

Contracaecum and Ligula Intestinalis Parasites in Oreochromis Niloticus and Labeobarbus Fishes in Lake Tana, Ethiopia

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Received: May 28, 2025; Accepted: June 09, 2025; Published: June 12, 2025

ABSTRACT

This study was held between December 2023 and July 2024, on the incidence of *Contracaecum* and *L. intestinalis* larvae in fish from the eastern Gulf of Lake Tana. In the study 253 *O. niloticus* and 131 *Labeobarbus* fishes were examined. The collective prevalence of these larval parasites in both fish species was determined to be 31%. Specifically, *O. niloticus* and *Labeobarbus* exhibited an infection rate of 31.2% and 30.5% respectively. *Contracaecum* larvae was infective in both species of fishes at its infective rate of 22.7% and *L. intestinalis* larvae was isolated only from *Labeobarbus* fish and its occurrence was 8.3%. Comparing the two parasites' larvae, *contracaecum* exhibited a statistically higher prevalence ($P = 0.000$) than *L. intestinalis*. The prevalence of *Contracaecum* and *L. intestinalis* larvae was notably greater in females (38.5%) compared to males (22.2%). Significance statistical variances were observed in fish length ($P = 0.022$) and weight ($P = 0.001$) categories, respectively that the longer and heavier fishes were more prevalent. The average parasite burden per fish was calculated as three *Contracaecum* larvae and 1.5 *L. intestinalis* larvae. As conclusion the two parasites burden was important predicament for fish production in Lake Tana. Thus, implementing parasitic control measures may help to control the problem.

Keywords: *Contracaecum*, Lake Tana, *Ligula Intestinalis*, *Labeobarbus*, *Oreochromis Niloticus*

Introduction

Lake Tana has relatively high fish density [1]. The primary fish species of commercial significance in Lake Tana include Nile Tilapia (*Oreochromis niloticus*), African Catfish (*Clarias gariepinus*), various barbs (genus *Labeobarbus*), and, in smaller quantities, Beso (*Varicorhinus beso*) [2]. These fish are subjected to numerous pollutants, including untreated sewage, agricultural runoff, and industrial waste. Farmers residing around the lake use it as a source of water for their and their animals, wash their clothes at the lake's edge using detergents, which increase heavy metal concentrations and adversely affect Fish's health and growth over time [3]. Together with these factors, the improper environment and management practices can directly and indirectly place fish to be vulnerable to a range of pathogens, like parasites [4].

Fish parasites play a crucial role in host biology, population dynamics, and overall ecosystem functioning [5]. The quantity of parasites needed to inflict damage on fish can vary widely depending on the fish species, size, and overall health. Fish can be affected *Platyhelminthes* (flatworms) and *Nemathelminthes* (roundworms). Specifically, flatworms from the class *Monogenea* are ectoparasites that target the skin and gills of fish, whereas *digenaeans* and *cestodes* are endoparasites that infect internal organs [6].

Larval stages of ascaridoid nematodes and cestodes, particularly from the genera *Contracaecum* and *Ligula*, are commonly found in the visceral cavities of various marine fish species, especially Nile Tilapia (*O. niloticus*) and *Labeobarbus*. Notably, the parasite *Contracaecum* has been documented to cause mortality in herring fry within aquaria [5]. Crustaceans serve as the primary intermediate hosts for these nematodes, while

Citation: Abraham A Tsegaye, Melkie Gebrie, Moges Maru Bimrew Admasu, Seid Kassaw, Gashaw Abebe. *Contracaecum* and *Ligula Intestinalis* Parasites in *Oreochromis Niloticus* and *Labeobarbus* Fishes in Lake Tana, Ethiopia. *J Infect Dis Treat*. 2025. 3(2): 1-7. DOI: doi.org/10.61440/JIDT.2025.v3.31

fish act as the secondary intermediate hosts. The larval stages of *Contracaecum* species that infect freshwater fish typically mature into adults in fish-eating birds, such as cormorants and pelicans [7]. Nematode infection is increase with age and length of fish. Thus, in contrast to *ligula intestinalis*, *contracaecum* nematodes are found in large fish [8].

Adult cestodes are morphologically characterized by a strongly flattened, dorsoventrally compressed body. They consist of three main parts: the scolex (head), the neck, and the strobila (body), with the strobila typically divided into multiple segments known as proglottids. These parasites are commonly found within the body cavity and internal organs of fish. Among them, cestode plerocercoides are particularly notable for being some of the largest and most damaging parasites affecting the body cavity of fish [9]. and among them the genus *Ligula* has a global distribution [10]. Adult parasites reside in the intestine, while plerocercoid larvae, which may belong to the same or different species, are present in the internal organs and muscles [11]. In these sites, severe infestations of these parasites can cause the host's body wall to bulge, muscles and reproductive organs to atrophy, growth to be slowed, and fecundity to be decreased [12].

The diagnosis of nematode parasites involves identifying the parasite either through visual inspection or under a microscope. Key characteristics used for identification include the roundworm shape, fusiform body, yellow capsule, and the structure of the muscular and glandular parts of the esophagus, as well as the site where the parasite is located [13].

Ethiopia's limited preference for fish as a food source, coupled with the underdeveloped fishing industry, has resulted in minimal investment in studying the region's rich diversity of fish species.

This has resulted in limited knowledge and research on the local aquatic ecosystem, which could otherwise support better management strategies and provides significant benefits to the fishing industry [14].

Only a limited number of studies have been conducted to assess the prevalence of disease-causing pathogens, particularly parasites, in fish from Ethiopian water bodies. The study of fish diseases, particularly those caused by helminth parasites, remains largely unexplored in the country. This study was conducted to determine the prevalence and perform the morphological identification of parasitic nematode (*Contracaecum*) and cestode (*Ligula intestinalis*) larvae found in two commercially significant fish species from the eastern gulf of Lake Tana.

Material and Methods

Study Area

The study had taken place at the eastern Gulf of Lake Tana, located in the Amhara Regional State, Ethiopia. Lake Tana, the largest lake in Ethiopia and the origin of the Blue Nile, is home to unique endemic cyprinid fish species and the commercially significant endemic subspecies of Nile Tilapia [15]. Lake Tana spans approximately 84 kilometers in length and 66 kilometers in width, with a maximum depth of 15 meters and an elevation of 1,788 meters a.s.l. It is fed by the Lesser Abay, Reb, Gumara, and several other small rivers. The lake's surface area fluctuates between 3,000 and 3,500 km², depending on the season and

rainfall. The water in Lake Tana is slightly alkaline, with a pH range of 6.98 to 9.97, and temperatures vary between 16.4°C and 31.3°C [2]. The fishery in Lake Tana is primarily focused on Nile Tilapia and *Barbus* species [12].

Figure: 1 map of the study area

Study Population

The fish populations in Lake Tana have been reported to include Nile Tilapia (*Oreochromis niloticus*), African Catfish (*Clarias gariepinus*), and various *Barbus* species (*Labeobarbus*) [16]. However, in the current study, only *Oreochromis niloticus* and *Labeobarbus* were collected from the eastern Gulf of Lake Tana in the South Gondar Zone.

Fish's Sex, lengths, weights, and larval counts from three selected landing sites were recorded to assess risk factors. The length of each fish was measured from the tip of the snout to the posterior tip of the caudal fin using a ruler, accurate to the nearest centimeter. The weight of each fish was measured to the nearest gram using a balance, following the procedures described by [17].

Fish weights were classified into three categories: 55 to 150 grams, 151 to 350 grams, and over 350 grams. Their lengths were also divided into three groups: 5 to 25 cm (short), 26 to 35 cm (medium), and over 35 cm (long). The sex of each fish was determined after dissection by identifying the presence of testes or ovaries, following the methodology outlined by [18].

Study Design and Sampling Methods

A cross-sectional study using a simple random sampling method from the captured fish was conducted from December 2023 to July 2024 to investigate the morphological identification and prevalence of major nematode (*Contracaecum*) and cestode (*Ligula intestinalis*) parasite infections in *O. niloticus* and *Labeobarbus* fish species in the eastern Gulf of Lake Tana, South Gondar. The fish were obtained from gill nets used by motorized and reed boat fishers. A total of 253 *Oreochromis niloticus* (Nile tilapia) and 131 *Barbus* (*Labeobarbus*) were assessed for the target parasites covering various risk factor categories.

Parasitic examination

All fish samples were clearly labeled, and essential parameters such as sex, length, weight, and larval count were recorded. Each fish was visually inspected and subjected to a postmortem examination using standard evisceration/incision techniques. During examination, the fish were kept wet throughout the process. To access the internal organs, an incision was first made along the ventral midline of the abdominal wall, starting from the anus (cut 1). A second incision was made from the anus to the lateral line, and then along the lateral line up to the gill cover (cut 2) as previously shown by Mbahinzireki. The detached portion of the abdominal wall was removed to allow examination of the internal organs. Each fish's internal organs were exposed by making an incision along the middle of the abdomen from the anus to the mouth. The entire alimentary canal was extracted, and the exterior of the gut was inspected for parasites, cleaned of any adipose tissue and mesenteries, and dissected with scissors. The gut's interior was examined under a microscope, and macro-parasites were removed using thumb-forceps. The visceral organs were thoroughly examined in situ, placed in a Petri dish,

and inspected for parasites. Any abnormalities were described and recorded according to the previous studies by [18].

The isolated parasites were preserved by fixing them in 70% alcohol, and then mixed with 1% glycerin as it was practiced by [19]. Paperna. for further morphological identification. The samples were transported to the Bahir Dar Regional Veterinary Parasitology Diagnostic Laboratory for further microscopic examination.

Data Management and Analysis

All data collected during the study were initially entered into Microsoft Excel 2007. Before statistical analysis, the data were thoroughly screened for errors and properly coded. Statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) software, version 16. Descriptive statistical methods, including tables, were employed to summarize and present the data. The prevalence of fish parasites was calculated as a percentage by dividing the number of infected fish by the total number of fish sampled. To examine the association between parasite infestation and various risk factors, such as fish species, sex, length, weight, and number of larvae per fish, a chi-square (χ^2) test was performed. A P-value of ≤ 0.05 was considered as significant, while a P-value > 0.05 was regarded as non-significant factors.

Results

Overall parasitic infection rates in the two fish species

Out of the 384 examined fishes in the study area, 119 (31%) were found to be infected with helminth parasites. The breakdown of infections by species is presented in Table 1. Specifically, *Oreochromis niloticus* (Nile tilapia) exhibited an infection rate of 79 out of 253 fish, which corresponds to 31.2%. Meanwhile, *Labeobarbus* showed an infection rate of 40 out of 131 fish, or 30.5%. The statistical analysis revealed that there was no significant difference in the prevalence of parasites between the two fish species, with a P-value of 0.890. This indicates that the rate of helminth infections was similar across both species, suggesting that neither species is more susceptible to helminth parasites than the other based on the data collected.

Table 3: prevalence of parasites in each fish species

Species of fish	Nº. of examined	prevalence of contracaecum	Nº. of xamined	Prevalence of L. intestinalis	χ^2	P-value
O. nilotocus	253	79(31.2%)	253		86.446	0.000
Labeobarbus	131	8(6.1%)	131	32(24.4%)		

Prevalence of parasites in relation to different risk factors

The overall prevalence of *Contracaecum* and *L. intestinalis* larvae was higher in female fish (38.5%) compared to males (22.2%), with this difference being statistically significant ($P < 0.05$). As detailed in Table 4, the infection rates were 39.3% in long, 28% in medium, and 23.8% in short length fishes. The variation in prevalence among these length groups was statistically significant ($P < 0.05$). Similarly, infection rates were 40.1% for fish weighing over 350 grams, 21% for those weighing between 151 and 350 grams, and 26.7% for fish weighing between 55 and 150 grams. Again, the differences in prevalence among these weight categories were statistically significant ($P < 0.05$). The analysis of the two fish species showed no significant difference in parasite prevalence between them ($p > 0.05$) as indicated in Table 4.

Table 4: Prevalence of parasites in relation to different risk factors

Risk factor	Category	Nº. Of examined (N=384)	Nº. of Positives	Prevalence (%)	χ^2	P-value
Sex	Female	208	80	38.5	11.8473	0.001
	Male	176	39	22.2		

Table 1: Overall prevalence of larvae infection in the two fish species

Types of fish	Number of examined	Number of positive	Prevalence	χ^2	P-value
O. niloticus	253	79	31.2%	0.0193	0.890
Labeobarbus	131	40	30.5%		
Total	384	119	31%		

In parasite wise observations, the prevalence of *Contracaecum* infections was observed in 87(22.7%) of the total sample. In the meanwhile, *L. intestinalis* larvae were found in 32 (8.3%) of the total sample (Table 2). The statistical analysis revealed a significant difference ($P=0.000$,) in the prevalence rates of *Contracaecum* and *L. intestinalis* infections. This suggests that the occurrence of *Contracaecum* was markedly higher compared to *L. intestinalis*, highlighting a notable disparity in the prevalence of these parasites within the fish populations studied.

Table 2: Overall prevalence of parasites larvae

Types of fish parasite	Number of infected fish	Prevalence	χ^2	P-value
contra caecum	87	22.7%	86.4460	0.000
L. intestinalis	32	8.3%		
Total	119	31%		

Over all prevalence of parasites in each fish species

The overall prevalence of *Contracaecum* larvae across both fish species was 22.7%. Specifically, *Oreochromis niloticus* (Nile tilapia) showed a higher prevalence of *Contracaecum* larvae, with 79 out of 253 fish infected, which equates to 31.2%. In contrast, *Labeobarbus* had a much lower prevalence of *Contracaecum* larvae, with only 8(6.1%) out of 131 fishes (Table 3). Regarding *L. intestinalis* larvae, the total prevalence observed in *Labeobarbus* was 24.4%, with 32 out of 131 fish being infected. Notably, *O. niloticus* did not show any infections of *L. intestinalis* larvae. These findings are detailed in Table 3, which illustrates the absence of *L. intestinalis* infections in *O. niloticus* and highlights the differing prevalence rates of *Contracaecum* and *L. intestinalis* across the fish species studied.

Length (cm)	Long	140	55	39.3	7.5954	0.022
	medium	139	39	28		
	Short	105	25	23.8		
Weight (gm)	>350	172	69	40.1	13.0583	0.001
	151-350	107	22	21		
	55-150	105	28	26.7		
Species of fish	O. nilotics	253	79	31.2	0.0193	0.890
	Labeobarbus	131	40	30.5		
Total		384	119	31		

Burden of Parasite (Number of Parasite per Fish)

For Contracaecum, among the 87 fish that tested positive, 39 fish (32.7%) were infected with a single larva each; 22 fish (18.5%) had three larvae each; 7 fish (5.9%) were found to have four larvae each; and 19 fish (16%) harbored six larvae each.

For L. intestinalis, among the 32 fish infected 26 (21.8%) had only one larva each; 4 fish (3.7%) had three larvae each; 1 fish (0.8%) had four larvae; and another 1 fish (0.8%) had five larvae (Table 5). Furthermore, the average number of larvae per fish showed distinct patterns for the two parasites. On average, Contracaecum larvae were present at a rate of about three per fish. These larvae were primarily located in the body cavity and mesenteries of the fish. In comparison, L. intestinalis larvae were found at an average rate of 1.5 per fish, with these larvae being observed both freely within the body cavity and attached to the fish's muscle. This information highlights the variation in parasitic load and distribution between the two types of larvae, as shown in Table 5.

Table 5: Parasite burden (Number of Parasite larvae per Fish)

No. of parasite larvae per fish	Spp of parasite	Number of infected fish	Percent (%) from the total	χ^2	P-value
1	Contracaecum	39	32.7%	17.9828	0.001
	Ligula intestinalis	26	21.8%		
3	Contracaecum	22	18.5%		
	Ligula intestinalis	4	3.7%		
4	Contracaecum	7	5.9%		
	Ligula intestinalis	1	0.8%		
5	Contracaecum	0	---		
	Ligula intestinalis	1	0.8%		
6	Contracaecum	19	16%		
	Ligula intestinalis	0	---		

Figure 2: Live ligula intestinalis larvae in the muscle of fish

Figure 3: Live ligula intestinalis larvae in the pericardial cavity of fish

Figure 4: Live Contracaecum larvae in the body cavity of fish

Discussion

The present study revealed an overall parasite prevalence of 31% across the fish species, with a P-value of 0.890, indicating that both *Oreochromis niloticus* (Nile tilapia) and *Labeobarbus* fish species are similarly susceptible to various parasitic infections. This finding is notably higher compared to previous studies by [20], who reported prevalence rates of 20.83% from Lake Ziway and by Senas who documented a prevalence of 9.5% in freshwater fishes in Japan. The higher prevalence parasites in the current study may be emanated from factors like the lack of effective waste disposal and management systems. In the studied area, post-harvest fish processing often leads to fish scraps and gastrointestinal contents being discarded into the lake and its shorelines. This improper disposal creates an environment conducive to parasite proliferation. Moreover, the stress on the fish population from nearby activities, such as animal grazing

and the presence of certain birds that may serve as intermediate or final hosts for these parasites, further exacerbates the issue.

On the other way, there are previous research reports showed a higher prevalence rates, including 66.3% by [20]. from Koka Reservoir and Lake Lugo (Hayke) in Ethiopia; and 47.8% by [21]. from various Ethiopian lakes. Additionally, [8]. found a prevalence of 37.07% in Lake Hashenge, and [22]. also observed a higher prevalence of 45.1% in selected lakes, including Lake Hayk, Ardibo, Golbo, and Maibar. The variations in these prevalence rates across different studies may be due to differences in the distribution of parasites definitive hosts, such as piscivorous birds, and primary intermediate hosts like copepods. Additionally, the season during which each study was conducted may affect the prevalence of parasites. Another potential factor is the level of immunity in the fish populations sampled during these studies; higher immunity levels could result in lower observed prevalence rates. Therefore, the observed differences in prevalence are likely influenced by a combination of ecological, seasonal, and biological factors affecting both the parasites and their hosts.

In this study, *Contracaecum* larvae were detected in 87 fish, translating to a prevalence rate of 22.7%. In contrast, *L. intestinalis* larvae were found in only 32 *Labeobarbus* fish, which represents a lower prevalence of 8.3%. The statistical analysis underscored a significant difference in the prevalence rates of *Contracaecum* compared to *L. intestinalis*, with a P-value of 0.000. This result highlights that *Contracaecum* was significantly more prevalent than *L. intestinalis* in the eastern Gulf of Lake Tana. The higher occurrence of *Contracaecum* larvae can be linked to the abundance of their definitive hosts, which support the life cycle of the parasite. This finding aligns with the results of [23], who reported similar patterns of parasitic prevalence in Lake Tana. Their research corroborates the idea that variations in the prevalence of parasitic species are influenced by ecological factors, such as the availability of hosts and the dynamics of the parasites' life cycles, as well as interactions within the host populations.

Prevalence infection of *contracaecum* parasites in *O. niloticus* was 31.2%, somewhat similar with [24]. (31.8%) at Lake Hawassa. However, it was higher than the values reported by [25]. (7.45%), Mokhtar (17.9%), [11]. This might be due to the maturation of parasites in large number of piscivorous birds around the Lake Tana which allow parasites to reproduce more and infect large number of fish hosts. Additionally, almost all fish caught were eviscerated along the shore and washed into the lake causing recontamination of the lake that in turn increases parasite burden in fish.

The presence of intermediate hosts and the prevailing physiochemical factors could also affect the prevalence of parasites. *O. niloticus* feeds mainly on phytoplankton and macrophytes although zooplankton and benthic organisms also contribute to the diet. Because zooplankton and benthic organisms act as intermediate hosts for several helminthes including *contracaecum*, their intake exposes the fish to infections. *O. niloticus* forms and defends territories along the shores. This territorial behavior increases the proximity to and maintains continuous exposure to the parasites (Mitiku et al., 2018), may be because of the availability of diver's host required to complete their life cycle [26]. reported 57.3% prevalence at Lake Tana of central Gondar. Much higher prevalence was also reported Marshet et al., in Gilgel Gibe I Dam (98.6%). This could be partly associated to the environmental and host-parasite relationship existing in the areas. Sampling periods and parasite fauna diversity could also be affected by environmental changes in the water bodies [27].

Total prevalence infection of *L. intestinalis* larvae in *Labeobarbus* fish was 32 (24.4%). The current study was higher than the values reported by Gulilat, et al., (3.09%) from koka reservoir of water body and Yimer reported at 12.4% from Lake Tana, Dagne and Nuru (8.6%) at Lake Tana tributaries.

The overall prevalence of larvae of *Contracaecum* and *L. intestinalis* in female was higher (38.5%) than male (22.2%) and there was statistically significant difference ($P < 0.05$) in the prevalence of these helminthes parasite between sexes. The finding agrees with [28,29,21]. that reported high percentages of infection in females than male fish. The influence of sex on the susceptibility of fish to infection could be attributed to

genetic predisposition and differential susceptibility owing to hormonal control. The physiological peculiarities of the female animals constituting stress and lowering the immunity make them vulnerable to infections, emaciation and event death, due to female fish especially the gravid once are susceptible to helminth infections that reduce their resistance Singh et al., [30,29]. However, the result was contradicted with the findings of Marshet, et al., [31-34].

From the fish's length ways analysis, the prevalence of infection in two fish species was 39.3%, 28 % and 23.8% for long, medium and short respectively. Similarly, the prevalence of parasitic larvae was 40.1%, 21 % and 26.7% for >350gm, 151-350gm and 55-150gm respectively. For all these fish species, the level of prevalence of parasitic larvae was significantly difference among the length group and weight categories. In both cases, the overall prevalence was increase in fishes which were longer length and higher (>350gm) weight categories. The significant difference in prevalence observed among length and weight groups of fish agreed with the previous studies carried in different lakes and paperna, [24].

This might be due to the difference in exposure periods of host to parasites. Moreover, longer and higher (>350gm) weight categories of fish consume a great variety of foods and hence liable to be infested more than others. Low ability of small fishes in catching the large and invasive copepods with parasites; and the larger and older fishes have been exposed for a longer period to the risk of being infected by the parasite from copepods. Some of these fishes may probably prey intermediate hosts for the helminthes parasite. Fishes might also be associated with the life cycle of the parasite. However, the result was contradicted with the findings of [35-37]

The *contracaecum* larvae burden was found that, out of 87 positive fishes 39 fishes (32.7%) were infected by one larvae of the parasite; 22(18.5 %) were infected by three larvae of the parasite; 7(5.9 %) were infected by four larvae of the parasite; 19(16 %) were infected by six larvae of the parasite; on an average, three *contracaecum* larvae were collected per fish. The results of the present study showed that the average number of larvae of *Contracaecum* parasite was much lower than to that of reported by [24,10]. report as on average seven larvae of *Contracaecum* were collected per fish. Moreover, high parasite level, up to 13.9 worms per fish, was reported by Marshet et al., and was found freely in the body cavities, mesenteries, and muscle of fish. The variation in prevalence could be associated with variation in distribution of definitive host, piscivorous birds and the primary intermediate host's copepods as well as on the season on which the studies are conducted and probably high level of immunity built up in fish sample that were sampled and examined during the study period.

On the other hand, *L. intestinalis* larvae were found that out of 32 positive fishes 26 fishes (21.8%) were infected by one larvae of the parasite; 4(3.7 %) were infected by three larvae of the parasite; 1(0.8 %) were infected by four larvae of the parasite; 1(0.8 %) were infected by five larvae of the parasite. In this study, an average, 1.5 larvae were collected per fish which is almost nearest to the work of [37]. on average 2 *Ligula plerocercoides* per fish were recovered and their main location

was abdominal cavity. High parasite level, up to 30 for *Ligula* infection, has been observed in European and North American cyprinid fish (Museth et al., 2001). The infection levels of *L. intestinalis* plerocercoid might depend on the length and weight of the host fish sampled and infection levels may continue to increase during the life of the fish as more plerocercoids are added to the plerocercoids already present in the fish (Baruš and Prokeš).

Conclusion and Recommendations

The study found that both *O. niloticus* and *Labeobarbus* were infected by *Contracaecum*, while *L. intestinalis* only affects *Labeobarbus*. Therefore, Fishermen should not dispose waste and offal at the shore of the Dam to avoid the existing recontamination of the lake and to break the life cycle of parasites of fish in order to avoid piscivorous birds. Further research should be conducted for correct identification of the parasite species by molecular techniques and their intermediate hosts may allow in future in defining preventive measures [38-42].

In this cross-sectional study the overall prevalence of parasites in fishes was significant. The longer and the heavier fishes were more prevalent for the parasite's infection. Moreover, the study found that both *O. niloticus* and *Labeobarbus* were infected by *Contracaecum*, while *L. intestinalis* only affects *Labeobarbus*. Therefore, Fishermen should not dispose waste and offal at the shore of the Dam to avoid the existing recontamination of the lake and to break the life cycle of parasites of fish in order to avoid piscivorous birds. Training should be provided to fisher men on proper postmortem and ante-mortem inspection of fish to decrease occurrence of zoonotic disease in the public. Further research should be conducted for correct identification of the parasite species by molecular techniques and their intermediate hosts may allow in future in defining preventive measures [43-45].

Acknowledgements

The authors like to acknowledge University of Gondar, College of Veterinary Medicine and animal sciences, Bahir Dar Regional animal disease investigation and research center, Fogera district agricultural office for their financial, logistics, and laboratory settings support respectfully.

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