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Assessing the Status of Cultured Fish Diseases through Identification of Important Fish Pathogen in Selected District of Oromia Region

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Abstract

This Study was conducted to assess the status of cultured fish diseases in selected district of Oromia region by collecting different fish species. Thus, a total of 351 specimens of *O. niloticus* (207), *C. gariepinus* (75) and *Lebeoburbus intermidus* (69) were examined. Different genera of fish parasites both from internal organs and external body surfaces of examined fishes were identified. The result revealed that 97(27.6%) from examined total fishes were infected with one or more genera of fish parasite. Among the three fish species examined of which ninety seven (97) were positive from the total (n: 351) sixty two (62) of them were infected by *Contracaecum* larvae (17.66% of the total and 63.9% of the infected) while thirty one (8.8% of the total and 31.9% of the infected), and sixteen (4.55% of the total and 16.5% of the infected) were *Clinostomum sp* and *Eustrongylides* respectively. These indicate higher prevalence of *Contracaecum* parasite during study period. On the other side from assessed external parasites of fish, *Dactylogyrus sp*. had the highest prevalence 11.3% followed by *Argulus sp*. (8.2%) and *Lernaea sp*. (5.1%). It is crucial to take the right precautions to avoid parasite infestation and to save significant fish species from extinction.

Keywords: Fish; Pathogen; Parasites; Prevalence

Background and Justification

As per FAO data, aquaculture has been the fastest growing food producing sector in the world, with an average annual growth rate of 8.9% since 1970, compared to only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems over the same period [1]. However, progress of aquaculture has caused some unwarranted activities both for the species and environment. At the same time, over exploitation of fisheries and anthropogenic stress on aquatic ecosystems has placed pressure on wild fish populations. The consequence has been the emergence and spread of an increasing array of new diseases change. As has been noticed in other food producing sector, aquaculture has been adversely affected due to frequent occurrence of disease outbreaks mostly due to intensive culture practices for higher economic gain [2]. Although aquaculture is the fastest-growing food production sector in the world, its production and trade is challenged by diseases of aquatic organisms [3]. The aquatic environment creates its own specific dares for disease control, with the spread of pathogens through water creating different patterns of disease to those observed in terrestrial animal production [4]. Disease epidemiology also varies globally, with outbreaks of aquatic animal disease at lower latitudes progressing more rapidly and resulting in higher mortality which require effective control systems to be developed [1].

Disease is one of the major constrains to aquaculture and may eventually become a limiting factor to the economic success of the fish farmers and it will result in production losses and public health problems. It also cause deterioration in the food value of fish and may even result in their mortality. Aquatic animal health therefore has major social and economic impacts on the people, businesses, communities and economies that rely on aquatic animal production [5]. Therefore, the knowledge of the diseases of major concern in a fish farming system is necessary in order to assess the risk factors influencing their introduction/spreading and defines the measures useful to their prophylaxis and control [6,7]. Losses due to mortality and retardation of growth of fish in ponds were estimated in different countries of the world. In China, it was estimated that around 10% culture area is suffering from disease, with annual losses of fish production is around 15% [8]. In Bangladesh the loss was estimated at about US$ 3.38 million in 1988 [9]. The finding in those two countries indicated the importance of assessing the impact of disease in aquaculture in order to develop farmers-oriented primary fish health management.

Objective

To investigate the status of parasitic fish diseases in different pond of Oromia Region.

Materials and Methods

Study Area

The study was conducted in four selected districts of two zones of Oromia region viz., Adama and Ada'a districts of East Shewa zone and Serbo and Omo Nada of Jimma zone due to abundance of fish resources and functionality of fish pond. Jimma Zone is divided in to 13 weredas (hosting a total population of over 2.2 million) with an agro-ecological setting of highlands (15%), midlands (67%) and lowlands (18%). The zone is one of the major coffee growing areas of Oromia region well-endowed with natural resources contributing significantly to the national economy of the country. Major crops grown, other than coffee are maize, teff, sorghum, barley, pulses (beans and peas), root crops (enset-false banana and potato) and fruits. Teff and honey production are another sources of cash after coffee. Enset is a strategic crop substantially contributing to the food security of the zone and is especially important in Setema and Sigimo weredas (highlands). Jimma Zone reliably receives good rains, ranging from 1,200-2,800 mm per annum. In normal years, the rainy season extends from February to October [10].

East Shewa is located at center of Oromia, connecting the western regions to the eastern ones. This zone is bordered on the south by the West Arsi Zone, on the southwest by the Southern Nations, Nationalities and Peoples Region, on the west by Southwest Shewa Zone and Oromia Special Zone Surrounding Finfinne, on the northwest by North Shewa, on the north by the Amhara Region, on the northeast by the Afar Region, and on the southeast by Arsi; its westernmost reach is defined by the course of the Bilate River. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), this Zone has a total population of 1,356,342, of whom 696,350 are men and 659,992 women; with an area of 8,370.90 square kilometers, East Shewa has a population density of 162.03. While 340,225 or 25.08% are urban inhabitants, a further 664 or 0.05% are pastoralists. A total of 309,726 households were counted in this Zone, which results in an average of 4.38 persons to a household, and 296,342 housing units [11].

Fish Collection

Samples of fishes were collected quarterly by hapa nets from the selected ponds. After capture, Total Length (TL) and Total Weight (TW) of each specimen was measured to the nearest 0.1cm and 0.1g, respectively. Each specimen was then dissected and its sex determined by inspecting the gonads. Maturity stages were rated visually and recorded. Five-point maturity scales were used for this purpose [10].

Pathological Examination

External Examination

The codes of each fish, species, sex, TL, TW, name of the site, date of sampling, organ of fish to be sampled, types of parasites observed, number of parasite per organ and different health related notes were recorded for each fish. Any abnormalities on the fish were recorded. A hand lens was used for quick identification of ecto-parasites on the skin and fins of the fish sample. Skin was also checked if there are capsules with metacercariae of trematodes in black dots and yellowish cysts which were sliced off the skin for further investigation. Parasites on gills were examined by removing the opercula using scissors; the gills were then placed in Petri dish containing normal lake water. Gill rakers were detached apart by forceps and examined under microscope for some worms. Protozoans were examined by scraping fish skin and taken with a cover slip (22 x 22mm) near the operculum to the caudal peduncle on the lateral side of the body length, dorsal part of the head, and ventral region of the fish from head to just after the anal point and from fins especially under pectoral and ventral fins. The mucus samples were smeared onto a clean microscope slide along with a drop of lake water and covered with a cover-slip and examined under compound microscope on 100x and 400x magnification.

Internal Parasite Examination

To examine internal parasite the coelom was opened by making a ventral surface cut from the anus forward to an imaginary line at the posterior portion of the operculum. Then, by following the digestive system from the esophagus to the anus and listing the number of parasite found on different organ. Finally, small and large intestines were cut out and wash bottle were used to flush out the inside so that parasite comes out the back end. The eye balls were taken out using scissors and forceps, then crushed; examined. Brain of African catfish was dissected longitudinally and the cranial cavity were washed away into Petri dish using water dropper and checked for parasites. Each parasite that gets each fish was kept in a plastic bag containing 4% formaldehyde solution.

Parasites Fixation, Preservation and Identification

With regard to the technique and method used in fixing, preserving and identification of each parasite specimens, the appearance and procedures of Paperna, et al. [12-14],were used as a guideline. Larva nematodes were fixed in 4% formalin and later stored in the saline solution. Adult nematodes were fixed in hot formalin to insure their relaxation and preserved in 4% formalin mixed in 1% glycerin to avoid accidental drying. Trematodes were fixed in AFA (Alcohol Formalin Acetic acid).

Data Management and Analysis

The whole data were processed by the MS Excel program and collected raw data was entered in to Microsoft excel data sheets and analyzed using SPSS-21 statistical software. Descriptive statistics, percentages and 95% confidence intervals were used to summarize the proportion of infested fish. Statistical significance was set at $p<0.05$.

pressure cuff. Used cuffs are rectangular (non D-Ring) or conical (with D-Ring) cuff type and are made for upper arm circumferences between 17 and 46cm (see Table 1). The cuffs are class I medical devices according to the Regulation (EU) 2017/745 (MDR) on medical devices and are developed and offered by PAR. In addition, an extension hose with a length of 3.0m was used.

Result and Discussions

Overall Prevalence

A total of 351 specimens of *O. niloticus* (207), *C. gariepinus* (75) and *Lebeoburbus intermidus* (69) were examined. Different genera of fish parasites both from internal organs and external body surfaces of examined fishes were identified. The result revealed that 97(27.6%) from examined total fishes were infected with one or more genera of fish parasite. The common endo-parasites were recorded as, *Contracaecum*, *Clinostomum* and *Eustrongylides* (Table 1). *Dactylogyrus sp*., *Argulus sp*., *Lernaea sp*., and *Ichthyophthirus sp*., are among assessed and identified ecto-parasites.

Prevalence of Each Parasite Species Identified During Study Period

The prevalence of each parasite shows different rates in fishes (*O. niloticus*, *Lebeoburbus intermidus* and *C. gariepinus*) that were recorded during the study period. Among the three fish species examined of which ninety seven (97) were positive from the total (n: 351) sixty two (62) of them were infected by *Contracaecum* larvae (17.66% of the total and 63.9% of the infected) while thirty one (8.8% of the total and 31.9% of the infected), and sixteen (4.55% of the total and 16.5% of the infected) were *Clinostomum sp* and *Eustrongylides* respectively. These indicate higher prevalence of *Contracaecum* parasite during study period. On the other side from assessed external parasites of fish, *Dactylogyrus sp*. had the highest prevalence 11.3% followed by *Argulus sp*. (8.2%) and *Lernaea sp*. (5.1%) respectively. From the examined and identified as infected fish species the prevalence of *Contracaecum* parasite was recorded as 39(62.9%) in *O. niloticus* and 21(33.8%) *C. gariepinus*. The result showed significant infection differences were occurred between the two fish species. On the other hand the prevalence of *Clinostomum sp* was higher in *C. gariepinus* and lower in *O. niloticus* (Table 1).

The present finding goes in line with the finding of study conducted in Sebeta ponds [15], which revealed from sampled *O. niloticus* about 91(71.9%) was infested with one or multiple parasites. In this finding about seven genera of fish parasites were identified but eleven genera of both external and internal parasites were identified in Sebata study site. In contradict with current finding which identified *Dactylogyrus sp*. (11.3) as the most prevalent external parasite, [15] identified *Trichodina spp*. from skin and gills and *Cichlidogyrus spp*. from gills as the most prevalent external parasites in Sebeta ponds with prevalence of 37.50 and 33.59% respectively.

Table 1: Prevalence of each parasite recorded among the examined fishes.

Parasites Observed	Number Observed	O. Niloticus	C. Gariepinus	L. Intermidus	% Age (Infected)
Contracaecum spp	62	39	21	2	63.9
Clinostomum	31	5	17	9	31.9
Eustrongylides	16	7	3	6	16.5
Dactylogyrus sp.	11	3	6	2	11.3
Argulus sp.	8	2	4	2	8.2
Lernaea sp.	5	0	5	0	5.1
Ichthyophthirus	3	0	3	3	3.09

x^2: 422.86 P< 0.05

Over all Prevalence in Male and Female Fishes

Sexes were assessed to observe their influence on the parasite infection results and the findings are presented in (Table 2). The result showed there were significant variation in the overall infection for sex difference of examined fishes (P<0.05). According to the result, out of infected fishes 61(62.8%) were males and the remaining 36(31.2%) were female (Table 2). Sexes were also assessed to observe their influence on the parasite infection results on basis of fish species examined. According to the findings from total examined male catfish 19 (46.34%) were found to be positive (Table 3).

There was slightly significant (p=0.021) difference between the infection of males and females in prevalence of infection. This observation agreed with the finding of (Ochieng et al., 2012) that male fishes were generally more susceptible than female to infections with nematodes, cestodes, acanthocephalan, crustacean and copepod parasites. This phenomenon is however still unclear but can be associated with the breeding habit of the fishes. During breeding, male fish takes care of a territory around the nest, keeping off other fish (females not spawning included). During this period the males spend more time than the females in the shallow waters where the snails harboring the cercaria of *Clinostomum* are mostly found, and this is probably why the males had higher prevalence.

Over all Prevalence in Relation to Different Length

The result shows that the prevalence of parasites in sampled fishes in relation to their total length (cm). Relatively high prevalence (35.7%) was recorded at 30-60cm total length of fish and the lower parasites prevalence (16.4%) was observed at 10-30cm total length of fishes. And there was statistical significant variation (p < 0.05) was obtained between the two total body length categories (Table 4). Lengths were also assessed to observe their influence on the parasite infection results on basis of fish species examined. According to the findings from total examined lower sized fish 39 (19.8%) were found to be positive which is lower when compared with higher size fish (Table 5).

Table 2: Prevalence of parasites on sex base of the host (n:351).

Sex	Number of Examined	Number of Infected	Percentage (Infected)
Male	191	61	62.8
Female	160	36	31.2

x^2: 6.54 P: 0.021

Table 3: Host sex-based prevalence of parasites for each fish species (n: 351).

Sex	Status	O. Niloticus	C. Gariepinus	L. Intermidus	Total
Male	Examined	117	41	33	191
	Infected	33	19	9	61
	% Infected	28.21	46.34	27.27	31.94
Female	Examined	90	34	36	160
	Infected	20	9	7	36
	% Infected	22.22	26.47	19.44	22.5
Total Infected	Examined	207	75	69	351
	Infected	53	28	16	97
	% Infected	25.6	37.33	23.19	27.64

Table 4: Prevalence of parasites in examined fish in relation to their total length.

Total length (cm)	Number of Examined	Number of Infected	% Age (Infected)
10-30	197	39	19.8
31-60	154	58	37.6

Table 5: Host length based prevalence of parasites for each fish species (n: 351).

Total Length (cm)	Status	*O. Niloticus*	*C. Gariepinus*	*L. Intermidus*	Total
10-30cm	Examined	129	21	47	197
	Infected	19	11	9	39
	% Infected	14.73	52.38	19.15	19.80
31-60	Examined	78	54	22	160
	Infected	34	17	7	58
	% Infected	43.59	31.48	31.82	36.25
Total Infected	Examined	207	75	69	351
	Infected	53	28	16	97
	% Infected	25.60	37.33	23.19	27.64

The prevalence of infection was positively correlated with host length ($p < 0.05$). This means that as fish grows, chances of infection increase for these parasites, because a long period of exposition to infective stages, and the amount of food it consumes, which including the larval stages of this parasite increased [16]. Larger fish have lived longer (as fish grow during all their life) and, therefore, have a higher probability of encountering parasites during their life span than smaller and shorter lived fish species. Moreover, feeding habits and wide diet put fish into contact with potential intermediate hosts of nematodes, cestodes, digenea, and acanthocephalan. Aho et al. [17] stated that the parasite species might accumulate among food chains, this could be particularly the case for endo-parasites. Szalai et al. [18] mentioned that the *Contracaecum spp*. larvae were absent in age 0 and age 1 bass (Micropterus salmoides) but prevalence and mean intensity increased with age, for bass age 2 or older. Poulin [19] stated that the increase in prevalence and intensity with the host length, could be related, not only to accumulation of parasites in the host during its life, but also to change of diet.

According to Luque et al. [20], correlation between the host total length and parasite prevalence and intensity is a pattern widely recorded in marine fish and documented with numerous cases in freshwater and marine fishes. Brutol [21] also indicates C. gariepinus with the ranges 30-70cm feed on large juvenile fish and crustacean parasites which also similar with this study. Paperna 1974 added that infection level increase significantly with size that also correlated with this finding. This finding agrees with the finding of Petchimuthu et al., (2018) which revealed that lengthy fishes are more susceptible to infections in comparison with small fish i.e. larger fishes were heavily parasitized than smaller ones. The highest prevalence value (23.71%) was found in length groups above 10cm and lowest prevalence value (14.28%) was found in 3-5cm length groups. The highest abundance value (3.32) was found in 5-10cm length groups but lowest (0.35) was bound in 3-5cm length groups.

Overall Prevalence in Relation to Different Body Weight.

It is clear from the result in (Table 6) that the prevalence of parasites in fish examined in relation to their body weight is higher in weight class 401-600gr (81.4%) while lower in class of 16-200gr (5.2%). Statistically significant variation was seen when the three weight categories were tested by Chi Square test.

Overall Prevalence in Relation to Sampling Sites

The prevalence of parasites in fish examined in relation to sampled sites were checked and the result showed significant variation were observed between the site ($P<0.05$) (Table 7). Study by Marishet et al. [15] revealed as there is a significant difference ($p<0.05$) in prevalence of parasite in the different study sites. This might be attributed to differences in the diversity and availability of invertebrate intermediate hosts and fish eating birds to complete its developmental cycle [22]. The prevalence of parasites in fish examined in relation to sampled zones was also checked and the result showed no significant variation was observed between the two zones (Table 8).

Table 6: Prevalence of parasites in examined fish in relation to their total body weight (gr).

Total weight (gr)	Number of Examined	Number of Infected	% Age (Infected)
16-200	83	5	5.2
201-400	127	13	13.4
401-600	141	79	81.4

x^2: 13.3 P: 0.03

Table 7: Prevalence of parasites in examined fish in relation to their sampling district.

Total weight (gr)	Number of Examined	Number of Infected	% Age (Infected)
Wonji	184	47	48.4
Godino	51	18	18.5
Serbo	49	11	11.3
Omo Neda	67	21	21.64

x^2: 13.3 P: 0.0173

Table 8: Prevalence of parasites in examined fish in relation to their sampling districts.

Zones	Status	*O. Niloticus*	*C. Gariepinus*	*L. Intermidus*	Total
East Shewa	Examined	134	32	69	235
	Infected	30	19	16	65
	% Infected	22.39	59.38	23.19	27.66
Jimma	Examined	73	43	0	116
	Infected	23	9	0	32
	% Infected	31.51	20.93	0.00	27.59
Total Infected	Examined	207	75	69	351
	Infected	53	28	16	97
	% Infected	25.6	37.33	23.19	27.64

Conclusion and Recommendations

Infestation with parasites is the biggest danger to fish culture systems. Every year, numerous fish species are infected by various forms of parasites, which have a considerable negative impact on fish productivity. The analyzed fish species in the study's chosen study sites contained parasites from a combined total of 7 genera, both external and internal. All of the fish that were sampled had either one or more parasite infections. Fish in every study site had external parasites such *Dactylogyrus sp*., *Argulus sp*., and *Lernaea sp*. on them. In order to stop the spread of fish parasites into the culture system, control techniques must be developed, especially when fingerlings from natural water bodies are used for stocking in other water bodies or intensive and small-scale fish farms.

On the other hand, further research should be done to identify parasites at the species level using molecular methods and parasite genomics of culture fish species. In studies of fish parasites, biotic parameters like stocking density and abiotic factors like water chemistry and water quality that can affect the abundance of parasites should also be taken into account. In general, appropriate measures should be implemented to avoid parasite infection and to save key fish species from extinction. In order to prevent the aquaculture business from suffering this significant economic loss on a farmer level each year, control of these parasite illnesses should be given top priority.

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