Prevalence of Fish Tapeworm – *Diphyllobothrium latum* in Commercial Fish Farms of Chitwan District, Nepal

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ABSTRACT

Diphyllobothrium latum, commonly known as "fish- tapeworm", has a zoonotic importance as it causes Diphyllobothriasis in humans. Fish, one of the highly consumed meat sources by human, also acts as second intermediate host for D. latum. There are several reports indicating consumption of D. latum infested poorly cooked fish causing Diphyllobothriasis in human. Thus, D. latum in fish can be considered as a major risk factor for Diphyllobothriasis in humans. Despite being a public health concern, no scientific data regarding the prevalence of D. latum in fish population of commercial fish farms of Nepal exists. The objective of this study was to bridge this knowledge gap by estimating the prevalence of D. latum in commercial fish farms of Chitwan district of Nepal. The study was conducted in six municipalities of Chitwan, Nepal. Out of 46 registered fish farms of Chitwan district, 42 farms were sampled in this study. The fish sampled from each farm were brought to the Histology laboratory of Department of Aquatic Resource Management of Agriculture and Forestry University, Nepal maintaining cold chain system and were dissected for feces collection. For qualitative parasitological examination of feces, sedimentation and centrifugal flotation method were used. Out of 42 farms sampled, 16 (38.10%) fish farms were found positive for presence of any kind of gastrointestinal parasites. Out 42 commercial fish farms examined, 9 (21.4%) farms were found positive for D. latum. Besides, D. latum fish farms were also found positive for Contracaecum sp. (19%), Coccidia (11.9%) and Eustrongyloides (2.4%). This research indicated that the prevalence of D. latum was more than 20% in commercial fish farms of Chitwan district showing possibility of zoonotic transmission to human population. Management practices of fish farmers must include ways to prevent D. latum infestation in fish population. Moreover, general public should be made aware about prevalence of D. latum in fish and its possible risk to human health along with its preventive measures for safe fish consumption.

Keywords: *Diphyllobothrium latum*, public health, zoonotic

INTRODUCTION

Total pond fish production in Nepal in 2020/2021 AD was 73,693 metric ton of which 3,580 metric tons was produced alone in Bagmati province (MOALD, 2022). Among 13 districts of Bagmati province, Chitwan district holds the highest percentage of fish production of around 84.77% (MOALD, 2022). The demand for fish is increasing in developing countries like Nepal due to its rich nutritional value (Ljubojevic et al.,2015). *Diphyllobothrium latum* (Phylum: Cestode, Family: Platyhelminthes), a parasite of fish, are zoonotic in nature and is a public health concern (Sampaio et al., 2005). The disease caused by fish tape worm *Diphyllobothrium latum* is known as Diphyllobothriasis. Although *Diphyllobothrium latum* was previously considered as a non-tropical disease, in recent years, its cases are also reported in tropical regions (Ramana et al., 2009). Cases of Diphyllobothriais has been reported in India and Bangladesh in both fish, animal and human population (Ramana et al., 2009; Samad2013). Definitive first and second intermediary hosts of *Diphyllobothrium* spp. include humans, mammals and birds that eat fish. In Nepal, parasitic zoonosis like Diphyllobothriasis has been considered as probable endemic (Devleesschauwer et al., 2014). Prevalence of *Diphyllobothrium latum* in Nepal was reported in pet and stray dogs of Rupandehi district (Yadav and Shrestha, 2017). Similarly, fecal sample from people of Bote and Darai communities of Tanahun district of Nepal which is adjoining district of Chitwan was also found to be positive for *D. latum* eggs (Thapa, 2000). *D. latum* has also been found in intestines of Common carp fingerlings in Kathmandu valley of Nepal (Rai, 2002) indicating possible prevalence of *D. latum* in commercial fish population of Nepal. Although being important topic of public health concern, no data is available regarding prevalence of *Diphyllobothrium latum* in commercial fish farms of Nepal and its zoonotic risk and not much studied have been done about it so far. The objective of this study is to estimate the prevalence of fish tapeworm, *D. latum*, in commercial fish farms of Chitwan district, Nepal.

MATERIALS AND METHODS

Study location

All six municipalities of Chitwan district i.e., Bharatpur Metropolitan, Ratnanagar, Madi, Kalika, Khairahani and Rapti Municipality were included in this study (Figure 1). Chitwan lies in the central part of the Bagmati province of Nepal. It lies at latitude 27.5291° N and longitude 84.3542° E. All study locations were situated in plain lands of Chitwan.

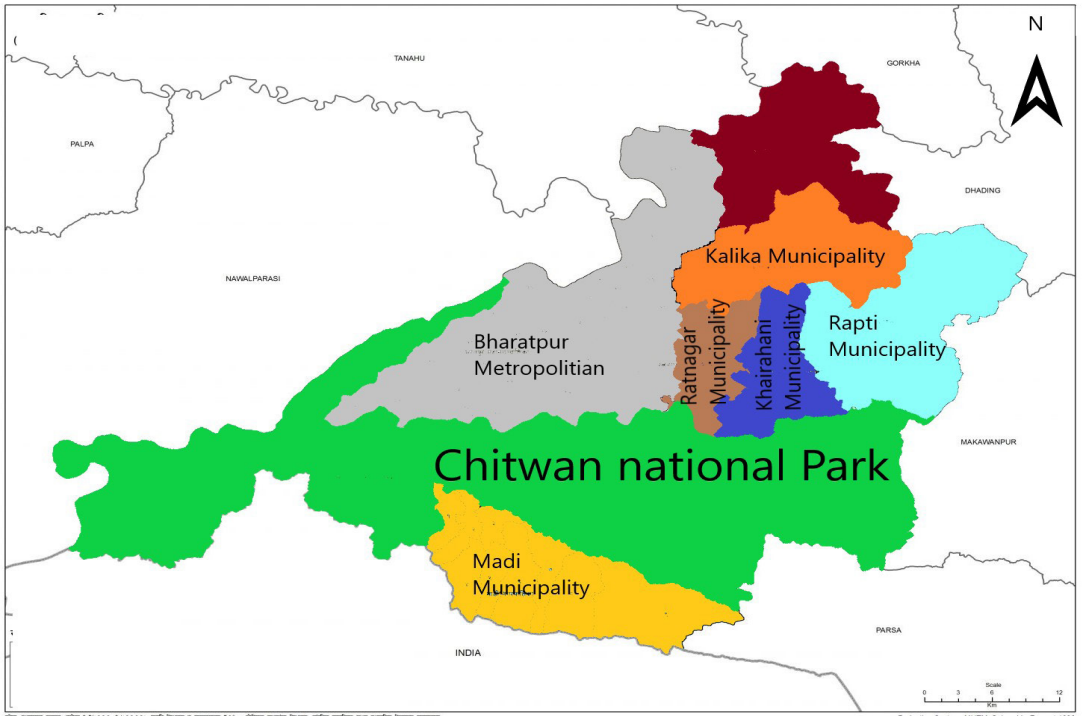

Figure.1. Map showing Chitwan district and its municipalities.

Sample size

to the data at the Veterinary Hospital and Livestock Expert Center (VHLEC), Chitwan, a responsible governmental organization for keeping records of registered farms, showed that the total number of registered commercial fish farm tin Chitwan in 2021 A.D. was 46. Sample size was calculated based on formula: n= N*X/(X+N-1), X=Z2P(1−P)/d2 (Daniel, 1999)

Where,

Z=value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI)

P= 50%, because no research of this kind has been done in Nepal.

d= 5% of precision

N=46, Total number of fish farms based on data provided by VHLEC, Chitwan.

Using above mentioned parameters, total number of fish farms to be sampled is 42 at 95% confidence level and 5% precision.

Sample collection

Sampling was done during mid-August to mid-September in 2021. Sample collection was done using simple random sampling method. Owners from selected commercial fish farms were shortly interviewed relating to fish disease and management before sample (fish) collection.

Out of total 42 commercial fish farm sampled, 13 was from Bharatpur Metropolitan city, 11 was from Madi Municipality, 10 from Ratnanagar Municipality, 1 from Kalika Municipality, 8 from Khairahani Municipality and 3 from Rapti Municipality as shown in Table1.

Table 1. Table showing location and number of commercial fish farm in the study

Location of fish farms	Number of fish farms
Bharatpur Metropolitan City	13
Madi Municipality	11
Ratnanagar Municipality	10
Kalika Municipality	1
Khairahani Municipality	8
Rapti Municipality	3
Total	42

Fish from each farm were selected according to that the number of ponds, one fish sample from 1-4 pond, 2 fish samples from 5-8 pond. Fish was brought to the Histology laboratory of Department of Aquatic Resource Management of Faculty of Animal Science, Veterinary Science and Fisheries, Agriculture and Forestry University, Chitwan, Nepal. Fish was then dissected aseptically for collecting fecal samples and each sample was labelled with unique identification number. Fecal sample was subjected to parasitological examination in the same day.

Fecal examination

Prevalence of parasite will be confirmed through two parasitological methods i.e., sedimentation and flotation. Both methods will be used for each fecal sample. For sedimentation method 3 gram of fecal sample will be mixed with 42 ml of water, macerated and passed through a strainer and sedimented for 15minutes. The supernatant was discarded and 2 drop of sediment will be poured to a glass slide for microscopy. Similarly, for flotation technique, the sediment will be mixed with 10ml saturated salt solution and centrifuged. The uppermost 2 drop of supernatant will be taken for microscopy. Parasite would be identified based on its morphology (Soulsby, 1982)

Data analysis

The data collected from questionnaire discussion and microscopic examination was analyzed using IBM SPSS version 26.0 and MS- Excel 2016.

RESULTS

Eight different species of fish were sampled from 42 commercial fish farms. Common carp (31%) was highest sampled fish followed by Pangas (19%), Naini (16.7%), Grass carp (11.9%), Bighead carp (7.1%), Rohu (7.1%), Catfish (4.8%) and Silver carp (2.4%).

Table.2. Different fish species sampled for fecal examination and their frequency

Fish Species	Frequency	Percentage (%)
Bighead carp (*Hypophthalmichthys nobilis*)	3	7.1
Catfish (*Clarias batrachus*)	2	4.8
Common carp (*Cyprinus carpio*)	13	31
Grass carp (*Ctenopharyngodon idella*)	5	11.9
Naini (*Cirrhinus mrigala*)	7	16.7
Pangas (*Pangasius sp.*)	8	19
Rohu (*Labeo rohita*)	3	7.1
Silver carp (*Hypophthalmichthys molitrix)*	1	2.4
Total	42	100

Out of 42 farms visited, 16 (38.1%), fish farms were found positive for presence of any kind of gastrointestinal parasites whereas 26 (61.9%) sampled fish farms were free from any kind gastrointestinal parasite prevalence.

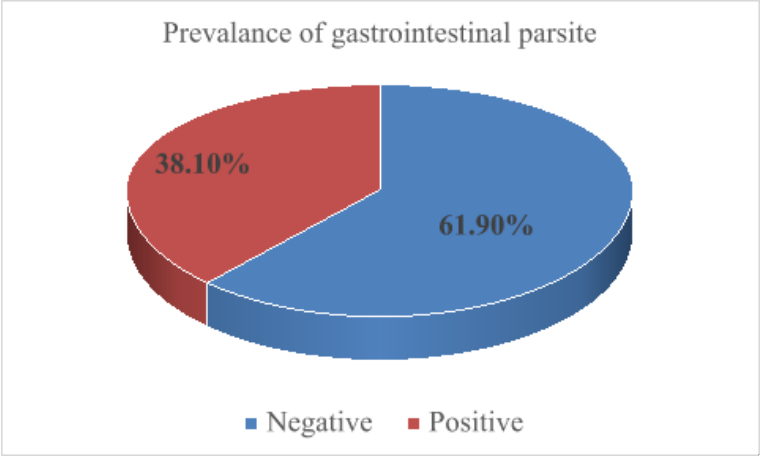

Figure 2. Pie chart showing prevalence of any kind of gastrointestinal parasite.

Through fecal examination of sampled fish, out of 42 fish farms, 9(21.4%) was found to be positive for *Diphyllobothrium latum,* 8 (19%) for *Contracaecum spp,* 5 (11.9%) for *Coccidia sp.* and 1 (2.4%) was found to be positive for *Eustrongyloides sp.*

Table.3. Gastrointestinal parasite and their prevalence percentage

Parasite species	Frequency	Individual parasitic prevalence (%)
Diphyllobothrium latum	9	21.4%
Contracaecum sp.	8	19%
Coccidia sp.	5	11.9%
Eustrongyloides sp.	1	2.4%

Table below shows fish species sampled, its sampling frequency and its parasite species wise frequency.

Table.4. Fish species with parasite species wise frequency

Fish Species	Sampling Frequency	***Diphyllobothrium latum***	***Eustrongyloides sp.***	***Contracaecum sp.***	***Coccidia sp.***
Bighead carp (*Hypophthalmichthys nobilis*)	3	**1**	**0**	**0**	**0**
Catfish (*Clarias batrachus*)	2		**0**	**0**	**0**
Common carp (*Cyprinus carpio*)	13	**5**	**1**	**4**	**2**
Grass carp (*Ctenopharyngodon idella*)	5	**0**	**0**	**1**	**0**
Naini (*Cirrhinus mrigala*)	7	**0**	**0**	**0**	**2**
Pangas (*Pangasius sp*.)	8	**0**	**0**	**2**	**1**
Rohu (*Labeo rohita*)	3	**3**	**0**	**1**	**0**
Silver carp (*Hypophthalmichthys molitrix)*	1	**0**	**0**	**0**	**0**
Total	42	9	1	8	5

DISCUSSION

Overall gastrointestinal parasite in fish population prevalence was greater than that reported by Bibi et al. (2018) who reported 26.3% overall gastrointestinal parasite prevalence from Chenab River Pakistan. Similarly, prevalence of *D. latum* was higher than that reported by Bibi et al. (2018) who reported 8% *D. latum* prevalence and Udechukwu et al. (2017) who reported 16.7% *D latum* prevalence in *Clarias gariepinus* (African catfish). *D latum* prevalence was lower than that reported by Uddin et al. (1980) who reported 82% prevalence of *D. latum* from *Harpodonnehereus* sampled from the Feni and Muhuri rivers at the confluent of the Bay of Bengal. *Contracaecum sp.* prevalence was lower than that reported by Naha et al. (2019) in Jew-

Fish (*Otolithoides Pama*). Similarly, prevalence of contracaecum was similar i.e,17.95% for *Contracaecum osculatum* in *Bliccabjoerkna* and lower i.e., 0.99% *for Contracaecum sp*. larva in *Iranocichlahormuzensis.* (Pajooki et al., 2011)

CONCLUSION

This research indicated that gastrointestinal parasite *Diphyllobothrium latum* is widely prevalent in commercial fish farms of Chitwan district. *D. latum,* being a zoonotic parasite*,* has a public health importance., Therefore, further studies need to be done with larger sample size to understand the true burden of *D. latum* in fish farms of Nepal. Public should be made aware about presence of *D. latum* in fish population and should also be made aware about safe fish cooking and consumption methods against *D. latum*.

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