

FOOD BORNE PARASITIC ZOONOSIS WITH SPECIAL REFERENCE TO METACERCARIAL INFECTION IN FISHES

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ABSTRACT

The study was conducted to evaluate the prevalence of food borne trematode infections in commercially important fish species of Pakistan such as, *Labeo rohita* (rohu), *Cirrhinus mrigala* (mori), *Catla catla* (thaila), *Hypophthalmichthys molitrix* (silver carp). For this purpose 222 different samples of fishes were collected from different water bodies i.e. Rawal Dam, Islamabad, Mangla Dam (Mirpur) and local fish markets, Lahore. The detection of infectious metacercariae of *Opisthorchis*, *Clonorchis* and *Heterophyid* were done by pepsin digestion technique. Overall infection rate was recorded as 28.37%. Species wise prevalence indicated that *Catla catla* and *Labeo rohita* were more frequently affected, whereas the lowest (23.5%) prevalence was reported in *Hypophthalmichthys molitrix*.

Keywords: Indian major carps, Trematode, Prevalence, Intensity.

INTRODUCTION

Food borne parasitic infections have been recently identified as an important public health problem having considerable economic impact in terms of morbidity, loss of productivity and health care costs. Poor sanitation and traditional methods of food preparation accelerated the spread of food borne trematode infection (Phan *et al.* 2010). Fish serve as second intermediate host of food borne trematode infections and serve as direct source of infection in humans (Nguyen *et al.* 2009; Phan *et al.* 2010). It has been estimated that over 40 million people throughout the world are infected with food borne trematodes (Chi *et al.* 2008; Keiser and Utzinger 2009). Majority of the food borne trematodal diseases results from the consumption of raw or undercooked fish containing infective metacercariae (WHO 2004; Chi *et al.* 2008). Food borne trematodes may affect liver, lungs, small intestine and other organs producing disease ranging in severity from mild to debilitating (Nguyen *et al.* 2009; Phan *et al.* 2010).

Trematodes are responsible for a variety of parasitic diseases, some of which are transmitted through fish and shellfish. The most important parasites transmitted by freshwater fish and shellfish belong to the genera *Clonorchis*, *Opisthorchis*, and *Paragonimus*. They all have similar life cycles involving a definitive host (snail) and two intermediate hosts (fish and shellfish). Man and animals are infected by eating raw or minimally processed, fish or shellfish (Nguyen *et al.* 2009; Phan *et al.* 2010).

Keeping in view the importance of these parasites the present study was therefore designed to study food borne parasitic zoonosis and to know about

commonly used food fishes serving as intermediate host of intermediate infection and to find out what is the prevalence of infection and number of metacercariae per gram of infected fish flesh and intensity of metacercariae infection.

MATERIALS AND METHODS

The study was conducted at postgraduate laboratory of the Department of Biology, Quaid-e-Azam University, Islamabad and postgraduate laboratory Department of Parasitology, University of Veterinary and Animal Sciences, Lahore.

Collection sites: Two hundred and twenty two samples (*Labeo rohita* (rohu) 66; *Cirrhinus mrigala* (mori) 58; *Catla catla* (thalia) 56; *Hypophthalmichthys molitrix* (silver carp) 42), were collected from Rawal Dam, Mangla Dam and local fish market. The detection of metacercariae of *Opisthorchis*/ *Clonorchis* and *Heterophyids* was made by pepsin digestion technique (Jackson *et al.* 1981; Qureshi *et al.* 2006). Parasites were isolated from the samples recorded as either live or dead or fragments. Live or frozen worms were fixed in glacial acetic acid and then preserved in small glass containing 70 percent ethanol.

Parasite identification: The metacercariae was identified by using standard keys. Xylene was used as clearing agent for microscopic examination of metacercariae. The recorded metacercariae were fixed and stained with carmine. Metacercariae of various trematodes were identified on the basis of their morphology (Hoffman 1977; Ditrich *et al.* 1992). About 200 gm of fish tissue was added to 750 ml pepsin solution in large beaker at 37°C. The beaker was placed at 37°C shaking water bath

so that level of water was within 1 cm of the fluid level in the beaker. The sample was shaken at low speed for about 15 minutes. The sample was then adjusted to pH 2 with 6 N HCL. The beaker was then covered with aluminium foil and continues shaking for 24 hours until the tissue was fully digested. Then poured the digested material through a sieve No. 18 into a suitable container, rinsed the sieve with normal saline and examined the washed remained by immersing the mash in clean normal saline. Forceps were used to transfer worm to a culture dish containing normal saline. Examined the material which passed through the sieve by transferring it to a clumped funnel allowed to settle for one hour and drained the sediment into a beaker. Then examined the worm using stereoscopic (120X) or magnifier. Alternatively, digested material was passes through nested No. 18 and No. 140 US standard sieve, which were then separated and the screened portion of the sieve, emerged in normal saline. All the collected parasites were transferred to a dish of normal saline, any of the larger worms caught in No. 18 sieve. Similarly transferred smaller worms caught in No. 140 sieve to normal saline.

RESULTS AND DISCUSSION

Out of these 222 fishes i.e. *Labeo rohita* (rohu), *Cirrhinus mrigala* (mori), *Catla catla* (thaila), *Hypophthalmichthys molitrix* (silver carp), 63 were found positive for various trematodal infections (Table 1). The prevalence was observed 28.37% (Table 1), similar result was reported by Razo and Leon (2001); Sandland and Goater (2001); Nithiuthal *et al.* (2002); Dzikowski *et al.* (2003); Chai *et al.* (2004); and Ondrackova (2004). It was observed that fish belonging to *Labeo rohita* (rohu) and *Catla catla* (thaila) were more commonly affected than others which was also reported by Mirza and Shaifq (2004). It was evident from the present study that in *Labeo rohita*, the prevalence of *opisthorchis* was 9.09% followed by *heterophyids* parasites 7.57% and lowest 6.06% for *clonorchis* trematode (Table 1). In *Cirrhinus mrigala*, the prevalence of both *opisthorchis* and *clonorchis* trematode parasite was 8.62% and 8.62% and *heterophyids* was 3.44% (Table 1). In *Catla catla*, the prevalence of *opisthorchis* and *heterophyids* was 8.92% and 8.92%, respectively whereas *clonorchis* parasite was 7.14% (Table 1). In *Hypophthalmichthys molitrix* (silver carp) the prevalence of *opisthorchis* was 9.52%, *clonorchis* and *heterophyid* were 4.76% and 2.38%, respectively (Table 1).

The trematodal infection was observed in all the four commercially important fish species during current study. The percentage of infection of different trematodes like *Opisthorchis*, *Clonorchis*, *Heterophyids* was observed both individually and as mixed infection. The

majority of trematodal infection occurs endemically in some countries of Eastern Asia, South America, Eastern Europe and West Africa and derives mostly from *Heterophyes* spp., *Metagonimus*, spp. *clonorchis sinensis*, *Echinostoma* spp. and *N. Salmincola*, could become a public health concern (Chi *et al.* 2008). The *Clonorchiasis opisthorchiasis* is an important zoonotic infection of man causing gastro intestinal disturbances, heavy infections and cause recurrent attacks of cholangitis, pancreatitis, etc. It is also considered an important risk factor for primary adenocarcinomes of liver in man. The intestinal flukes causing inflammation, ulceration, haemorrhage, persistent diarrhea and other enteropathic conditions leads to malabsorption and protein loss. Human pathogenic parasites occur in several species of fish that may be cold-smoke, including gadoids, salmonids, grouper, halibut, herring, mackerel, mullet, sablefish, small tunas and turbot (Mirza and Shafiq 2004; Kumchoo *et al.* 2005). However, no information is available on species of food fishes that are infected with trematodes zoonotic importance and the prevalence of infection in these species. The reason that fish-borne trematodes are problem in some countries comes from the tradition of eating raw or uncooked (typically fermented) fish products. These are very popular in South East Asia and are a traditional food in rural areas. Properly cooked fish poses no risk, but the encysted flukes are quite durable, rendering to remain viable in fresh fish and surviving the salty, acid environment of fermented fish for many days. Deep freezing leads to a rapid loss of viability but is rarely used in rural areas. Changes in irrigation and particularly the development of large-scale irrigation systems are associated with increasing risk of infection, probably due to the ideal habitat they form for snails. Sewage-fed aquaculture also has a role as it provides the perfect opportunity for completing the life cycle, especially where latrines are placed over ponds. In rather odd twist, the increasingly wealthy urban consumers are now more at risk from fish-borne trematodes due to the increasing trend to eat raw fish. Thus, the problem is moving from the rural areas into the cities.

In Pakistan people do not use raw or undercooked fish, so do not get infection with metacercarial trematodes but in some countries like Thailand, Korea, Germany, China and Canada, etc., where people used raw fish and problem of trematodal infection is very high (Chai *et al.* 2004; Lin *et al.* 2001; Kumchoo *et al.* 2005).

In conclusion it was evident that fishes belonging to *Labeo rohita* (Rohu) are more commonly affected with metacercarial infection where as the lowest prevalence was reported in *Hypophthalmichthys molitrix* (silver carp).

Table 1. Comparative prevalence of various parasitic infections in four species of fish

Species of fish	No. of fish Examined	<i>Opisthorchis</i>	<i>Clonorchis</i>	<i>Heterophyids</i>	Mixed infection	Over all Infection
<i>Labeo rohita</i> (rohu)	66	6 9.09%	4 6.06%	5 7.57%	5 7.57%	20 30.30%
<i>Cirrhinus mrigala</i> (mori)	58	5 8.62%	5 8.62%	2 3.44%	4 6.89%	16 27.58%
<i>Catla catla</i> (thaila)	56	5 8.92%	4 7.14%	5 8.92%	3 5.35%	17 30.55%
<i>Hypophthalmichthys molitrix</i> (silver carp)	42	4 9.52%	2 4.76%	2 2.38%	2 2.38%	10 23.50%

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