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Prevalence of *Ligula intestinalis* Larvae in Barbus Fish Genera at Lake Tana, Ethiopia

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Abstract: There are many parasitic diseases of fishes in the world. Among them the disease caused by larvae of *L. intestinalis* is one of the most common parasitic diseases which cause huge economic losses. A cross sectional study with simple random sampling technique was conducted from October 2013 to March 2014 to determine the prevalence of *L. intestinalis* larvae in *Barbus* fishes found in Lake Tana. Three hundred (300) *Barbus* fishes with different length categories (short, medium and long); weight categories (small, medium and large) fishes were collected from fish supplier associations and using Minnow trap and Scoop net and eviscerated in laboratory to investigate the parasite (*L. intestinalis*), the parasite burden in each fishes. A total of 87 Fishes were parasitized by *L. intestinalis* and the results showed that the overall prevalence of the disease was 29.00 % in *Barbus* fishes in the study area. Variation in parasite detection and identification between sex groups was not observed; and it was 0.85% and 2.00% in male and female fishes respectively. The rest 84 fishes out of the total 87 positive fishes were difficult to determine their sexes as the sex organs were affected by the parasite; so that the 84 positive fishes were either male or female. The parasite burden (number of parasite per fish) was also determined and it was found that out of 87 positive fishes 83 (94.50%) fishes were infested by one parasite larvae and the rest 4 (5.50%) by two parasite larvae. The rate of parasite detection and identification was different in different length categories of fishes. The parasite was detected and identified with prevalence of 40.60%, 24.40% and 21.15% in short; medium and large length categories of fishes respectively. There was statistically significant difference (χ^2 = 10.90, P = 0.004) in different length categories of fishes. The rate of parasite detection was also different in different weight categories of fishes. It was found that the prevalence of the parasite was 40.56%, 25.50% and 13.33% in small, medium and large fish categories respectively. There was highly statistically significant difference (χ^2 = 13.13, P=0.001) in different weight categories of fishes. There have been economic losses in fishery production due to *L. intestinalis* larvae. So the prevalence of the disease should be studied further to set appropriate control measures.

Key words: Barbus Fish · Lake Tana · *L. intestinalis* · Scoop Net and Minnow Trap

INTRODUCTION

Globally, fish provides for more than 1.5 billion people with almost their entire average per capital intake of animal protein and 3.0 billion people with at least 15 percent of such protein [1]. Global demand and consumption of fresh fish have increased [2, 3] for instance a significant growth in fresh fish market has been observed in the United Kingdom (UK) during recent years. Fish is important to human population in trade and economy; it is of importance in the diet of different countries especially in the tropics and subtropics where malnutrition is a major problem [4, 5]. As the human population inevitably increases, the demand for fish as source of protein will grow [6]. In recent times, there has been tremendous increase in the development of fish farming and culture attributable to the increased need for affordable animal protein especially in the tropics [7].

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Ethiopia covers a land area of 1.13 million km^2, of which 99.3 percent is a land area and the remaining 0.7 percent is covered with water bodies and comprises 10 major lakes in the country [8]; from these inland water bodies for fish supply, Lake Tana is the leading and the biggest lake with potential of about 13,000 to 15,000 tons of fish per year [2] Current annual per capita fish production is less than 240g in Ethiopia [9]. Despite this, based on only a single factor; population current annual demand for fish in the country is estimated at 65,344 tones, equivalent to 1 kg/person. Future demand at the present population growth rate will reach 94,526 tons in 2015 and 117,586 tons in 2025 [10].

Diseases rarely affect fish under ideal circumstances but when something in the environment is not right the stress weakens the fish thus become susceptible to many diseases. Parasitic diseases are very common in fish all over the world and are of particular importance in the tropics and subtropics [11]. Parasitic diseases are an essential part of the aquatic environment and represent a significant proportion of aquatic biomass. Their presence in their host is generally at equilibrium in aquatic organisms and the most common life style on the planet [12]. Parasitic infection and diseases are some of the factors hindering high productivity in fish farming [13, 14]. Fish parasites result in huge economic losses as they increase mortality; increase farm inputs via increased treatment expenses and cause reduction in growth rate and possibly weight loss during and after the period of parasitic disease outbreak. All these militate against the expansion of aquaculture [14].

Together with nematodes and flukes, tapeworms (Class: cestoda) are among the most important helminthes parasites of fish [11]. These cestodes are internal (endo) parasitic platyhelminthes that parasitize fish, amphibians and reptiles [15]. Cestodes (tape worms) are ribbon like flat worms in which all adults inhabit the vertebrate intestine. They required at least one intermediate host to complete their life cycle. Helminthe parasites frequently occur within the body cavity and viscera of fish and some of the largest and the most harmful parasites of the body cavity of fish are cestode plerocercoides [16]. Among the plerocercoides the genus *Ligula* has a global distribution, mainly as parasites of cyprinid fish. The parasite causes compression and distortion of the viscera and inhibited gonadal maturation. Infected fish do not enter spawning shoals, often swim poorly and more susceptible to predation. Often infected fish may be recognized by their swollen abdomen [17].

Ligula intestinalis is a cosmopolitan tapeworm that infects cyprinid fishes and fish eating birds and is indigenous to southern Africa [18]. The eggs of the parasite shed together with the birds' faces and hatch in water to form infective coracidia, which are ingested by copepods [19]. Cyprinid fish become the second intermediate hosts when they eat parasitized copepods and are infected by the second stage larva called plerocercoid [19]. The plerocercoid is matured in intestine of the third host, birds and laid eggs are released and hatched into the water and again are ingested by copepods [20]. Fish ligulosis is a food borne zoonosis. There have been recently reported a number of human infestations with this parasite especially when raw or not properly cooked fish is utilized [21].

Many research works in different countries of the world show that the larvae of *Ligula intestinalis* is prevalent and causes high economic loss in fish production due to high morbidity, mortality, loss of production and high treatment cost, but the prevalence of the disease has not been quantified in Ethiopia. So the present study was planned with the following main objectives:

- To determine the current prevalence of the larvae of *Ligula intestinalis* in genera of *Barbus* fish found in Lake Tana.
- To study severity of infection (burden of the parasite) and organs of fish that are affected by larvae of the parasite
- To study the association of different risk factors with prevalence of the parasite.

MATERIALS AND METHODS

Description of the Study Area: The study was conducted from October 2013 to March 2014 in Bahir Dar town, at Lake Tana. Bahir Dar is located in Amhara Regional State about 565 km North West of Addis Ababa at an altitude range of 1562 meters to 2341 meters above sea level (Fig. 1). It is found at 11° 29' N latitude and 37° 29` E longitudes. The annual minimum and maximum temperature and rainfall ranges from 15 °C to 22.5 °C and 120 mm to 180 mm respectively. Lake Tana is one of the highlands lakes and is the source of the Blue Nile River and is the largest lake in Ethiopia(Figure 1). Lake Tana is located nearer to Bahir Dar city and it has approximately 84 kilometers long and 66 kilometers wide, with a maximum depth of 15 meters and it covers the area that ranges from

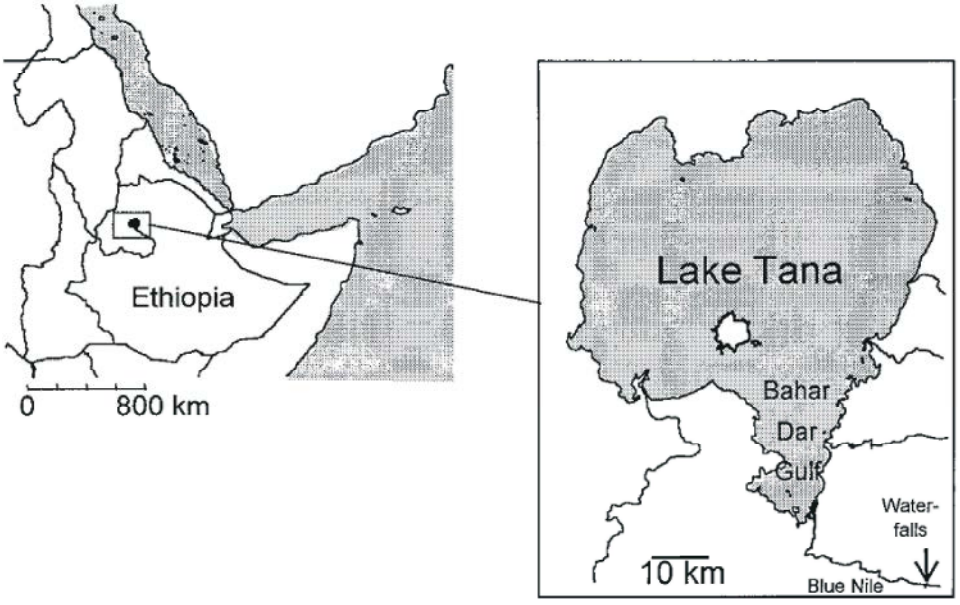

Fig. 1: Map of the study area [30].

3000 Km^2 up to 3500 km^2 depending on seasonal variation. Lake Tana is located at an altitude of 1788 meters above the sea level [22]. The sources of water for Lake Tana are different rivers including the small Abay, Reb and Gumara rivers. The water temperature fluctuates depending on the season. It has an average minimum temperature of 19 °C from July up to March and an average maximum temperature of 24 °C from May up to June. The lake level has been regulated since the construction of the control weir where the lake discharges into the Blue Nile. This controls the flow to the Blue Nile Falls (Tis Abbay) and hydro electric power station. Lake Tana is an important source of fish both for the people immediately around the lake and elsewhere in the country. Its unique and isolated landscape includes forested islands, immense and varied wetlands and high mountain areas. The region is renowned for its biodiversity and it is also the home of churches and monasteries dating from the 14th to the 16th century. Ninety percent of the area's rapidly growing population of 2.5 million people depends on subsistence agriculture for their livelihoods. The productivity and sustainability of mixed farming practices depend on ecosystem goods and services, which rely on the functional integrity of the watershed's ecosystems, rivers, wetlands, lake, forests, pastures and soils [23]. The phytoplankton of Lake Tana is dominated by Cyanobacteria and Bacillariophyceae; however Chlorophyta and the green algae are less abundant [24] while Papyrus (*Ceprus papyrus*) and water lilies are the predominate flora. They are only loosely rooted and can also be found in larger quantities, sometimes floating freely in the water column [25]. Since there are no inflows that link the lake to other large water ways and the main outflow, the Blue Nile, is obstructed by the Blue Nile Falls, the lake supports a highly distinctive fish fauna, which generally is related to species from the Nile Basin [26]. About 70% of the fish species in the lake are endemic [26] This includes one of only two known cyprinid species flocks (the other, from Lake Lanao in the Philippines, has been decimated by introduced species) which consists of fifteen relatively large which has the length of 1 m is *Labeo barbus barbs* [26, 27]. Eight of these are piscivorous and an important prey is the small Barbus tanapelagius, another endemic of the lake are *B. humilis, B. pleurogramma* also occur in Lake Tana, but neither of them is endemic [27, 28].

Lake Tana supports a large fishing industry, mainly based on the *Labeo barbus barbs* (formerly in genus Barbus), Nile tilapia and sharp tooth catfish (a large catfish that is widespread in Africa). According to the Ethiopian Department of Fisheries and Aquaculture, 1,454 tons of fish are landed each year at Lake Tana which is 15% of its sustainable amount [9]. Among other fauna, the lake supports relatively few invertebrates. There are fifteen species of molluscs, including one endemic and also an endemic freshwater sponge [26]. Numerous wetland birds such as the great white pelican and African darter, reside at Lake Tana. It is an important resting and

Fig. 2: Study populations and equipments (scoop net and minnow trap) that were used to trap small sized fishes

feeding ground for many migrant water birds [26]. There are no crocodiles, but the African soft shell turtle has been recorded near the Blue Nile outflow from the lake [29].

Study Population: The study animals were fishes of one genus Barbus that were collected from Lake Tana. Large, medium and small sized fishes were included in my study. Large and medium sized fishes were selected by simple random sampling from the two fish supplier associations that are found in Bahir Dar, while the small sized fishes were collected from the lake and were selected by simple random sampling. The number of large and medium sized fishes for each association was divided by considering their daily production (Kg of fish/day). The study population of large, medium and small sized fishes, with the techniques of trapping of small sized fishes in the lake is showed in Figure 2.

Study Design: The study was cross sectional with simple random sampling technique to determine the prevalence of *Ligula intestinalis* on the Barbus fish genera.

Sample Size Determination: The sample size required for this study was determined depending on the expected prevalence of the parasite and the desired absolute precision. The sample size was computed using the formula given in Thrusfield [31] as follows

$$N = \frac{1.96^2 \times Pexp\ (1-Pexp)}{d^2}$$

Where:
N = required sample size
Pexp = expected prevalence
d = desired absolute precision

There is one previous study which indicates the prevalence of *Ligula intestinalis* in genus *Barbus* fish in the study area which was 22.5%. Therefore, an expected prevalence 22.5% was used to estimate the sample size. Using desired 95% confidence interval, 5% precision and 22.5% expected prevalence, the necessary numbers of fishes needed to determine the prevalence, severity of infection (burden of parasite) and the affected organs of fish by the parasite were 268 fishes. However, 32 more fishes were added and 300 fishes were included in my study to improve the precision.

Study Methodology: A total of 300 different sized fishes were included in my study. Big and medium size fishes were collected from two fish supplier associations found in Bahir Dar; while the small size fishes were collected directly from the lake by using Scoop net and Minnow trap. All samples of fishes were clearly labeled with the date of sampling and the name of associations [32]. Thus, all samples were transported immediately in ice box to Bahir Dar Fishery and To Aquatic Life Research Center Laboratory. In the laboratory, the necessary parameters including length and weight were taken and sex of the fishes was determined [33].Finally each sample of fish was evaluated visually and postmortem examination was done using appropriate postmortem kits using standard evisceration/incision technique [33]; to assess the parasite; affected organs of fish, the burden the parasite and the sex of the fishes were determined. Based on the above procedures fishes were categorized in different groups. In respect to their length, fishes with the length of 5-20 cm, 21-29 cm and 30-40 cm were categorized as small, medium and large fishes respectively. In addition, the weights of the fishes were measured and fishes with the weight of 3-100 gm, 101-299 gm; 300-675 gm were categorized in different groups. Sex of the fishes was identified after post mortem evisceration of fishes; and they were categorized as male, female and undetermined sex (Sex organ of the fishes are affected by parasite and difficult to identify the sex).

Data Management and Analysis: The data were entered and managed in Microsoft Excel. All the data analysis was done by Statistical Package for Social Science (SPSS) software version 16. Descriptive statics such as percentages and frequency distribution were used to describe the nature and the characteristics of the data. The prevalence of *L. intestinalis* was analyzed using percentages. The association of different risk factors with prevalence of parasite was computed by Chi-square (χ^2) test.

RESULTS

Prevalence of *L. Intestinalis* in Barbus Fish: A total of 87 samples out of 300 fishes were positive for *L. intestinalis* larvae. The overall prevalence *L. intestinalis* larva in Barbus fish was found 29.00 %.

Prevalence of *L. intestinalis* in Respect to Length of Fishes: The prevalence of *L. intestinalis* was determined in relation to their total length; and it was found different with different length groups. The prevalence of the parasite was 40.60%, 24.40% and 21.15% in small, medium and large fishes respectively as shown in Table 1.

Prevalence of *L. intestinalis* in Respect to Body weight of Fishes: The prevalence of *L. intestinalis* was determined in different weight categories of fishes; and it was found that the prevalence of the parasite is different in different weight categories of fishes; and it was 40.56%, 25.50% and 13.33% with their 3-99 gm, 100-299gm and above 300 gm weight categories respectively as shown below in Table 2.

Prevalence of *L. intestinalis* in Respect to Sex of Fishes: The prevalence of *L. intestinalis* was determined in different sex groups of fishes; and it was found that the prevalence of the parasite is almost similar with different sex groups; and it was found that 0.85% and 2% in male and female fishes respectively. The higher prevalence was found in sex undetermined groups of fishes (84.00%) as shown in Table 3. The reason why the prevalence of the parasite was higher in sex undetermined group is that the parasite affects the sex organs of fishes and it was difficult to determine their sex; however sex undetermined fishes were either male or female.

Table 1: Prevalence of *L. intestinalis* in *Barbus* fishes with different lengths

Length of fish in cm	No of samples	No of infected fish	Prevalence (%)	95% of CI
5-20	106	43	40.60	31.25-49.95
21-29	90	22	24.40	6. 40-42.35
30-40	104	22	21.15	20. 84-21.46
Total	300	87	29.0	24.00-34.00

Table 2: Prevalence of *L. intestinalis* in Respect to Body Weight of Fishes

Body weight (gm)	No of samples	No of positives	Prevalence (%)	95% of CI
3-99	106	43	40.56	31.25-49.95
100-299	149	38	25.50	11.65-39.35
300-675	45	6	13.33	13.87-40.52
Total	300	87	29.00	24.00-34.00

Table 3: Prevalence of the Parasite in Respect to Sex of Fishes

Sex of fishes	No of samples	No of positive	Prevalence (%)	95% CI
M	118	1	0.85	0.79-0.91
F	98	2	2.00	0.04-3.96
Sex Undetermined groups	84	84	#	*
Total	300	87	29	24.00-34.00

and * = No need of determination as they are either male or female groups

Table 4: Parasite burden (Number of Parasite larvae per Fish)

No of parasite larvae per fish	No of infected fishes	Percent (%) from the total
1	83	94.50
2	4	5.50
Total	87	100

Table 5: Association of the prevalence of *L. intestinalis* with different risk factors

Body weight (gm)	No of samples	No of positives	Prevalence (%)	95% of CI
3-99	106	43	40.56	31.25-49.95
100-299	149	38	25.50	11.65-39.35
300-675	45	6	13.33	13.87-40.52
Total	300	87	29. 00	24.00-34.00

Burden of Parasite (Number of Parasite per Fish): The parasite burden of the affected fishes was assessed by counting the number of *L. intestinalis* larvae. It was found that out of 87 positive fishes 83 fishes(94.50 %) were infected by one larvae of the parasite; while 4(5.50 %) were infected by two larvae of the parasite as shown in Table 4.

Association between Prevalence of *L. intestinalis* with Different Risk Factors: Further the association of the prevalence of *L. intestinalis* with different risk factors was assessed; and it was found that the association of the prevalence of the parasite with different length categories of fishes was statistically significant difference ($P<0.005$) as shown in Table 5. Association of the prevalence of the parasite with different weight categories of fishes was also assed; and it was found it had highly statistically significant difference ($P<0.005$) in different weight categories of fishes as shown in Table 5.

DISCUSSION

There are many parasitic diseases of fishes in the world. Among them the disease caused by larvae of *L. intestinalis* is one of the most common parasitic diseases of fishes. Fish parasites result in huge economic losses as

they increase mortality; increase farm inputs via increased treatment expenses and cause reduction in growth rate and possibly weight loss during and after the period of parasitic disease outbreaks. In this study, attempts were conducted to determine the prevalence of *L. intestinalis* in genera of *Barbus* fishes at Lake Tana. The results of the present study showed that the prevalence of *L. intestinalis* in genera of Barbus fishes in Lake Tana was found to be (29.0%). Similar results had been reported by Gholami *et al*. [34] which was (22.22 %); the research work that was done in Mehran River of south of Iran. Barbus fishes are zooplankton feeders most of the time and harbor the parasite by ingesting copepods which are the first intermediate host of *L. intestinalis* [35]. The prevalence of *L. intestinalis* was also determined in different sex groups of fishes; and it was found 0.85%, 2.00 %, in male and female fishes respectively. The prevalence of the parasite was 84.00 % in undetermined sex groups of fishes; this is the fact that the parasite affects the sex organs of fishes and it was difficult to determine their sex; however sex undetermined fishes were either male or female, as the *Ligula intestinalis* affects both sexes without preference. This result totally agrees with the finding of Mehmet *et al.* [36]; the research work that was done at Lake Mogan. *Ligula intestinalis* hampers the gonadal development and cause infertility of infected fish [37].

The parasite burden of the affected fishes was assessed by counting the number of *L. intestinalis* larvae. It was found that out of 87 positive fishes 83 fishes (94.50 %) were infected by one larvae; while 4 (5.50 %) were infected by two larvae of the parasite. The result agrees with the finding of Gholami [34]. He found that out of 63 dissected fish only two fish were infected with 4 and 3 parasites respectively and the others had only one parasite per fish. While it contrasts with the finding of Dejen *et al*. [38]; who found only one parasite per fish in his study. This may be due to the increase in the final host (large white pelicans) population.

This study was also focused to know the presence or absence of association between the prevalence of *L. intestinalis* with different risk factors. The prevalence of the parasite was 40.60 %, 24.40 % and 21.15% in small, medium and large length fishes respectively. The difference was statistically significant ($P<0.05$). This could be due to the fact that small and medium fishes are found on the shores of the lake where the birds and copepods are more. Small length (juvenile) fishes were highly susceptible to parasite infection due to the fact that their immune system is not fully developed [39]; which was in total agreement with the finding of Shargh *et al.* [40] and Oguz *et al.* [41]. However, it contrasts with the finding of Dejene [35] who found that small size fishes have a low ability in catching the infected copepods than large fishes; and large size fish have been exposed for a longer period to the risk of being infected by the parasite. The association of the prevalence of the parasite with different weight categories of fishes was assessed and it was found that the prevalence of the parasite varies with different weight categories of fishes; and it was 40.46 %, 25.50% and 13.33% in small, medium and large size fishes respectively. The difference was statistically significant ($P< 0.05$). This could be due to the fact that the copepods were the major part of small fishes' diet; and low weight is one of the susceptible factors for *L. intestinalis* [40]. The low infection level of large fishes was the fact that large fishes are older in age and they less consume copepods than small fishes, which was totally disagrees with the work of Dejen [35] and Geraudi *et al.* [42] who found the 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.

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