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Original Paper

Prevalence and intensity of *Bothriocephalus acheilognathi* Yamaguti, 1934 in *Tor putitora* in Mahakali River, Nepal

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Bothriocephalus acheilognathi Yamaguti, 1934 is a predominant parasite of cyprinid fish all over the world. This study was conducted to investigate the prevalence and mean intensity of Asian fish tapeworm (*Bothriocephalus acheilognathi*) in Golden Mahseer *(Tor putitora*) in Mahakali River, Nepal from October 2021 to September 2022. A total of 149 *Tor* fish were collected from the four 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, prevalence and intensity of the *Bothriocephalus acheilognathi* in *Tor putitora* were 4.69% and 2.14 respectively. Size-wise, higher prevalence and intensity were found in 0.1-0.2 m long fish (5.71%, 2.5) than >0.2 m long fish (4%, 1.66). Weight-wise, highest prevalence was found in 0.101-0.15 kg weighted fish (8.82%) and highest intensity in 0.051-0.1 kg weighted fish (4). Month and sex-wise, highest prevalence and intensity were observed in April (17.39%, 2.5) followed by July (8.33%, 2), January (6.66%, 1) and June (5.88%, 2) in female host fish (5%, 2.14). Higher prevalence and intensity were found in spring season (10.52%, 2.5) followed by summer (4.87%, 2) and winter season (2.32%, 1). Statistically, the prevalence and intensity of the parasite were insignificant ($p > 0.05$). This study reveals stable low infection of the Asian fish tapeworm in Golden Mahseer but increases the risk of dissemination of parasites from the Mahakali River to nearby fish farms and also zoonotic risk to human.

Keywords: Asian fish tapeworm, Golden Mahseer, Mean intensity, Prevalence, Season

1 Introduction

Asian fish tapeworm (*Bothriocephalus acheilognathi* Yamaguti, 1934*)* infects more than 300 species of fish worldwide (Scholz, 1997; Dove & Fletcher, 2000; Nie' et al., 2000; Choudhury et al., 2006; Zargar et al., 2012; Salgado-Maldonado et al., 2015; Luque et al., 2017; Kuchta et al., 2018; Pérez-Ponce De León et al., 2018; Palermo et al., 2021). This tapeworm has significant economic importance in fisheries (Košuthová et al., 2015). Cyprinid fish, especially *Tor putitora,* are the most important fish species in Mahakali River due to their dominance in the river (Joshi & Joshi, 2020) and high demand in the local markets (Nautiyal, 2014).

Several studies have shown that fish of natural water body acquire more parasitic infections than those from culture system (Nnadi et al., 2011) due to the access of the piscivorous birds acting as definitive hosts for some helminthic parasites (Ortega-Olivares et al., 2008; Murugami et al., 2018) to open water lotic ecosystem. But information is lacking regarding the *Bothriocephalus acheilognathi* and other parasites in *Tor putitora* in Mahakali River. So, the present study was conducted to determine the prevalence and mean intensity of *Bothriocephalus acheilognathi* in *Tor putitora* in

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Mahakali River, Nepal. This study will become the basis for further investigations of fish parasites in the river and adjoining rivulets in future.

2 Material and methods

2.1 Fish sample collection, identification and morphometric measurements

Tor fish (n=149), estimated as Endangered (Jha et al., 2018), were collected month-wise randomly from four sites: Machheti-Khalla (29º63''17.28'N 80º89''17.16'E), Brahmdev (29º37''49.8'N 80º77''26.16'E), Sarada Barrage (28º58''45.44'N 80º69''48.96'E) and Mahakali Suspension Bridge Areas (28º55''35.11'N 80º65''25.8'E) in Mahakali River, Nepal (Fig. 1) covering a total distance of 22.5 kilometers between October 2021 to September 2022 using gill nets with the help of local fishermen. The fish were also purchased, if assured they were from the Mahakali River from local markets.

The collected fish specimens (Fig. 2) were brought to the laboratory of Central Department of General Science, Far Western University, Nepal for further processing. The fish were identified as described by Jayaram (2010). The photograph of the collected fish were captured using Canon camera (model number: Cannon 8000 D) in the laboratory. Fish standard length and body weight were measured using a metal ruler calibrated in centimeters/inches and electronic weighing balance respectively. Their Sexes were determined by manual pressing on the genitalia under the lower abdominal region and confirmed by visualization of the gonads after dissection.

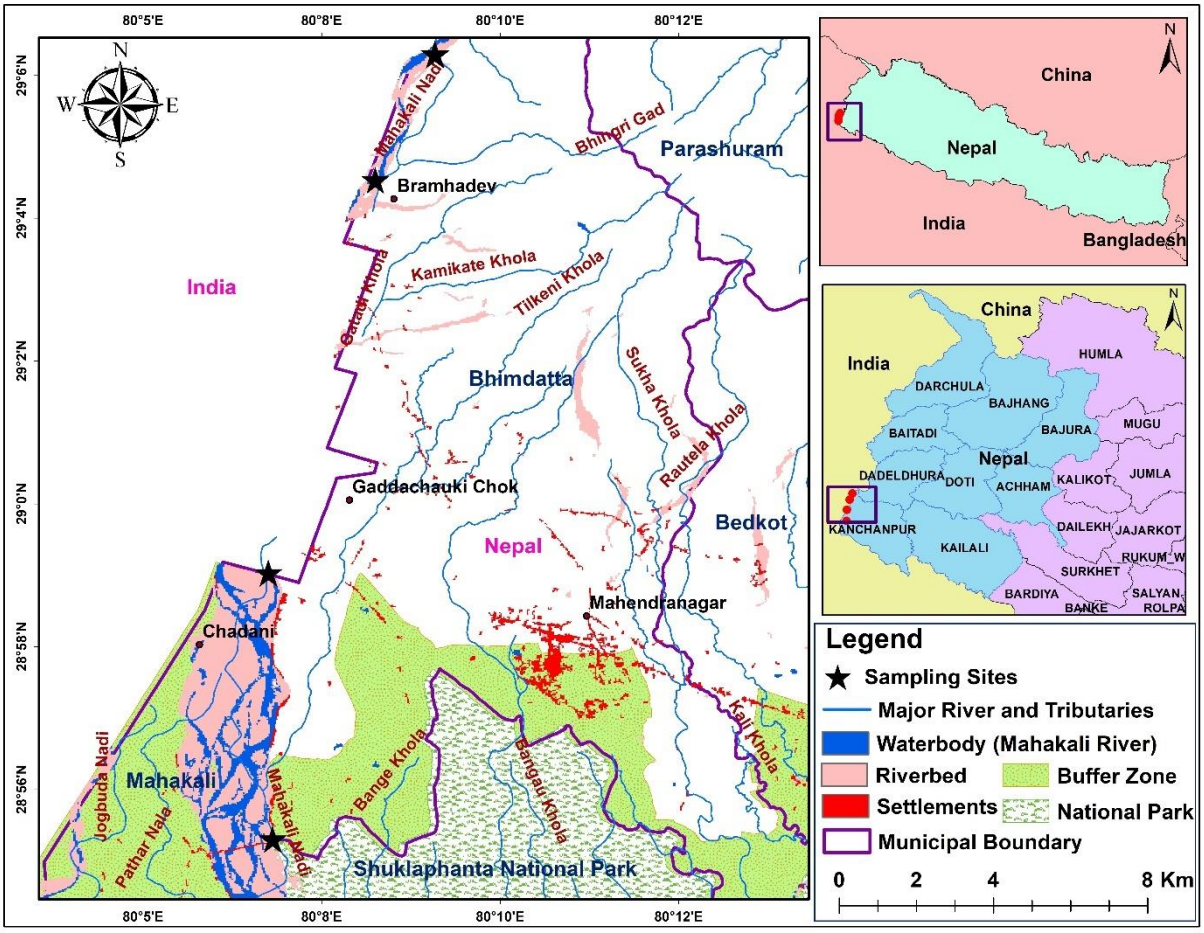

Fig. 1 Sampling sites in Mahakali River, Nepal

Fig. 1 Golden Mahseer *(Tor putitora) (*Asghar Nawab/WWF- India)

2.2 Examination and recovery of parasites

After morphometric measurements, external body surface (skin) and organs such as fins, gills, eyes, nostrils and buccal cavity of the fish were examined with the help of magnifying glass for the presence of parasites. Then, skin, gill, nostril and fin smears were prepared according to Thatcher (2006) and Whittington & Chisholm (2008). Fish were dissected through the abdomen by making a longitudinal slit on the ventral surface from the cloaca up to the level of pectoral fins using a surgical blade. The alimentary tract was isolated, stretched, removed out of the body, put in a Petri dish and cut into parts (stomach and intestine). These sections were placed into two separate Petri dishes containing physiological saline solution (0.9% NaCl Solution), split longitudinally, washed and examined thoroughly first under magnifying glass and then binocular microscope (model: Tech+ Inspected) 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 examined under physiological saline solution with the help of magnifying glass for the presence of parasites. The helminth parasites, recovered from each site, were processed for identification according to Thatcher (2006).

2.3 Identification of parasites

The helminth parasites were identified according to standard references of Chubb et al. (1987) and Thatcher (2006). The photograph of the parasites were captured using 12 MP 1/2.3'' Sony CMOS Sensor Microscope Camera (Model number: COSUSB12000) under 10X magnification.

2.4 Data analysis

Infection indices (prevalence and mean intensity) were determined using following formulae as per 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 ($p \leq 0.05$) of prevalence of the Asian fish tapeworm with body size, weight and sex of host fish, months and season of the year.

$$\text{Prevalence} = \frac{\text{Total number of hosts infected} \times 100}{\text{Total number of hosts examined}}$$

$$\text{Mean intensity} = \frac{\text{Total number of parasites recovered}}{\text{Total number of infected host examined}}$$

3 Results and discussion

A total of 7 *Tor putitora* fish were found infected by *Bothriocephalus acheilognathi* tapeworms. Fifteen *Bothriocephalus acheilognathi* tapeworms (Fig. 3) were recovered from anteror part of intestine in infected fish. Overall, prevalence was 4.69% and mean intensity was 2.14 (Table 1).

Infection of the *Bothriocephalus acheilognathi* was not observed in small sized fish. Both the prevalence and mean intensity of the tapeworm were higher (5.71% and 2.5) in 0.1-0.2 m sized fish than in other sized fish (Table 1).

Fig. 2 *Bothriocephalus acheilognathi* (a) Scolex with proglottids, (b) Proglottids

Table 1 Host size-wise prevalence and mean intensity of *Bothriocephalus acheilognathi*

Size of the examined fish (m)	Number of fish examined	Number of infected fish	Number of parasites recovered	Prevalence [%]	Mean intensity
Less than 0.1	4	0	0	0	0
0.1-0.2	70	4	10	5.71	2.5
More than 0.2	75	3	5	4	1.66
Total	149	7	15	4.69	2.14

SiImilarly, prevalence of the *Bothriocephalus acheilognathi* was highest (8.82%) in 0.101-0.15 kg weighted fish and lowest (2.89%) in 0.051- 0.1 kg weighted fish but intensity was highest (4) in 0.051-0.1 kg weighted fish and lowest (1) in up to 0.05 kg weighted fish (Table 2).

Table 2 Host weight-wise prevalence and mean intensity of *Bothriocephalus acheilognathi*

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	31	2	2	6.45	1
0.051- 0.1	69	2	8	2.89	4
0.101-0.15	34	3	5	8.82	1.66
0.151-0.2	9	0	0	0	0
0.201-0.25	2	0	0	0	0
More than 0.25	4	0	0	0	0

Further, infection of the *Bothriocephalus acheilognathi* was not observed in male fish and year-round. The prevalence of the tapeworm was highest in female fish in April (17.39%) and lowest in June (5.88%) but the mean intensity was highest in female fish in April (2.5) and lowest in January (1) (Table 3).

Table 3 Host sex and month-wise prevalence and mean intensity of *Bothriocephalus acheilognathi*

Months	Number of fish examined			Number of infected fish		Number of parasites recovered	Prevalence [%]	Mean intensity
	Male	Female	Total	Male	Female			
10/2021	2	10	12	0	0	0	0	0
11/2021	0	4	4	0	0	0	0	0
12/2021	0	20	20	0	0	0	0	0
01/2022	0	15	15	0	1	1	6.66	1
02/2022	0	8	8	0	0	0	0	0
03/2022	0	9	9	0	0	0	0	0
04/2022	0	23	23	0	4	10	17.39	2.5
05/2022	0	6	6	0	0	0	0	0
06/2022	0	17	17	0	1	2	5.88	2
07/2022	0	12	12	0	1	2	8.33	2
08/2022	2	10	12	0	0	0	0	0
09/2022	5	6	11	0	0	0	0	0

Moreover, infection of the *Bothriocephalus acheilognath i*was not recorded in autumn season possibly due to not capturing of parasitized fish during random sampling. Both the prevalence and mean intensity of the tapeworm were highest (10.52% and 2.5) in spring season and lowest (2.32% and 1) in winter season (Table 4).

Table 4 Seasonal prevalence and mean intensity of *Bothriocephalus acheilognathi*

Season	Number of fish examined	Number of infected fish	Number of parasites recovered	Prevalence [%]	Mean intensity
Autumn	27	0	0	0	0
Winter	43	1	1	2.32	1
Spring	38	4	10	10.52	2.5
Summer	41	2	4	4.87	2

The results of Fisher's Exact test were presented in Table 5. It indicated that statistically, the prevalence of the *Bothriocephalus acheilognathi* was not significant ($p > 0.05$) with the size, weight and sex of the host fish and with months and seasons of the year at 5% significance level indicating equal chance of parasitism in the categorical groups.

Table 5 Fisher's Exact Significance value for infection level of *Bothriocephalus acheilognathi*

Categorical variables for prevalence of *Bothriocephalus acheilognathi*	Fisher's Exact Test
	Exact Sig. (2-sided)
Host fish size	0.763
Host fish weight	0.658
Host fish sex	1.000
Months	0.533
Season	0.439

Thus, *Bothriocephalus acheilognathi* has variable prevalence and mean intensity (Dove & Fletcher, 2000; Pullen et al., 2009; Archdeacon et al., 2010). In this study, there was low overall prevalence (4.69%) and mean intensity (2.14) of *Bothriocephalus acheilognathi* in *Tor putitora* due to cold water (Choudhury et al., 2006) in Mahakali river system. The observed differences in prevalence and mean intensity of *Bothriocephalus acheilognathi* with the size, weight and sex of host fish, and months and seasons of the year were statistically insignificant due to low burden of the tapeworm (Vincent and Font, 2003) indicating no difference in between the categorical groups other than due to chance. The apparent higher prevalence and mean intensity in 0.1-0.2 m sized female host fish (Figure 4a and Figure 4b) might be due to more feeding habit (Brouder, 1999) or missing of infected male host fish during random sampling. However, some studies have shown the prevalence is increased with increasing size and weight of the host fish due to ageing factors irrespective of host sex (Tekin-Özan et al., 2008; Zargar et al., 2012). In this study, the tapeworm showed higher prevalence and mean intensity in the month of April and spring season followed by July and summer season, and January and winter season (Figure 4c to 4f). The monthly and seasonally differences in prevalence and mean intensity of *Bothriocephalus acheilognathi* (Tekin-Özan et al., 2008; Archdeacon et al., 2010; Zargar et al., 2012) were attributed to variations in the intermediate copepod host (Marcogliese & Esch, 1989; Košuthová et al., 2015), fluctuation of water temperature as the parasite is thermophilic (Marcogliese, 2008) and visiting of seasonal migrating piscivorous birds to the river.

In the present study, few (1 to 6) *Bothriocephalus acheilognathi* tapeworms were detected unattached by bothria with the intestinal mucosa in the anterior part of the host fish intestine in each infected fish (Pullen et al., 2009). Some studies have reported 2 to 600 tapeworms causing blockage, ischemia and perforation of intestine (Košuthová et al., 2015; Palermo et al., 2021). The infection of tapeworm *Bothriocephalus acheilognathi* was found more in the fish of lower reaches of the river than upper reaches (Pullen et al.,2009) due to decrease in river flow increasing the colonization of intermediate host but Nie' et al. (2000) reported the tapeworm from highlands rather than low land flood plains due to cosmopolitan distribution of its both definitive and intermediate host.

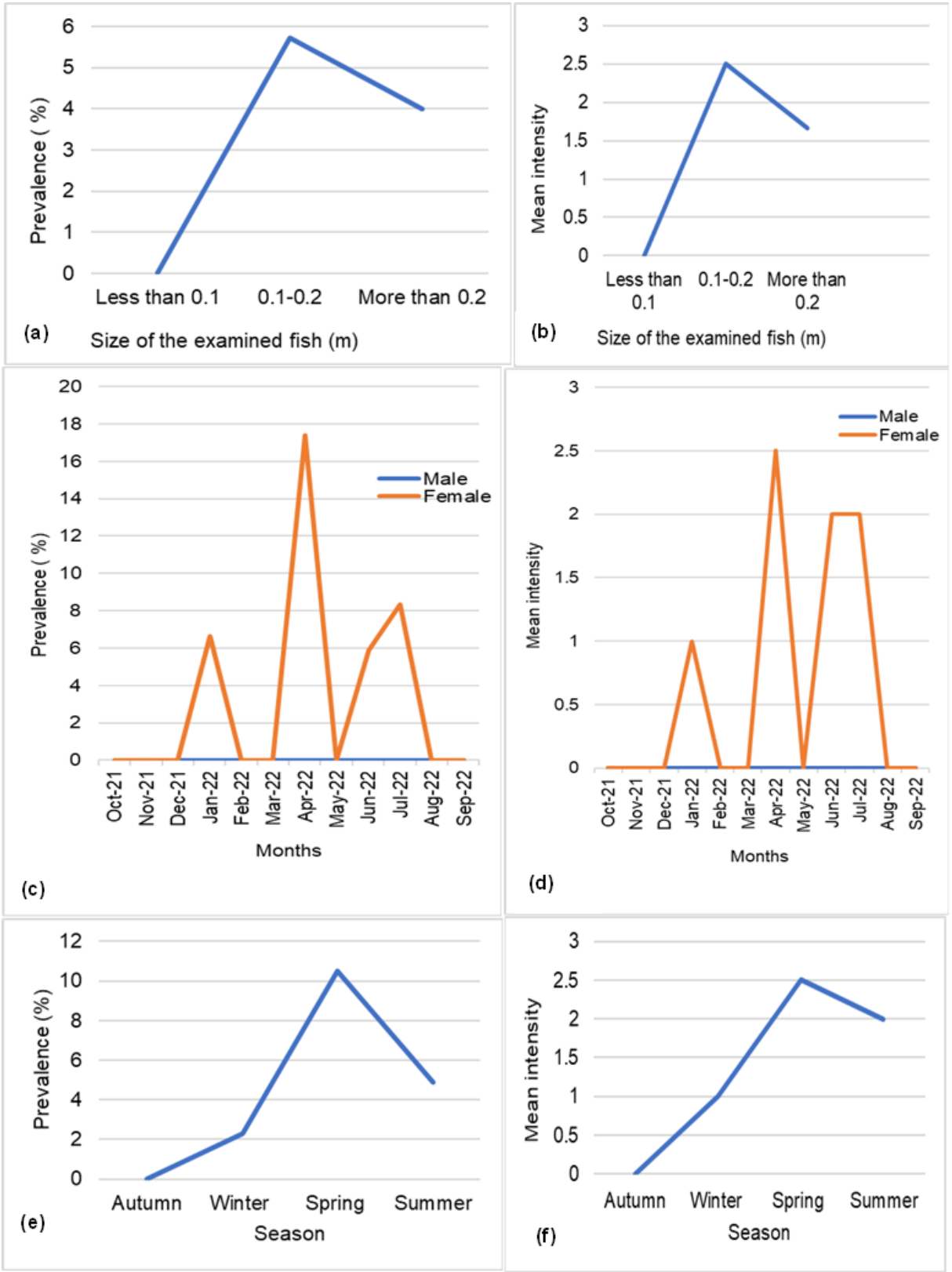

Fig. 3 *Bothriocephalus acheilognathi:* prevalence and mean intensity (a) and (b) host size-wise, (c) and (d) month-wise, (e) and (f) season-wise

4 Conclusions

The present study documents the infection of *Bothriocephalus acheilognathi* tapeworm in the *Tor putitora* fish in Mahakali River first time. There was low prevalence and mean intensity of the Asian fish tapeworm. Supply of Mahakali River water to nearby fish farms of the Kanchanpur district and frequently visit of piscivorous birds from the river would increase the risk of dissemination of tapeworms to the cultivated cyprinid fish in fish farms and, also accidental zoonoses in human who consume the gastointestinal tract of fish in raw forms containing copepods with procercoid. This needs further intensive investigation.

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