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Prevalence and Diversity of Internal Cestode Parasites Infected Nile Tilapia (*Oreochromisniloticus*) and African Catfish (Clariasgariepinus) in Farmers Fresh Water Ponds in Kenya

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Abstract

This study is one of series of paper expected to occur in this journal (Part of my PhD work Moi University2014-17). The study conducted between January 2016--June 2016 involved three counts, Kirinyaga, Kisii, and Uasingishu of Kenya, with the aim to investigate problems of stunted and mortality of 520 fishes of these two species of fresh water from farmer ponds were randomly sampled and examined for internal cestode parasites. 100 fish were C. gariepinus and 420 O. niloticus. These specimens were killed by scissors or a knife and then dissected internal organs placed in Petri dish mixed well with sodium chloride solution different parasites observed and identified using common characteristics and standard keys. The over roll results show that 240 (46.2%) of the total fish examined were infected by different parasites. C. gariepinus had a higher infection rate 55(55.0%) out of the 100 fish sampled compared to O. niloticus 185 (44.1%) of the 420 fish sampled. Prevalence and diversity of internal parasites. Diphyllobothriumlatum(34.59%), Proteocephalus spp(49.62%), Caryophyllidea spp(13.53%) and mixed infection (2.26%) Statistically Significance was tested at 0.05, The study findings show t that there was a significant relationship (p=0.0002) between the type of fish and the number of parasites Also insignificant Relationship observed between internal cestodeparasites and sampled fish on length (0.06657), weight(0.1690) and sexes(0.3668). The study concluded that the C. gariepinus are more attacked by internal cestode parasites compared O. niloticus. Our study suggest that farmers to keep more species with higher tolerance with parasites.

Key words: Internal cestode parasites; stunted and mortality.

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1. Introduction

The assessment of the prevalence of internal cestodes parasites on Nile tilapia (Oreochromisniloticus) and African catfish (Clariasgariepinus) is very important to the understanding of the functional role and effect of parasites on the two warm water fish species within their living environment [1].

Fish serves as an important source of energy, protein and a range of essential nutrients, accounting for about 17% of the global population's intake of animal protein [1; 16]. In the natural situation where the fish live only few reach adult stages, while majority are predated upon or die due to parasitic infections and other diseases [2]. Further studies by[14] indicated that many fish die under culture conditions than would in the wild due to stress caused by lack of sufficient oxygen, predators and parasitic infection[12; 20] Low oxygen levels, increased organic matter load and poor aquatic environmental conditions are conditions known to enhance parasitic infections in aquatic systems[2;19].

Despite the fact that fish in their natural environment experience parasitic infections just like other animals, the presence of fish parasites plays a major role on the fisheries and the fishing industry [7; 11; 32] There is literature on diagnosis of epizootic infection in both cold and warm water fish species[7; 11; 29]. Even though there are few reported cases of epizootic fish parasites, parasitized fish are more likely to experience mass mortality, retarded growth and weight loss and eventually low market value [2]. Despite fish being a good source of animal protein, fish parasitic infections pose a major threat to aquaculture sub-sector that has recently demonstrated tremendous growth in fish production in mitigating dwindling stocks of global capture fisheries[22;34] Studies have indicated that environmental factors have a major impact on fresh water biota and thus play a major role in determining the health of fish [20; 32].

Fish parasites, specifically cestodes are known to inhibit nutrient absorption and is more pronounced in cultured species due to overstocking coupled with poor water quality management [14;15]. Parasites are important components of any ecosystem and play key roles in fish population dynamics, community structure, and also provide important information on environmental stress, food web structure, function and biodiversity [31;33] Parasites take a significant proportion of list of fish diseases (80%) and are among the major causes of economic losses in the fish industry and in aquaculture [14;15]

Kenya's fish farming has experienced great growth since 2009 as a result of the Economic Stimulus Program [10] Increasing fish production without improving management of the fish ponds may result in bacterial, viral, fungal and parasitic infestations. On average 80% of fish diseases are parasitic especially in warm water fish, and severe fish mortalities as a result of parasites have been reported elsewhere [5;7] but few studies have been carried out in Africa[15; 23]. The effect of parasites on the economic value of fish is greater than their impact on human health [35]. Parasites affect the fish host through destroying tissue, removing blood and cellular fluids, diverting part of its nutrient supply and allowing secondary infections to develop easily [4;24]Factors that enhance parasitic infection include nature of ponds ,quality of feed, culture systems, and low oxygen levels resulting from poor pond management [2]. This knowledge will help fish growers improve their productivity, fish and fish product value, enhance incomes and thus alleviate poverty and malnutrition

Objective of study

To assess the diversity and prevalence of internal parasites in O. niloticus and C. gariepinusunder different culture systems

Research Hypothesis

 H_o There is no significant difference on the diversity and prevalence of internal cestodes parasites in O. niloticus and C. gariepinusunder different culture systems in fresh water ponds.

Significance of the study

The findings in the current study have provided empirical data and will address the challenges pose by internal cestodes parasites of O. niloticus and C. gariepinus which are important to fish farmers, fisheries and policy makers

2. Materials and methods

Study Area

This study was conducted in three counties in Kenya: Kirinyaga, Kisii and UasinGishu . Kirinyaga County is located in the central region of Kenya approximately 117km Northeast of Nairobi .It lies at latitude $N0^0 S37^0$ and an altitude of 1030 metres above sea level [21; 25]

UasinGishu County is located in the north rift region of Kenya. It lies at latitude $N1^0 E34^0$ and an altitude of 2000 meter above sea level..

Kisii County is located in Nyanza region 64 km from Kisumu city. It lies at latitude 0.6773° S and Longitude 34.7796° E, at an altitude of 2000 meters above sea level.

Study design and sampling method

The study conducte cross sectional and experimental for data collection, Sampling directed by financial and time constains.Purposive used ie calculated samples randomly taken with manimum error hence can be generalised.

520 live fish comprising of (Kirinyaga=144O. *niloticus* and 40 *C. gariepinus*,), (Kisii= 162O. *niloticus* and 30 *C. gariepinus*) and (UasinGishu= 114O. *niloticus* and 30 *C. gariepinus*) were sampled for parasites analysis. The box like containers (1MX1Mx1M) half filled with water was used for transported alive fish sampled from field to laboratory for parasites examination. The sample size for this field study was calculated using the formula by [25].

Measurement and morphological description of fish

The study involved morphological description of fish means used data obtained from body of fish samples (sexes, length, weight and maturity).

The weights of all sampled fish were measured using an electronic balance TX4202L and their length measured using a meter ruler and recorded. The sex of sampled fish was determined by observing the genital organs and the observation was recorded for each fish specimen

.Maturity was determined by pressing the abdomen for sperms for adult male fish and eggs, sometimes spawned (youngs) in mouth for adult female fish .Absence of sperms or eggs were an indication that the fish were immature.

The sex of the fish was also determined by observing the testes and ovaries (gonad) after dissecting the specimen. [14; 36]

Parasitological examination (gastrointestinal parasites)

Fish specimens were dissected from the anus to the lower jaw using a pair of dissecting scissors. All visceral organs i.e., stomach, intestines and the liver were extracted and placed on a Petri dish and bathed with sodium chloride solution 9 gms/l for parasites recovery.

The stomach and entire intestine were dissected longitudinally with a pair of dissecting scissors and then placed in sample bottle which was shaken vigorously for about 1-2 minutes thereafter the upper part of the mixture was discarded. This process was repeated until the remaining liquid was transparent the parasites that were visible to the naked eye were placed on a glass slide and transferred to the SZ-ST Olympus stage microscope for examination and identification. Photos of the observed parasites were taken using an Olympus camera PM-6 230154 mounted on the dissecting microscope. The parasites that were identified were recorded. The parasites were identified by observing common characteristics and by the support of standard keys [31;36;37] The parasites were then placed in labeled sample bottles (vials) containing 70% alcohol for preservation.

Data analysis

The data of this study of all counts collected from field were coded and stored in Microsoft excel. Finally these data were analyzed using MINITAB- Statistic software, 14 Statistical Significance was tested at 0.05, Fisher Exact test were used to test for significance between length, weight, and sexes.

3. Results

A total of 520 live fish comprising of 420 O. niloticus and 100 C. gariepinus were sampled and examined for internal cestodes parasites. 144 O. niloticus and 40 C. gariepinus were sampled in Kirinyaga, 162 O. niloticus and 30 C. gariepinus in Kisii while in UasinGishu, a total of 114 O. niloticus and 30 C. gariepinus were sampled and examined for internal cestodes (Figure 1)

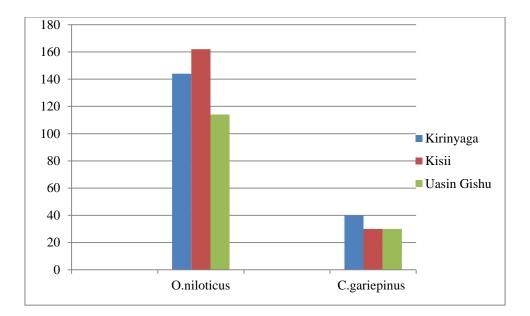


Figure1: showing fish species and total number of fish sampled per County.

		Internal cest	odes Parasites		
Fish species	None infected	Between 1 and 4	Between 5 and 8	Between 9 and 12	Total infected
O. niloticus	235	173	12	0	185
	56.0%	41.2%	2.9%	0.0%	44.1%
C.	45	42	10	3	55
gariepinus	45.0%	42.0%	10.0%	3.0%	55.0%
Total	280	215	22	3	240
	53.8%	41.3%	4.2%	0.6%	46.2%

The results show that 240 (46.2%) of the total fish examined were infected by different parasites. C. gariepinus had a higher infection rate 55(55.0%) out of the 100 fish sampled compared to O. niloticus 185 (44.1%) of the 420 fish sampled.

The results in table 2 indicated a significant difference (p = 0.0002) for Fisher Exact test between the type of fish and the number of parasites in the fish. In this case C. gariepinus had more likelihood of attacks by parasites compare to O. niloticus

Tests for association of variable	s: Fisher and Chi-S	quare Tests	
	Value	Df	statistical
Fisher Exact test	-	-	0.0002
Chi-square test:			
Likelihood Ratio	19.567	15	0.1630
Linear-by-Linear Association	12.684	15	0.9230
N of Valid Cases	520		0.7200

 Table 2: Statistical relationship showing diversity and prevalence of internal cestode parasites between O.

 niloticus and C. gariepinus.

Table 3: Diversity and prevalence of internal cestodes parasites in examined fish species

			Cestod	es		Total
	Fish species	Caryophyllaeidea species	Proteocephalus species	Diphyllobothrium species	2 Cestodes	
	0.	15	55	37	2	109
	niloticus	13.8%	50.5%	33.9%	1.8%	100%
	C.	3	11	9	1	24
	gariepinus	12.5%	45.8%	37.5%	4.2%	100%
		18	66	46	3	133
Total		13.5%	49.6%	34.6%	2.3%	100%

Table 3: indicates that Caryophyllaeidea species was only found in O. niloticus. The study also indicated that, the Proteocephalus species was highly distributed in C. gariepinus compared to O. niloticus while the distribution of Diphyllobothrium species was almost the same in both O. niloticus and C. gariepinus i.e., 33.90% and 34.60% respectively.

The results show that. Infection was higher in immature fish (49.0%) compared to mature fish (45.4%) as shown in table 4.

The results of this finding indicated that internal cestodes parasites were more prevalent in fish of length 21cm and below compared to those of length \geq 22cm.Generally infection decreases with increasing length across all internal cestodes species as shown in table.6.

	Maturity		Total	
Infection Status	Mature	Immature		
None	227	53	280	
	81.1%	18.9%	100.%	
Infected	189	51	240	
	45.4%	49.0%	46.2%	
Total	416	104	520	
	80.0%	20.0%	100.%	

Table 4:Distribution of internal cestode parasites by maturity of the fish examined.

Table5: Diversity and prevalence of internal cestode parasites in relation to length of fish examined.

		L	ength of Fis	h		
Internal cestode	< 14cm	14cm≤21cm	22≤28cm	29≤35cm	>35cm	Total
Caryophyllaeidea species	3	6	6	3	0	18
	16.70%	33.3%	33.3%	16.%	0.0%	100%
Proteocephalus species	8	43	7	7	1	67
	12.1%	63.6%	10.6%	10.6%	1.5%	100%
Diphyllobothrium species	5	30	3	8	0	46
	10.9%	65.2%	6.5%	17.4%	0.0%	100%
2 Cestodes	0	2	0	0	1	3
	0.0%	66.7%	0.0%	0.0%	33.3%	100%
Total	16	81	16	18	2	133
	12.0%	60.2%	12.0%	13.5%	1.5%	100%

 Table 6: Statistics showing diversity and prevalence of internal cestode parasites in relation to length of fish examined.

Tests for association of variables	: Fisher and	l Chi-S	Square Tests
	Value	df	statistical
Fisher Exact test			0.06657
Chi-square test:			
Likelihood Ratio	+20.23	15	0.163
Linear-by-Linear Association	0.009	1	0.923
N of Valid Cases	520	-	-

The results indicated that there was no significant relationship (p = 0.06657) between the length of fish and diversity and prevalence of internal cestode parasites. Although it was observed that infection with internal cestode parasites tailed -off with increasing length (table.6), this was not statistically significant as shown in table8.

Table 7: Distribution and prevalence of interna	l cestode parasites in relation	to weight of fish examined.
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			Fish Wei	ght (grams)			Total
Parasites	<50	50≤100	101≤150	151≤200	201≤250	≥250	
Counts							
None	48	98	71	35	21	7	280
	17.1%	35.0%	25.4%	12.5%	7.5%	2.5%	100%
Between 1	26	79	48	31	24	7	215
and 4	12.1%	36.7%	22.3%	14.4%	11.2%	3.3%	100%
Between 5	0	5	3	7	6	1	22
and 8	0.0%	22.7%	13.6%	31.8%	27.3%	4.5%	100%
Between 9	0	0	0	3	0	0	3
and 12	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	100%
Total	74	182	122	76	51	15	520
	14.2%	35.0%	23.5%	14.6%	9.8%	2.9%	100%

The results of this finding indicated that internal cestodes parasites were more prevalent in fish of weight 200grams and below compared to those of weight \geq 201grams.Generally infection decreases with increasing weight across all internal cestodes species examined. It was also observed that fish that weight less than 50grams only harbor between 1 and 4 internal cestodes parasites. The occurrence of between 9 and 12 internal parasites was only recorded in fish that weight between 151 and 200 grams as shown in table

 Table 8: Statistics showing distribution and prevalence of internal cestode parasites in relation to weight of fish examined.

Tests for association of variabl	es: Fisher	and	Chi-Square Tests	
	Value	df	statistical	sign(p=)
Fisher Exact test	-	-	0.169	
Chi square test				
Likelihood Ratio	34.37	15	0.163	
Linear-by-Linear Association	14.134	1	0.923	
N of Valid Cases	520	-	-	

The results indicated that prevalence and diversity of internal cestode parasites in relation to weight of fish do not differ significantly (p = 0.196). Although fish of weight 200grams and below recorded more infections compared to those of weight ≥ 201 grams this was not statistically significant. Generally infection decreases with increasing weight across all internal cestodes species examined as shown in table .

Table 9: Sex-related divers	sity and prevalence of internal ce	estode parasites species in examined fish.

internal cestodes	Sex		Total	
	Male	Female		
Caryophyllaeidea	15	3	18	
species	83.3%	16.7%	100%	
Proteocephalus species	49	17	66	
	74.2%	25.8%	100%	
Diphyllobothriumlatum	27	19	46	
species	58.7%	41.3%	100%	
2 Cestodes	2	1	3	
	66.7%	33.3%	100%	
Total	93	40	133	
	69.9%	30.1%	100%	

The study results indicated that Caryophyllaeidea species occur most frequently in males (83.30%) with the least frequently occurring internal cestodes parasites to be Diphyllobothriumlatum (58.7%). Whereas in females, Diphyllobothriumlatum (41.30%) had the highest prevalence with the lowest by Caryophyllaeidea species (16.7%) as shown in table 9

Table 10: Sex-related distribution and prevalence of internal cestode parasites in examined fish.

Tests for association of variable	Value	df	. oqu	Statistical Sign (p=)	sign(p=).
	value	uı		Statistical Sign (p=)	sign(p=).
Fisher Exact test	-	-		0.3668	
Chi square test:					
Likelihood Ratio	12.286	15		0.056	
Linear-by-Linear Association	1.996	1		0.158	
N of Valid Cases	520	-	-		

The study findings indicated that there was no significant relationship between the prevalence and diversity of internal cestode parasites and the sex of fish with Fisher Exact test (p=0.3668) and Linear by Linear Association (p=0.158) as shown in table 10. Even though female fish harbor more diverse parasites than the male, this was not statistically significant.

4. Discussion

The findings indicated that 240 (46.2%) of the total fish examined were infected by different internal cestodes parasites .These are Proteocephalus species, Diphyllobothriumlatum, and Caryophyllaeidea species. This percentage prevalence is consistent with studies conducted by (38;8) in fresh water ponds in Lahore Pakistan in which they reported a prevalence of 44.45% infection by internal cestodes parasites in nine (9) fish samples . In India,(8) have reported a slightly lower prevalence of internal cestodes parasites of between 26.31% and 36.84% at different sampling sites during the monsoon season. Similarly (3) have reported a prevalence of gastrointestinal parasites infection of 38.7% in Nigeria. They attributed these to poor hygienic conditions of the ponds which tend to favour the existence and propagation of many parasites. Decaying of the organic matter in the bottom of the ponds, depletes dissolved oxygen and water quality parameters are altered. The too could be the case in the present study where most of the ponds used were not properly maintained Related studies conducted by (40) in Maiduguri, Nigeria have recorded similar parasites prevalence of 38.6% in C. gariepinusthough fish sampled were purchased from local fish sellers within the metropolis. In Kenya (25), carrying out studies at KMFRI Sagana Aquaculture Research Centre and river Tana and, (17) in Lake Baringo have reported higher percentage prevalence (65.8% and 68.33%) of helminthes parasites. Helminthes infestation at a prevalence of 77.7% and 60.3% in Gandoman Lagoon, Iran and river Niger of Nigeria respectively have been reported (29:16). This high cestode prevalence could be attributed to the fact that these studies were carried out in rivers, lake and lagoon which are apparently dirty and have free materials deposits caused by eroded substances that may make the environments conducive for parasite development. The difference could also be that these studies recorded all helminthes of internal parasites whereas in the current study, only internal cestodes parasites were reported. The current study showed that C. gariepinus had the highest prevalence and diversity of worm burdens (internalcestodes parasites) of 55(55.0%, n=55) compared to O. niloticus 185 (44.1%, n=185). This difference could be attributed to the habitat favoured by C. gariepinus which consists of turbid environments and shore areas that are usually covered with vegetation (27), as was the case at many of the ponds used for this study. These habitats favour the intermediate hosts of cestodes as well as other digenetic trematodes (40). Further, C. gariepinusare omnivorous fish that feed on both aquatic plants and animals including copepods (28). Copepods act as first intermediate host of most internal parasites that infest freshwater fish (26;30). The larval stage of internal cestode parasites gains entry into copepods through ingestion as the copepod seeks food within its habitat. Copepods carrying the larval stage of cestodes are thereafter fed on by fish which act as second or final host that complete the lifecycle of cestodes (17). The large size of C. gariepinus also predisposes this particular species to parasites infestation compared to those of O. niloticus(27). The presence of fewer internal cestodes parasites in O. niloticus could be attributed to its resistance to parasitic infections. This may be explained by the fact that O. niloticus species rarely succumb to disease epidemics and have a remarkable power of recovery from infections (35). Therefore this could account for the low infection rates recorded during this study regardless of poor management to some of the ponds. These cestodes are widely reported around the world. For instance Proteocephalus, and Caryophyllaeidea species have also been reported in India (7) and Kenya (25) in fish sampled from rivers, lakes and fish ponds. Studies conducted in Zimbabwe by (27) have reported Polyonchobothriumclarias and Proteocephalusglanduliger. The tapeworm species (Diphyllobothriumlatum) reported in this study also had recorded current study in Africa.

Diphyllobothriumlatum was found in C. gariepinus in Maiduguri, Nigeria (40). The possible reasons for the recovery of these fish tapeworms in Africa of recent is that most small fish farmers use rivers water in their ponds, this water may carry first intermediate host copepods hence circulate from country to country. Another possible reason is distribution through the intermediate host (definitive or final host) of cestode e.g. piscivorous bird, mammals or reptiles (17).Researcher of present study recommended that further molecular and phylogenetic be conducted to confirm Diphyllobothriumlatum species reported. This could be that more researches are being done in the areas of fish parasites of recent in Africa. The findings in relation to fish maturity clearly show that infection was higher in immature fish (49.0%, n=51) compared to mature fish (45.4%, n=189) in O. niloticus and C. gariepinus in all the three (3) counties of Kirinyaga, UasinGishu and Kisii. The possible reason for this relationship immature fed less amount of foods hence gained less immunity compared the mature fish. This is in agreement with study conducted in Nigeria (3) who reported that smaller (immature) fish were more infected compared to larger (mature) probably due to their nature of acquired immunity with age. In contrast, the present study disagrees with findings reported (41;16) who both reported that bigger (and therefore possibly mature) fish have more parasites compared to small fish because they feed more on diverse food sources thereby exposing them to more parasitic infestation. The present study findings in relation to fish length indicated that internal cestodes parasites were more prevalent in fish of length 21cm and below compared to those of length \geq 22cm. Generally infection decreases with increasing length of fish to all types of internal cestodesparasites investigated. Internal cestodes parasites were more prevalent in fish of weight 200 grams and below compared to those of weight \geq 201 grams. The results obtained in the current study is in agreement with those reported (42) They reported that infestation was lowest in fish with body length of between 23.00 to25.75 cm and highest in fish with 25.76 to31.25 cm body length. Similarly, the parasitic infestation increased with lower body weight of between 160-258 g, while almost no parasites were observed in heavier fish (>553 g). This may be attributed to the fact that fish of a shorter length and lower weigh, and therefore possibly immature may not have acquired immunity compared to fish with large length and heavier weight fish (mature). Larger fish, in the current study which had experienced exposure to different internal parasites might have acquired stronger immunity hence their higher capacity to resist infestation of parasites (.42). Contrary to the present reports, (3) in Nigeria and (30) in Egypt have both reported that the smaller fish are relatively less infected than larger ones. This they also attributed to long time accumulation of parasites by larger and matured fish compared to the smaller/young ones. In relation to the sex of the fish, more males were infected with internal cestodes parasites (68.3%) compared to the female fish (31.5%) in both O. niloticus and C. gariepinus. However no variation or significant different in prevalence and diversity of cestodes was observed between the sexes. A high rate of infection was observed in males which may be attributed to activeness and aggressiveness of males compared to female fish which gives them an upper hand in the search for food hence increasing their chance of acquiring more internal cestodes parasites compared to female fish. Similar studies by Omejiand his colleagues2013 had also reported that male fish from earthen pond had a higher parasitic infestation (64.29%) than the female fish (57.69%). Similarly, (3) also recorded higher infection levels in male fish (37.8%) than female fish (23.5%). On the other hand, in Female fish cultured in concrete ponds in Nigeria were reported to have a higher percentage of parasitic infestation (22.73%) than the male (16.67%)(28). Abdel-gaber and his colleagues(2015) in Egypt also recorded higher prevalence rate 72 (90%) in female fish than males 58 (48.33%) although the difference was not significant (P > 0.05). This was attributed to the

physiological state of the female fish, as most gravid females fish could have had reduced resistance to infestation by parasites. In addition, their increased rate of food intake to meet their food requirements for the development of their eggs might have exposed them to more contact with the parasites, which subsequently could increased their chance of being infected

5. Conclusion and recommendation

The results of this study recorded high incidence of infections of the internal cestode parasites prevalence and diversity in both species sampled O .niloticus and C. gariepinus. Therefore stake holders should train the farmers effect of these parasites before starting keeping fish. The researcher of this study suggests more studies on internal parasites to be conducted

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