EPIDEMIOLOGICAL STUDIES OF NEMATODES IN FISHES

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ABSTRACT: Three hundred fresh water fishes of six species were collected from six different fish farms of Lahore for the prevalence of nematodes. Out of 300 fishes examined, 12 were found to be infected with the helminthes, majority of them were isolated from the stomach and intestines. The following two species of nematodes were recorded; *Capillaria spp.* and *Eustrongylides spp.* The overall prevalence of intestinal nematodes was recorded as 4%(12/300). The prevalence of nematodes was recorded on monthly basis which ranged from 0-8%. The highest prevalence of nematodes was 8% (4/50) during March, while the lowest prevalence was noted in June 0%.Singharee (*Sperata sawari*) showed the maximum infestation of nematodes of 8% (4/50), whereas in Silver Carp (*Hypopthaimichthys molitrix*) minimum prevalence of nematode (0%) was noted. The prevalence of different nematode in a particular fish specie was also recorded, and it was stated that overall prevalence of *capillaria spp.* was 6% in Rahu (*Labeo rohita*) and Saul (*Channa marullius*). Similarly overall infestation of *Eustrongylides sp.* was recorded as 4% in Singharee (*Sperata sawari*) and Silver carp (*Hypopthaimichthys molitrix*). The nematode intensity might be linked with the genetic makeup, intestinal vigor, and other managemental and environmental factors.

Key words: Epidemiology; *Labeo rohita* (Rahu), *Sperata sawari*, (Singharee), *Channa marullius* (Saul); *Ctenopharyngodon idella* (Grass Carp), *Oncorhynchus mykiss* (Rainbow Trout); *Hypopthaimichthys molitrix* (Silver Carp)

INTRODUCTION

One of the most harmful worm parasites which are frequently regarded to affect fish are nematodes. By serious mechanical harm from excessive movement which puts a strain on the host, they can kill the host fish (Moravec, 1994; Woo, 1995 Niaz*et al.*, 2010 and Pazooki*et al.*, 2012). Including oxygen, enzymes, nutrients and even developmental stimulus, in fish nematodes, for various requirements are metabolically dependent on the host. By outright ingesting undigested food, tissue exudates into the gut, disintegrated tissues and enzymatic fluids from food material in the lumen of the digestive tract, they deprive the host fish of nutrients and cause diseases in fishes (Kaddumukasa *et al.*, 2006).

Nematodes, or roundworms, infect many species of aquaculture and wild fish. Small numbers of nematodes often occur in healthy fish, but high numbers cause illness or even death. In aquaculture systems, brood stock infected with a small number of nematodes may not even show signs of illness, but they often have reduced reproductive capacity. On the other hand, juvenile fish infected by small numbers of nematodes are more likely to show signs of illness and also have reduced growth rate. In aquaculture situations, fish become infected with nematodes if they are fed on live foods containing infective life stages or if they are raised in culture settings that promote the growth of other animals that carry the infective stages of the nematode (vector or paratenic host) or allow nematodes to complete their life cycle (intermediate hosts). Some nematodes can be transmitted directly from fish to fish. Adult nematodes are typically found in fish digestive tracts. However, depending upon the species of nematode and the species of infected fish, adult and other life stages of nematodes can be found in almost any part of the fish, including the coelomic (body) cavity, internal organs, the swim bladder, deeper layers of the skin or fins, and external muscle layers (Yanong, 2011).

This study is aimed at establishing the magnitude of prevalence of parasites in different fish species. Parasites are a natural occurrence, not contamination. They are as common in fish as insects are in fruits and vegetables. They become a concern when consumers eat raw or lightly preserved fish. They cause little apparent damage in feral fish populations, but can become causative agents of diseases of great importance in farmed fish, leading to pathological changes in the fishes or cause decrease in fitness or reduction in the market value of fish. Despite considerable progress in fish parasitology in the last few decades, major gaps still exist in the knowledge of taxonomy and biology.

The present study was designed to study the prevalence and identification different species of nematodes form different locations.

MATERIALS ANDMETHODS

Duration of study: The study of epidemiology of nematodes in fishes was conducted for a period of six months from January 2012 to June 2012.

Sample size: To study the nematode infestation 300 fish samples were collected on monthly basis over throughout the conduct of research.

Sampling location: The samples were collected from six different freshwater fish farms of Punjab. These farms were located in and around district Lahore. The selected farms were Military Fish Farm No.1, Military Fish Farm No. 2, Military Fish Farm No. 4, Military Fish Farm No.10, Minar Pakisatn Fish Jheel and Muhammad Javed Fish Farm.

Fish collection method: Fishes were collected every other alternative day from the fish ponds by using fishing nets. Total fifty fishes were collected from the each farm. The collected specimens were put in seal bags and labeled with the necessary information (species, weight, and age) and were brought in an ice box to the GC University Department of Zoology for further processing.

Fish identification: A total of six fish species were sampled and identification of each fish was done by following the keys described by Jayaram (2010).

Clinical examination: The length and weight of each fish was measured. The external body surface was examined through magnifying glass for the ecto-parasites. For internal examination the fish was dissected and opened. The internal organs like intestines were cut and placed separately in a petri dishes. The intestines were then cut and inner lumen was exposed. Rafique*et al.*,2003; Genc, *et al.,* 2005; and Binky*et al.*,2011.

Parasitological examination: The detection of nematode egg was done by the using direct wet mount fecal technique (Johannes, 1996). For direct fecal smear, a small quantity of fecal material from the distal end of the gut was placed on a slide, mixed with water droplet and a cover slip was placed on the fluid. Then, the slide was thoroughly examined under microscope (Hendrix, 2006).

Identification of parasites egg: All nematode eggs were identified based on their morphological characteristic described by Thienpoint *et al.* 1998 and Taylor *et al.* 2007.

RESULTS AND DISCUSSION

Identification of the fish: The six fish species were identified as; *Labeo rohita* (Rahu); *Sperata sawari* (Singharee); *Channa marullius* (Saul); *Ctenopharyngodon idella* (Grass carp); *Oncorhynchus mykiss* (Rainbow Trout) and *Hypopthaimichthys molitrix* (Silver Carp). Out of 300 fishes examined, 12 were found to be infected with the helminthes, majority were isolated from the stomach and intestines. The species of nematodes recorded were *Capillariaspp.* and *Eustrongylides spp.* The overall prevalence of intestinal nematodes was recorded as4% (12/300). During the study period, most frequently observed nematode parasite specie in fish intestine was *Capillaria spp.* The point prevalence was calculated by the following formula:

$$\text{Point Prevalence \%} = \frac{\text{No. of existing cases during specified time period}}{\text{Population at risk during specific time}} \times 100$$

Month wise prevalence of nematodes: The monthly prevalence of nematodes was recorded and is shown in Table 1. The range of monthly incidence was 0-8%. The highest prevalence of nematodes was recorded during March 8% (4/50), while the lowest prevalence was noted in June 0% (0/50).

Fish species wise prevalence of nematodes: The fish species wise prevalence of the nematode was recorded and shown Fig-1**.** The range of incidence of the nematodes in various fish species was 0-8%. *Speratasawari* showed the maximum infestation of nematodes of 8% (4/50), whereas *Ctenopharyngodon idella* and *Hypopthaimichthys molitrix* showed no nematode infestations.

Nematode species prevalence among different fishspecies: Fish wise prevalence of different nematode species was recorded and shown in the Table- 4.3. The prevalence of *capillaria sp.* nematode was 3.33% (10/300) whereas *Labeo rohita* and *Channa marullius* showed maximum infestation of the 6% (3/50). Similarly infestation of *Eustrongylides sp.* was recorded as 1.33% (4/300) and infestation was showed by the *Sperata sawari* and *Hypopthaimichthys molitrix*.

Nematode species infestation prevalence: 10 samples out of 300 showed infestation with the Capillaria spp. which give a prevalence of 3.33% whereas the point prevalence of Eustrongylides is 1.33% and shown in Table 1 and Fig 1.

Table-1 Showing Prevalence of Nematodes in relation to month, fish species and Nematode species.

Prevalence	Total samples collected	Total samples collected	Positive samples	Prevalance (%)
Month Wise	January	50	2	4
	Feburary	50	2	4
	March	50	4	8
	April	50	3	6
	May	50	1	2
	June	50	0	0
Fish Species Wise	Labeo rohita(Rahu)	50	3	6
	Sperata sawari (Singharee)	50	4	8
	Channa marullius (Saul)	50	3	6
	Ctenopharyngodon idella (Grass Carp)	50	0	0
	Oncorhynchus mykiss (Rainbow Trout)	50	2	4
	Hypopthaimichthys molitrix (Silver Carp)	50	0	0
Parasite Species wise	Capillaria	300	10	3.33
	Eustrongylides	300	4	1.33

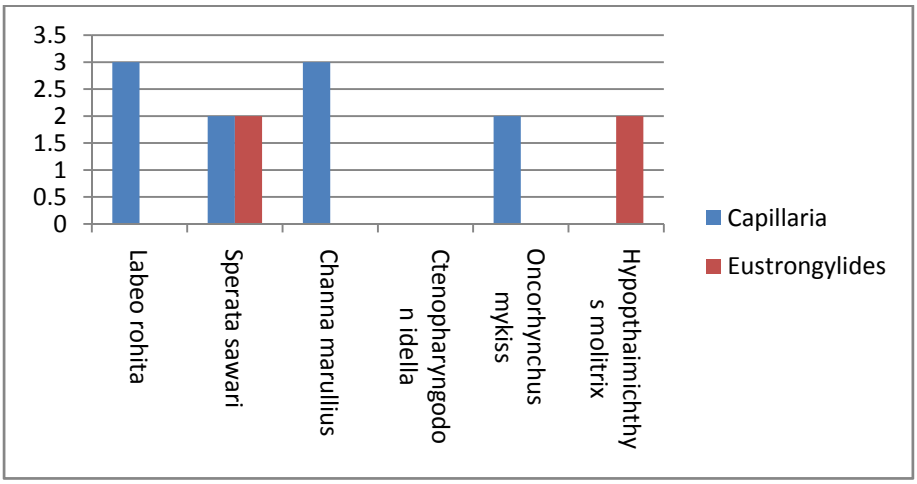

Figure 1: Comparison of two nematodes prevalence in different fish species.

The results of present study revealed that different fishes examined showed 4% (12/300) nematodes infestation. The value of our results agreed with the results of nematode prevalence as documented by (Rafique *et al.,* 2003) and Ahmed e*t al.,* 2007. However, some other researchers reported 25.3% and 33.3% of helminthes infestation (Genc*et al.,* 2005; Bichi, 2006). The low infection rate of 4% in the present study indicated that life cycle was not continuous, which means factor for the spread of this nematode was not appropriate. Leningrad, 1999 pointed out that an increase in temperature to certain limits accelerate the fission and larval development of intermediate stages of helminthes parasites. The increase in temperature probably caused the eggs degeneration, destroyed many parasites before becoming adults.

Large body size, long life span and slow reproduction may be a cause of high infestation (Morris*et al.,* 2000). The study of (Amundsen *et al*. 2003) indicated that parasite carrying capacity might be higher in predator hosts. Environmental conditions may also contribute to the parasite infestation. (Genc *et el.,* 2005) documented that warm water conditions resulted in an unusually heavy infestation of philometrid nematodes which can function as a threat to the serranid fish population as these parasitic nematodes decreased the spawning capacity of serranids. (Khan *et al.,* 2008) reported richness of and overall status of native fishes along with exotic fishes and our results are comparable to their studies.

In the present study the prevalence of *capillaria spp.* infestation was 3.33% (10/300). The highest prevalence of infestation was reported by the *Labeo rohita* and *Channa marullius* (6%). The present study result for *capillaria spp.* infestation disagreed with results documented by (Binky *et al.*, 2011).*Capillaria* females were easily identified carrying the brown, barrel-shaped eggs with a plug-like structure on each end. *Capillaria* species have direct life cycles, and can spread from one fish to another by ingestion of infective larvae. It may take *Capillariapterophylli* eggs up to three weeks at 68-73°F (less time at warmer temperatures) before embryos developed enough to be infective when ingested by a fish. The length of time required from infection until the mature adult parasites are producing eggs or larvae was approximately three months at these temperatures. Even though *Capillaria* species have direct life cycles, a tubifex worm may act as a paratenic (alternative) host and "carry" infective stages of *Capillaria* to the fish that consumed them.

The present study reported that 1.3% prevalence of fish nematodes namely *eustrongylides spp.Speratasawari* and *Hypopthaimichthys molitrix*. *Eustrongylides* species were found in muscle, free, within the body cavity, or encapsulated on the liver and other organs. *Eustrongylides* species have complex, indirect life cycles. Adult *Eustrongylides tubifex* andother Eustrongylides nematodes are found in fish-eating birds. The eggs were shed by the birds into ponds, where they developed into a life stage that was consumed by an oligochaete worm, such as the tubifex worms. Within these tubifex worms, the nematodes develop still further into a third larval stage, known as an "L3", which is the life stage of the *Eustrongylides* group that could infect fish when eaten. *Eustrongylides* were typically very long, coiled and red due to presence of hemoglobin and an infected fish often has more than one nematode in its body cavity. The highest nematode prevalence was observed as 8%.The nematode intensity might be linked with the genetic makeup, intestinal vigor, and other managemental and environmental factors.

In relation to the seasonal variation, March and April had the greatest infestation records. These results agreed with that recorded by (Nadia, 1991). These results may be attributed to the different types of fishes, the presence of intermediate hosts and the suitable temperature for the survival of these intermediate hosts.

Other helminth parasites such as trematodes, cestodes and acanthocephalans were not found in these fish species. The intermediate hosts for these parasites were probably not being used as food for these species. Moreover, water birds (Piscivorus birds), which are very important factor for the spread of the trematodes, cestodes and acanthocephalans were not seen visiting this pond. It indicated that the dispersal of parasites and completion of their life cycle was not adequate resulting into disappearance from these fishes (Rafique*et al.,* 2003).

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