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Research article

Infection indices of *Camallanus cotti* Fujita, 1927 in *Tor putitora* in Mahakali River, Nepal

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Abstract

Red worm (*Camallanus cotti* Fujita, 1927) is an intestinal parasite both in wild and cultured fish all over the world. This study aimed to investigate the prevalence and mean intensity of red worms in Mahseer *(Tor putitora)* in Mahakali River, Nepal. The study was conducted from October 2021 to February 2023. Mahseer fish (n=211) were collected from two sites of the river using gill nets and also purchased from the local markets of respective sites assuring the fish were from the Mahakali River. Parasites were recovered and processed using standard helminthological methods. Overall, the prevalence and mean intensity of the red worms in Mahseer were 3.79% and 1.37, respectively. The highest prevalence was found in more than 0.2 m long (4.38%), 0.051–0.1 kg weighted (6.42%) female host fish (4.32%) in March (22.22%) and spring seasons (5.26%). Similarly, the highest intensity was found in 0.1–0.2 m long (2), 0.051–0.1 kg weighted (1.42) female host fish (1.37) in January (4) and winter season (2). Statistically, the variation in prevalence and intensity of the parasite were insignificant (p> 0.05). This study reveals low infection of the red worm in Mahseer but increases the risk of dissemination of parasites from the Mahakali River to nearby fish farms.

Keywords: Mahseer *(Tor putitora)*, Mean intensity, Prevalence, Red worm (*Camallanus cotti*)

1 | Introduction

Red worm (*Camallanus cotti* Fujita, 1927), an intestinal nematode (Wu et al. 2007), parasitizes more than 58 tropical freshwater fish species (Moravec & Justine 2006), both in natural water and aquarium culture (Rigby et al. 1997). This nematode causes haemorrhage, congestion, oedema and extensive erosion in the rectal mucosa with marked thickening of the wall in cultured ornamental host fish (Menezes et al. 2006) and thus has significant economic importance in fisheries (Košuthová et al. 2015). Mahseer (*Tor putitora*), a dominant fish species in the Mahakali River (Joshi & Joshi 2020), has high demand in the local markets (Nautiyal 2014).

Several studies have shown that fish of natural water bodies acquire more parasitic infections than those from culture systems (Nnadi et al. 2011) but information is lacking regarding the parasitic indices of red worms and other parasites in Mahseer of Mahakali River. So, the present study was conducted to determine the prevalence and mean intensity of red worms (*Camallanus cotti*) in Mahseer (*Tor putitora*) in Mahakali River, Nepal. This study will become the basis for further investigations of fish parasites in the river and adjoining rivulets in future.

2 | Materials and methods

2.1 | Fish samplings and measurements

Mahseer fish (n=211), identified as described by (Jayaram 2010), were collected month-wise randomly from two sites: Khalla (29°63′17.28″N 80°89′17.16″E), Brahmdev (29°37′49.8″N 80°77′26.16″E) and Bhujela (28°58′-45.44″N 80°69′48.96″E), Mahakali Suspension Bridge (28°55′35.11″N 80°65′25.8″E) of Mahakali River, Nepal between October 2021 to February 2023 using gill nets with the help of local fishermen. The fish were also purchased assuring they were from the Mahakali River from local markets.

The collected fish specimens were brought to the laboratory of the Department of General Science, Far Western University, Nepal for further processing. Their morphometric measurements were made using a metal ruler calibrated in centimeters/inches and an electronic weighing balance. Sexes were determined by visual and microscopical examination of the gonads after dissection.

The water temperature of the fish sample collected sites was recorded once a month between 8–10 AM throughout the study period with the help of a mercury thermometer calibrated in degrees Celsius (˚C) to support the results.

2.2 | Examination and recovery of parasites

After morphometric measurements skin, fins, gills, eyes and buccal cavity of the fish were observed for the presence of parasites. Fish were dissected through the ventral surface of the abdomen using a surgical blade. The alimentary tract was isolated, removed from the body, put in a Petri dish and cut into parts (stomach and intestine). These sections were placed into two separate Petri dishes, split longitudinally, washed with physiological saline solution (0.9% NaCl solution) and examined thoroughly first under a magnifying glass and then binocular microscope (model: Tech+) for the presence of parasites. Further, the surfaces of the internal organs (heart, liver, gall bladder, spleen, swim bladder, kidneys and gonads) and body cavities and mesenteries were also examined under physiological saline solution with the help of magnifying glass for the presence of parasites. The recovered parasites were processed according to Thatcher (2006) for identification. The photographs of the parasites were taken using a Samsung Galaxy S9 (SM-G960N) camera under 100× magnification in a binocular microscope. The collected specimens are stored at the Department of General Science Museum of Far Western University.

2.3 | Identification of parasites

The parasites were identified based on number of longitudinal ridges in buccal capsule, amphids, cephalic papillae, sublateral sclerotic plates and basal ring; prongs of tridents; size of muscular and glandular oesophagus; position of dierids, nerve ring, excretory pore and vulva; length of tail and its caudal mucrones; striations of cuticle and body size as described by Rigby et al. (1997), Menezes et al. (2006), Moravec and Justine (2006) and Thatcher (2006).

2.4 | Data analysis

The prevalence and Mean Intensity of the red worms were determined by following Margolis et al. (1982) and Bush et al. (1997). Fisher's Exact Test was computed using IBM SPSS Statistics Version 25.0 (IBM Corp 2017) to analyze the significant differences ($p \leq 0.05$) in the prevalence of the nematode with body size, weight and sex of host fish, month and season of the year and sites of the river. Measurements were converted into the international system of units. Data, collected from October 2021 to February 2023, were amalgamated monthly during analysis to express the valid results in terms of months and seasons.

3 | Results

A total of eight Mahseer fish were found infected by red worm nematodes (Fig. 1). Eleven nematodes were recovered from infected fish. Overall, the prevalence was 3.79% and the mean intensity was 1.37 (Table 1)).

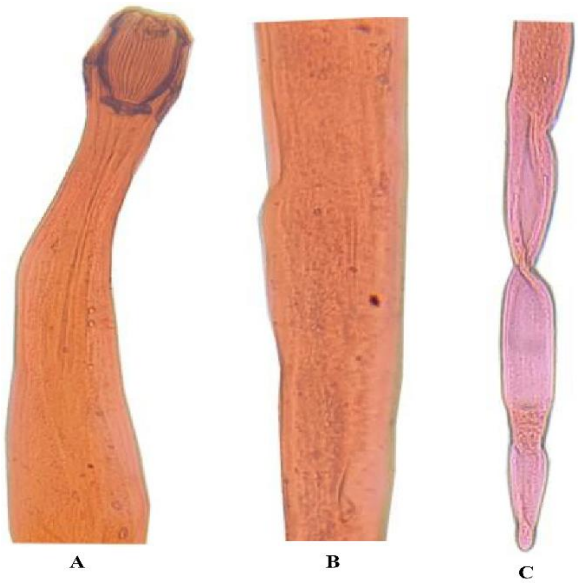

Figure 1. *Camallanus cotti* (×100). A. Anterior end, B. Middle part, C. Posterior end

3.1 | Host size-wise prevalence and mean intensity

Table 1. Host size-wise prevalence and mean intensity of *Camallanus cotti*

Size of the fish (m)	Number of fish examined	Number of infected fish	Number of parasites recovered	Prevalence (%)	Mean intensity
Less than 0.1	4	-	-	-	-
0.1–0.2	93	3	6	3.22	2
More than 0.2	114	5	5	4.38	1
Total	211	8	11	3.79	1.37

Table 2. Host weight-wise prevalence and mean intensity of *Camallanus cotti*

Weight of the examined fish (kg)	Number of fish examined	Number of infected fish	Number of parasites recovered	Prevalence (%)	Mean intensity
Up to 0.05	35	-	-	0	-
0.05–0.1	109	7	10	6.42	1.42
0.10–0.15	44	1	1	2.27	1
0.15–0.2	12	-	-	-	-
0.20–0.25	6	-	-	-	-
More than 0.25	5	-	-	-	-

Infection of the red worms was not observed in small-sized fish. The prevalence was highest (4.38%) in more than 0.2 m sized fish followed by (3.22%) in 0.1-0.2 m sized fish. But, the mean intensity of the nematode was higher (2) in 0.1–0.2 m sized fish than in more than 0.2 m sized fish (1) (Table 1). Statistically, the prevalence was insignificant with the size of the host fish ($p>0.05$).

3.2 | Host weight-wise prevalence and mean intensity

Prevalence and mean intensity of the red worms were highest (6.42%, 1.42) in 0.051–0.1 kg weighted fish followed by (2.27%, 1) in 0.101–0.15 kg weighted fish (Table 2). Statistically, the prevalence was insignificant with the weight of the host fish ($p>0.05$).

3.3 | Host sex and month-wise prevalence and mean intensity

Infection of the *Camallanus cotti* was not observed in male fish and year-round. The prevalence of the nematode was highest in female fish (4.32%) in March (22.22%) and lowest in October (3.84%) but the mean intensity was higher in female fish in January (4) than in other months (Table 3). Statistically, the prevalence of the parasite was insignificant with host sexes and months ($p>0.05$).

3.4 | Seasonal prevalence and mean intensity

Infection of the *Camallanus cotti* was recorded in all seasons. The prevalence of the nematode was highest (5.26%) in the spring season but the mean intensity was (2) in the winter season (Table 4). Statistically, the prevalence was insignificant with seasons ($p>0.05$).

3.5 | Site-wise prevalence and mean intensity

Infection of the *Camallanus cotti* was recorded only in the fish of lower reaches of the river with prevalence (6.89%) and mean intensity (1.37) (Table 5). Statistically, there was a significant difference in the prevalence of the nematode in the fish of the upper and lower reaches of the river ($p<0.05$).

3.6 | Month-wise water temperature

The water temperature of the river was recorded at a minimum of 11 °C in January in Khalla-Brahmdev and a maximum of 24 °C in July in the Bhujela-Mahakali Suspension Bridge (Fig. 2).

Table 3. Host sex and month-wise prevalence and mean intensity of *Camallanus cotti*

Months	Number of fish examined			Number of infected fish		Number of parasites recovered	Prevalence (%)	Mean intensity
	Male	Female	Total	Male	Female			
October	3	23	26	-	1	1	3.84	1
November	1	19	20	-	1	1	5	1
December	8	26	34	-	2	2	5.88	1
January	1	20	21	-	1	4	4.76	4
February	6	14	20	-	-	-	-	-
March	-	9	9	-	2	2	22.22	1
April	-	23	23	-	-	-	-	-
May	-	6	6	-	-	-	-	-
June	-	17	17	-	1	1	5.88	1
July	-	12	12	-	-	-	-	-

Table 4. Seasonal prevalence and mean intensity of *Camallanus cotti*

Seasons	Number of fish examined	Number of infected fish	Number of parasites recovered	Prevalence (%)	Mean intensity
Autumn	57	2	2	3.5	1
Winter	75	3	6	4	2
Spring	38	2	2	5.26	1
Summer	41	1	1	2.43	1

Table 5. Site-wise prevalence and mean intensity of *Camallanus cotti*

Study site	Number of fish examined	Number of infected fish	Number of parasites recovered	Prevalence (%)	Mean intensity
Khalla-Brahmdev	95	-	-	-	-
Bhujela-Mahakali Suspension Bridge	116	8	11	6.89	1.37

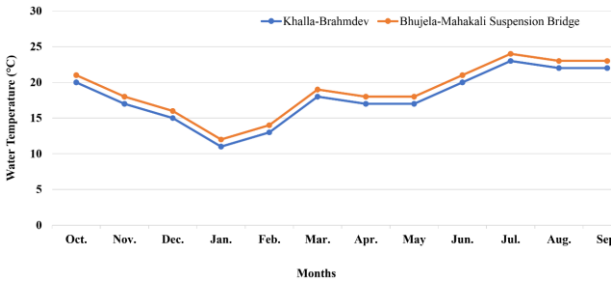

Figure 2. Month-wise water temperature of sampling sites in Mahakali River

4 | Discussion

Red worm (*Camallanus cotti*), a nematode parasite of fish, has higher prevalence and mean intensity in cultured ornamental fish (Kim et al. 2002; Levsen & Berland 2002; Menezes et al. 2006; Wu et al. 2007; Tavakol et al. 2017) and some natural water body fish (Moravec & Justine 2006; Gagne et al. 2015) due to stable climatic conditions and more host specificity.

In this study, overall, there was a low prevalence (3.79%) and mean intensity (1.37) of red worms on Mahseer probably due to the cold water of the Mahakali River system and the occasional parasitic nature of the host fish. The observed differences in prevalence and mean intensity of *Camallanus cotti* with the size, weight and sex of host fish, and months and seasons of the year were statistically insignificant due to the low burden of the nematode (Kennedy 1968; Vincent & Font 2003) indicating infection occurs in categorical groups due to chance. The apparent higher prevalence in more than 0.2 m-sized female host fish might be due to ageing factors irrespective of host sex (Tekin-Özan et al. 2008; Zargar et al. 2012) and long-range migration in the river. Higher mean intensity of the nematodes in 0.1-0.2 m sized female host fish might be due to more feeding habits (Brouder 1999). Stromberg and Crites (1975), Kim et al. (2002) and Wu et al. (2007) also reported no preference for *Camallanus* nematode for host sex in white bass, cultured guppies and Chinese hook snout carp respectively due to the equal opportunity for both the male and female fish to consume the infected copepod hosts.

In this study, the observed infection of the nematode *Camallanus cotti* only in female host fish but not in male fish year-round might be due to either escape of the nematode from the male host fish before sample collection or chancily exclusion of infected male host fish during random sampling. Further, the nematode showed higher prevalence in the month of March and Spring season due to favourable temperatures for rapid completion of its life cycle (Levsen & Berland 2002) thereby dissemination in more and more fish and also more availability of intermediate copepod hosts in that season, But, the mean intensity was higher in January and winter season. This might be due to colonization of the nematodes in a few host fish for reproduction, as the female carrying several larvae were observed at this period. This finding is consistent with the Kennedy (1968) and Wu et al. (2007). But, Vincent and Font (2003) and Gagne et al. (2015) reported steady infection year-round due to low climatic changeability with slight raised incidence in summer months in Hawaiian gobies. Stromberg and Crites (1975) and Wu et al. (2007) also reported high incidence in summer months. Thus, the monthly and seasonally differences in prevalence and mean intensity of the *Camallanus cotti* were attributed to variations in the number of intermediate copepod host (Stromberg & Crites 1975; Marcogliese & Esch 1989; Košuthová et al. 2015) and fluctuation of water temperature of the river.

In the present study, a few (1 to 4) *Camallanus cotti* nematodes were detected freely as well as embedded with the stomach and intestinal mucosa of the host fish in each infected fish. Some studies have reported 1 to 307 nematodes (Moravec & Justine 2006; Wu et al. 2007; Gagne et al. 2015; Tavakol et al. 2017). The infection of the nematode *Camallanus cotti* was found only in the fish of lower reaches of the river due to decrease in river flow and comparatively higher water temperature thereby increasing the colonization of intermediate hosts, which increased the probability of parasite transmission.

5 | Conclusions

The present study documents the low prevalence and burden of red worm (*Camallanus* cotti) nematodes in the Mahseer (*Tor putitora*) fish of Mahakali River. Supplying Mahakali River water to nearby fish farms of the Kanchanpur District from the river would increase the risk of dissemination of nematode to the cultivated fish in fish farms.

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Authors' contributions

Y.R.J. conceptualized and designed the research, collected and analyzed data and wrote the manuscript. P.J. supervised the research and finalized the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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