifuge tubes or Eppendorf tubes, and with 3 minutes of sedimentation time at the rate of 4000 rpm, supernatant was discarded. After this, with the help of a micropipette, using blue tips, drop pellets liquid onto a plane slide. For staining, slides must be dry.

A) 

B) 

Figure 4: Filtration Process A) Figure 5: Centrifugation Step In Lab In B)

Staining and Microscopy

All slides become dry after the drying process. Further, the slide is stained with Giemsa stain for 2 to 5 minutes for sample to be effectively absorbed by the stain and for easy visualization of eggs or cyst of parasites. The slide was then placed on the stage and examined under x 10 and x 40 objective of the light compuned microscope as described by Goselle et al. 2008. Microscopy of parasites should be examined within 15 minutes of preparing a microscope slide. The microscope must be attached to a digital camera to capture images of cysts or eggs of the parasites by Goselle et al. (2008). The microscope connected to a digital camera for pictures of cysts or eggs of parasites.

A) 

B0 

Figure 6: Staining and Microscope Step for Seeming of Parasites

Analysis of Data

Numbers of fish and parasites caught at the different sampling stations were analyzed using simple percentage according to Marcoglies*et al. [* 25]and Bush *et al.* [26] as follows:

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1) Parasite prevalence (P%) =NO. of infected fishes ×100 ÷ Total number of fish examine
2) Intensity= Number Positive ÷ Number of Infected Host
3) Mean Burden=Total Parasites Found ÷ Total number of fish examine
4) Confidence Interval for Prevalence (Wilson Score Interval).

$$p \approx \hat{p} \pm \frac{z_\alpha}{\sqrt{n}} \sqrt{\hat{p}\,(1-\hat{p})}\,, \qquad \hat{p} \equiv \frac{n_s}{n}$$

5) Binomial Test.

$$\Pr(X = k) = \binom{n}{k} \pi_0^k (1-\pi_0)^{n-k}$$

RESULTS

The present study is based upon checking of the prevalence of Caprillaria pterophylli in freshwater fishes of river Indus collected at D.I. Khan. In the research, a total of 360 numbers of Oreochromis niloticus, Cyprinus Carpio, and Labeo rohita fishes were caught randomly from the study sites, which were examined for the presence of parasites from December 2022 to April 2023. These involve: Oreochromis niloticus, n=120; Cyprinus Carpio, n=120; Labeo rohita, n=120. Different species of fishes have been identified for Caprillaris pterophylli, an endoparasite parasite. Out of the 360, 23 (6.3%) of them were infested with this parasite belonging to nematodes. Capillaria pterophylli is characterized by smooth, cylindrical, large and relatively long roundworms commonly found in the gut of the fish. Among the species, the prevalence of infection was maximum in Oreochromis niloticus (7.5%) and in Cyprinus Carpio (6.6%). The minimum prevalence was, however, recorded in Labeo rohita (5%). In total, 6.4% of fishes were infected, indicating that only 23 fish species were positive for parasites out of the total 360 fishes. These results agree with those reported by R.R. Dewi et al., 2018, Abay H 2018, Francis Sikoki et al., 2013, Hassan Borji et al., 2011, Jayti Upadhyay et al., 2011 and dissimilar to Shafqat Nawaz Qaisrani et al., 2018 and Anthony Ekata Ogbeibu et al., 2014.

Table 1: Overall Prevalence of Capillaria pterophylli Among Oreochromis niloticus,Cyprinus Carpio and Labeo rohita Fishes in Indus River, D. I. Khan.

Freshwater Fish Species	Number Examined	Number Positive	Prevalence (%)	Mean Intensity (Intensity)	Mean Burden (Burden)	95% Confidence Interval (Lower)	95% Confidence Interval (Upper)	Binomial Test p-value
Three Fishes Species	360	23	6.3	1.0	0.064	4.29%	9.40%	0.225

Table 2: Prevalence On Based of Gender Rohu (Labeo Rohita)

Study	**Location**	**Sample Size**	**Average Length (cm)**	**Average Weight (g)**	**Number Positive**	**Prevalence (%)**
Male	**River indus**	60	32.4	1400	4	**3.33**
Female	**River indus**	60	38.4	1500	2	1.66

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Table3: Prevalence of Gender Common Carp (Cyprinus carpio)

Study	Location	Sample Size	Average Length (cm)	Average Weight (g)	Number Positive	Prevalence (%)
male	**River Indus**	60	40.2	1300	5	4.16
Female	**River indus**	60	42.5	1350	3	2.5

Table 4: Prevalence On Based of Gender Nile Tilapia (Oreochromis niloticus)

Study	Location	Sample Size	Average Weight (g)	Average Length (cm)	Number Positive	Prevalence (%)
Male	River indus	60	300	18.5	4	3.33
Female	River indus	60	280	14.5	5	4.16

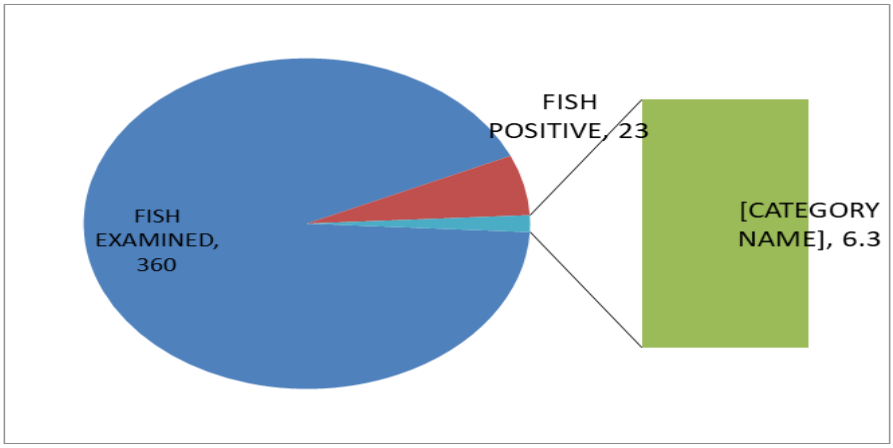

Figure 7: Overall Prevalence of Capillaria pterophylli Among Oreochromis niloticus, Cyprinus Carpio and Labeo rohita Fishes in Indus River, D. I. Khan.

Table 5: Quality of the water of Indus River at that time of experiment.

Species	Sex	Season	Total Fish Examined	Total Infected Fish	Prevalence (%)	Mean Water Temperature (°C)	Mean Dissolved Oxygen (mg/L)
Labeo rohita	Male	Summer	60	3	2.5	28	6
Labeo rohita	Female	Summer	60	1	0.83	28	6
Labeo rohita	Male	Winter	60	1	0.83	15	8
Labeo rohita	Female	Winter	60	1	0.83	15	8
Cyprinus carpio	Male	Summer	60	3	2.5	28	6
Cyprinus carpio	Female	Summer	60	2	1.66	28	6
Cyprinus carpio	Male	Winter	60	2	1.66	15	8

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Cyprinus carpio	Female	Winter	60	1	0.83	15	8
Oreochromis niloticus	Male	Summer	60	2	1.66	28	6
Oreochromis niloticus	Female	Summer	60	3	2.5	28	6
Oreochromis niloticus	Male	Winter	60	2	1.66	15	8
Oreochromis niloticus	Female	Winter	60	2	1.66	15	8

Table 6: Prevalence,Mean Intersity and Mean Burden of Capillaria pterophylli among Oreochromis niloticus, Cyprinus carpio and Labeo rohita Fishes in Indus River, D. I. Khan

Freshwater Fish Species	Location Of Capture	Number Examined	Number Positive	prevalence (%)	Mean Intensity	Mean Burden	95% CI (Lower)	95% CI (Upper)	Binomial p-value
Oreochromis niloticus	**Indus River, D.I. Khan**	**120**	**9**	**7.5**	**1.0**	**0.075**	**0.0399**	**0.1364**	**0.2049**
Cyprinus Carpio	**Indus River, D.I. Khan**	**120**	**8**	**6.6**	**1.0**	**0.0667**	**0.0342**	**0.1261**	**0.3968**
Labeo rohita	**Indus River, D.I. Khan**	**120**	**6**	**5**	**1.0**	**0.0500**	**0.0231**	**0.1048**	**1.0000**
Total		**360**	**23**	**6.3**					

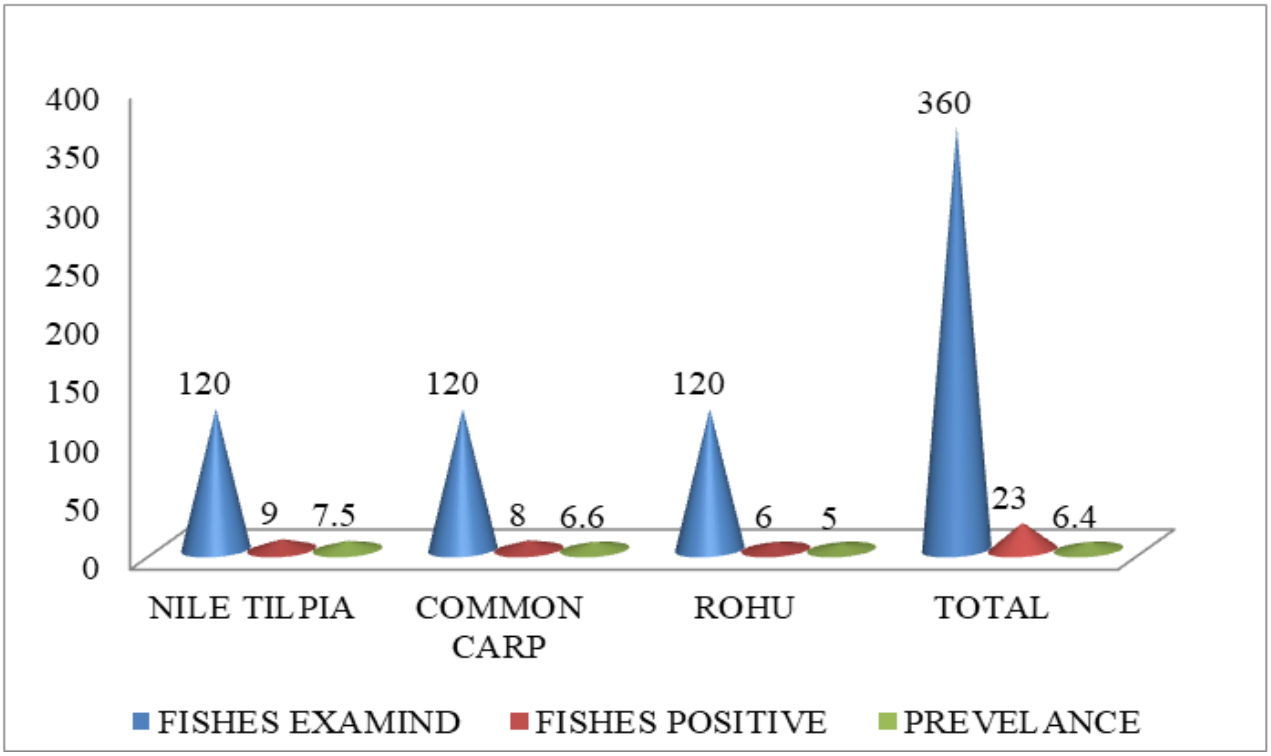

Figure 8: Prevalence of Capillaria pterophylli among Oreochromis niloticus, Cyprinus carpio and Labeo rohita Fishes in Indus River, D. I. Khan

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DISCUSSION

Fish parasitism is one of the serious threats to fish productivity and increasing demand for fish as a ready and safe source of protein for human beings should trigger further studies on fish fauna and their parasites.

Oreochromis niloticus

In the present study, 120 samples of Nile Tilapia were obtained from Indus River, D. I. Khan. These samples were caught with proper care and the prevalence of Capillaria pterophylli was about 7.5%. Nine fishes were infested with these endoparasites which gave higher results among all other species. The reason for the high prevalence rate in Nile tilapia was due to their feeding nature, as they ingest a wide variety of natural food organisms that include plankton, some aquatic macrophytes, planktonic and benthic aquatic invertebrates, larval fish, detritus, and decomposing organicmatter.

Natural feed generally accounts for 30 to 50 percent of tilapia growth during weight gain. Tilapias are sometim es considered filter feeders due to their ability to capture plankton from the water. They naturally live in fresh or slightly brackish water with a temperature range of 3.0°C in temperate climates. Our results also matched with Abay H 2018 and Francis Sikoki et al,2013 with a prevalence of 6.2% and 11% respectively, and opposite to Shafqat Nawaz Qaisrani et al., 2018.

Cyprinus carpio

Sample common carp were 120 from the River of D.I. Khan. Out of 120, 8 samples were positive with Capillaria pterophylli, an endoparasites of Nematodes. The prevalence was 6.6 % in common carp that was lower than niletilpia. Hassan Borji, et al. 2012.

The main difference in expansion is due to fish consumption and some environmental problems. They normall y live in warm climates, in fresh or polluted waters with a pH of 6.5-
9.0, a salinity of up to 0.5% and a temperature of 3 to 35 °C. They can feed on herbivorous water plants, but they generally prefer to scavenge at the bottom for insects, crustaceans, including zooplankton, crawfish, and benthic worms. Their findings are in line with Hassan Borji et al. 2011, and below Anthony EkataOgbeibu et al., 2014.

Labeo rohita

The sample size of Rohu was equal to other fish species in this study. In 120 fishes, only six were infested with endo-parasites that lead to the lowest prevalence rate of parasites. The maximum parasitic load recorded in Rohu—only 5%. In Rohu, the rate of endo-parasite was low due to living habitat and feeding behavior of the fish. Three. Labeo rohita does not thrive at temperatures below 14°C. Most studies agree that rotiferis in nature have a partial preference for zooplankton, consisting mostly of rotifers and cladocerens, in the early st ages of their lives, and that phytoplankton is their food. Rice is important. Niche distribution of L. rohita durin g growth. Its total length at finger level is 0-20 cm. South Asian wildlife preys exclusively on zooplankton. At >20 cm in total length, it ingests both zooplankton and phytoplankton, while at the matured adult stage, the fish ingests exclusively on phytoplankton. Rohu is an omnivore, column feeder fish and is used in composite fish culture. It shows the presence of plant materials in digestive tract analysis. The results are up to JaytiUpadhyay et al., 2011, and opposites to Shafqat Nawaz Qaisrani et al., 2018.

CONCLUSION

Fish are strictly aquatic, coldblooded vertebrates with streamlined bodies and lateral body lines. Research sho ws that there are many types of parasites in freshwater fish. Therefore, taking precautions against nematodes th at infect the intestinal system of fish is always better than treating them. As the use of fish as readymade food i ncreases, the overall health of fish in the body becomes more beneficial. Fish parasitism poses a threat to fish p roduction, and the increasing demand for fish as a readily available and safe source of protein for humans shou ld lead to further research on fish fauna and worms.

ACKNOWLEDGMENTS

First and foremost, I would like to sincerely thank my supervisor Assistant Professor **INAYAT UR REHMAN** for his guidance, understanding, patience and most importantly, he has provided positive encouragement and a warm spirit to finish this, Paper. It has been a great pleasure and honour to have him as my supervisor.

I would sincerely like to thank **Muhammad Hasnain** to support me through thick and thin.

RECOMMENDATIONS

- Sample size should be increased up to 800 or plus.
- PCR should be used to work at a molecular level.
- Proper management should be carried out to capture fish at the spot.
- Proper protocol should be followed because endoparasites can be harmful to you.

Water bodies should be increased to overcome biasness

Conflict of interest declaration: *There* is no conflict of interest or otherwise

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