PREVALENCE OF HELMINTH PARASITES IN FARMED FISH IN NYERI COUNTY, KENYA

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DECLARATION

I declare that this research project is my original piece of work and it has not been submitted by any other individual in any institution as part of an academic course. No part of this work may be produced without written permission from the researcher

SIGN……DATE…….

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I, as the university supervisor do hereby approve the submission of this research project for examination

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DEDICATION

To all my future clients and to my caring parents, friends and lecturers

ACKNOWLEDGEMENT

I would like to thank God for life and the protection He has given me to enable me complete my university studies and more so this research project. I am also grateful to my family for the moral and material support they gave me as I pursued this degree.

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ABSTRACT

This research was carried out to determine the prevalence rates of helminths in farmed fish in Nyeri County; Kenya.Nyeri County is located in central Kenya about 164 Kilometers from Nairobi with a suitable climate for agriculture due to the high rainfall and good soil.

The objectives of the study were to establish the types of helminths affecting fish kept under aquaculture in Nyeri County, Kenya and to determine the prevalence rates of helminthes affecting fish kept under aquaculture in Nyeri County, Kenya

Tetu constituency was picked for sampling and 50 fish farms sampled using a stratified sampling technique.150 samples were collected and postmortems done at the site before harvesting the gastrointestinal tracts which were then stored in 70% alcohol and transported to the University of Nairobi's Department of Veterinary Pathology Microbiology and Parasitology for further processing. Worms were isolated and observed under a microscope for identification using morphological features 9 samples tested positive with a prevalence rate of 18 %(n=50) with trematodes (*Heterophylus* spp and *Clinostomum* spp) isolated with the following prevalence rates 8% and 10 % respectively(n=50). *Gyrodactylus* was also isolated but only in *Oreochromis* spp and *Clarias* spp. This was considered an incidental finding as this worm is generally found in the gills.

This therefore was evidence of prevalence of gastrointestinal fish helminths in farmed fish in Nyeri county of Kenya. Trematodes are the most prevalent with Heterophylus spp and Clinostomum spp isolated.

This prevalence rates called for intervention strategies and carrying out of more research studies to document prevalences, intensities and distribution of helminths in fish in Africa

CHAPTER ONE INTRODUCTION

1.1Background

Agriculture forms the backbone of the Kenyan economy with 75% of the population dependent on it for food and income (Orodho, 2006; FAO, 2006). This sector also contributes 26% of the gross domestic product (GDP) and 60% of the foreign exchange (FAO, 2006)

Fish farming has recently emerged as a lucrative venture due to its provision of an alternative source of animal proteins. As the number of people embracing fish farming and adoption of modern techniques such as aquaculture rises, so has the number of challenges giving need for comprehensive studies on a number of problems facing this sub-sector of agriculture.

The need for diversification of protein sources to meet the demand is real so as to feed the growing population estimated at 40 million (GOK, 2010). Despite the fact that the natural fishing grounds such as Lake Victoria have been overexploited (Greboval et al.,1994),the supply still falls short of the demand hence bringing into focus the need for fish farming.

The increase in the uptake of aquaculture and subsequent increase in its production has been a factor of the aforementioned increasing population growth and the need to commercialize farming.

Of all the regions, central Kenya has been at the forefront in adopting aquaculture leading to an astonishing rise in the statistics on aquaculture in these parts of the country. Nyeri County is one of the leading fish-producing regions in the country. The County is located on the southwest flank of Mt Kenya and covers an area of 3,556 square kilometers, with a population of 661,156 people (GOK, 2010). The county's main economic activities include tea farming, dairy farming and aquaculture (FAO, 2006). Nyeri has 2097 fish farmers most under the Fish Farming

Enterprise Productivity Programme (FFEPP) of the economic stimulus programme (ESP) (Fisheries department, 2015)

The main species of fish kept under aquaculture are tilapines-which form 90% of all farmed fish in the country, catfish, common carp, largemouth bass and rainbow trout (Balarin, 1985)

Fish helminths are a big concern to farmers not only due to the economic losses but also due to a decrease in the aesthetic value of the products. Aquaculture, while helping in increasing the volume of the produce also plays a big role in establishing fast spread of helminthiasis in a pond due to the confinement of the parasites

1.2 Hypothesis

The following hypotheses were tested during the study:

HO: fish kept under aquaculture in Nyeri county do not have helminth infestation.

H1: fish kept under aquaculture in Nyeri county have helminth infestations.

1.3Objectives

1.3.1General objective

To investigate and document the occurrence of helminthes in fish kept under aquaculture in Nyeri county

1.3.2 Specific objectives

The specific objectives of this study were to:

1.0 To establish the types of helminths affecting fish kept under aquaculture in Nyeri county, Kenya

2.0 To determine the prevalence rates of helminthes affecting fish kept under aquaculture in Nyeri County, Kenya

1.4 Justification

There is very little research and documentation on fish helminths despite the enormous importance of the subsector to the economy. The knowledge gap has scared off potential farmers and led to many economic losses for those who ventured into aquaculture

There is therefore need for more research on fish helminths to bridge the gap and provide farmers with solutions to one of the biggest challenges facing this subsector

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 AQUACULTURE IN KENYA

The main species farmed in aquaculture (trout, common carp and largemouth bass) were introduced into the country by European settlers in 1910 through the Kenya angling association (Achieng, 1994).with the establishment of the Sagana and Kiganjo fish culture stations in 1948 followed by the setting up of the Kenya Department of Fisheries in 1954(World Bank, 1985), aquaculture picked up pace

According to (FAO, 2003) annual intensive aquaculture in Kenya has been taken up by more farmers resulting in an upshot of production with the year 2007 registering 1500 tonnes of fish produced from aquaculture with volume rising to 15,000tonnes in 2010(**FAO, 2015**).Nyeri county produced 2180 metric tonnes of fish from the farm in 2012 putting it in front of all the other counties in the country

2.2 FISH SPECIES

The main species of fish kept in Kenya are *Oreochromis niloticus(*Tilapia*),Clarias gariepinus(*North African Catfish),*Cyprinus carpio*(Common Carp),*onchorhyncus mykiss(*Rainbow Trout*)* and,*Micropterus salmoides(*Largemouth Bass*)(*Balarin,1985;Nyandat,2004;FAO,2009*)*

Tilapia is endemic to the central and North Africa as well as the Middle East being a native of Israel (Anonymous, 2008; Picker& Griffiths, 2011).According to Mathenge C.M. (2010); it is one of the most common species in the tropics due to its tolerant nature being able to thrive in any environment apart from temperatures below11.1 degrees Celsius. Tilapia is found in

freshwater and estuarine habitats preferring shallow and still waters hence its abundance on the edges of lakes, in ponds, and shallow streams (Picker& Griffiths 2011). Their main source of feed consists of phytoplanktons, periphytons, aquatic plants, detritus, bacterial fauna and other fish and fish eggs (FAO, 2012)

The North African catfish (*Clarias gariepinus*) is one of the most important tropical catfish species and it's distributed widely almost covering the whole of the African continent as well Israel, turkey, and Syria (FAO, 2012). These fish inhabit calm waters hence preferring lakes, streams, flood plains and swamps and as Burton (1979a) and Clay (1979) pointed out, they are found in flood plains which later dry out and their accessory air breathing system helps in their survival. Their feeding pattern has not been clearly studied but they are generally considered omnivores (Micha, 1973; Burton, 1979b) feeding on insects, fish, plants, fruits and mollusks. This fish is a seasonal breeder, fawning during the rainy season (FAO 2012)

Onchorhyncus mykiss (rainbow trout) has a fusiform body with a coloration ranging from olive green to blue in freshwater (FAO, 2012). It's a cold water fish that is a native of North America from Alaska to Mexico. This species primarily inhabits the freshwater with little pollution and abundant supply of oxygen hence preferring fast flowing streams and open lakes according to (Picker & Griffiths, 2011). *O.mykiss* predates on small fish and their eggs, and invertebrates and spawns once per year in the wild (Skeleton, 2001). The favorite fawning grounds are gravel beds hence explaining their low distribution in calm smooth river beds and dams.FAO (2012) explains their tolerance to high temperature (they can survive in temperatures as high as 30 degrees Celsius) with the only caveat being their inability to reproduce in temperatures outside the 9-19 degrees Celsius

*Cyprinus carpio (*common carp*)* evolved in the Caspian Sea before migrating to the black and Arial seas (Balon, 1995). This species generally inhabits lakes, ponds and lower sections of rivers with moderately flowing or standing water(Barus et al,2001)adults feed on aquatic plants,detritus,small insects and planktons while larvae feed on zooplanktons(US Dept. of Interior,2015). Currently, as many as four subspecies have been identified even though other literature point to two. The four according to (Jhingran & Pullin, 1985) and they are *Cyprinus carpio carpio,* c.carpio*aralensis, c.carpio haematopterus,* and *Cyprinus carpio viridiviolaceus.* This species spawn in 2-3 periods annually in the tropics with these periods linked to rainy seasons and floods (FAO, 2012)

*Micropterus salmoides(*largemouth bass*)*is a native of North America inhabiting rivers and still waters of lakes and dams(Wheeler and Allen 2003).according to (Garcia-Berthou,2002),its distribution is worldwide with this species gaining popularity due to their role as sports fish(Brown et al,2009). Their distinctive feature is a very large mouth extending past the eye(Brown et al,2009) with their optimal temperature ranging from 24-30 degrees Celsius(Venables et al,1978;stuber et al,1982).spawning takes place between 13-26 degrees Celsius(Kelley,1868) with an optimum of 20-21 degrees Celsius(Clugston,1964). This occurs from late spring to early summer (Scott and Crossman, 1978) a t dusk or dawn. Their diet consists of insects, larvae, small fish and eggs (Brown et al, 2009)

2.3 FISH HELMINTHS

The helminthes parasitizing fish are classified as follows

2.3.1 Phylum Platyhelminthes

According to Myers (2002), this phylum consists of flatworms, which are dorsoventrally flattened and bilaterally symmetrical in addition to lacking an anus, skeletal, circulatory and respiratory systems. Most of them also are monoecious. This phylum consists of:-class Trematoda, class cestoda, class Turbellaria and class Monogenea (Smith, 2000)

Class Turbellaria consists of free living; tiny inconspicuous worms that mostly affect marine and freshwater fish (Smith, 2000). Due to their importance in fish, much attention is placed on the other three classes

-Class Monogea

These are commonly referred to as flatworms or flukes (Klinger and Floyd, 2002). These worms are found in the gills, skin and fin of freshwater and brackish fish especially the teleostei family species (Khalil, 1971; Paperna, 1979; Whittington et al, 2000). According to Klinger and Floyd (2002), they have a direct live history due to their host and size specificity

These worms are all hermaphrodites with apical sensory structures, with or without accessory suckers and clamps in addition to the mouth at the anterior ends (FAO, 1996). They are subdivided into other major taxa but the subclasses monopisthocotylea and Polypisthocotylea form the most important part of fish parasites (Noble and Noble, 1982). These parasites gain importance as internal helminthes due to the few specialized ones that inhabit the nasal passages, stomach and urethra of freshwater fish (Du Plessis, 1948, Ergens, 1988)

Subclass monopisthocotylea

These are primarily fish helminths occupying the gills, fins and skin of fish (Cone, 1995). They are either viviparous or oviparous with direct life cycles. Dactylogyroidae are oviparous with

most of them infesting the gills(FAO,1996)while Gyrodactyloidea consisting of Gyrodactylus, and Gyrodactyloidea are viviparous, host specific and are found on the skin and fins (FAO,1996b).*Dactyogyrus,Cichlidogyrus* and *Gyrodactylu*s are the main genera affecting fish from this subclass(FAO,1996b)

Subclass Polypisthocotylea

Unlike the Monopisthocotylids, this subclass consists of gill dwelling blood suckers (Cone, 1995). The genus *Diclidophora* is found in marine and primitive freshwater fish such as sturgeons and paddle fish while *Protopolystom*a are found in aquatic clawed toads (Cone, 1995)

Class Trematoda

Order Digenea

This order is characterized by two suckers; on the anterior and antero-ventral parts of their bodies (Mathenge C.M, 2010) there are over 50 species affecting freshwater fish in Africa (Khalil, 1971). These parasites have complex life histories with larval stages affecting juveniles, bottom dwellers and shallow inhabitants in Africa (Fahmy and Selim 1959; Williams and Chaytor, 1966; Van as and Bassoon 1984). Information on this group is limited due to the little research o their life cycles (FAO, 1996). They have multiple host life cycles with mollusks as the intermediate hosts (FAO, 1996). Fish can also act as the primary or intermediate host with these parasites affecting both the internal and external parts of the organs of hosts (Klinger and Floyd, 2002)

Trematode metecercariae the most commonly observed life stage in fish and normally affect different tissues of the same host according to Shaw et al(2005) but only extra intestinal species are harmful to the host(FAO,1996). Thrombosis may result from blockage of blood vessels by

adults and subsequent ischemia (Hoffman et al, 1985) while Evans (1974) noted the destructive nature of miracidia that cause anaemia due to blood loss during migration these worms also modify the morphology and hence the physiology of fish by disrupting the heart, brain and eye lens (Sandland and Goater, 2003; Coleman, 1993, Hoffman and Hoyne, 1958, Larsen, 1965).

Trematodes include *Posthodiplostomnum, Clinostonum, Heterophylus* (encyst in the gill tissue causing lots of mortalities). Family Heterophyidae, genus *Heterophyes* and *Haplorchis spp* encyst in internal organs while the family Clinostomatidae, genus *Clinostomum* is found on the skin and internal organs. Family Diplostomatidae, genus *Diplostomum spp* and *Naescus spp* and *Posthodiplostomum spp* are found on the skin, gills and eyes. On the other hand, *Sanguinicola spp* are found in blood vessels as adult worms (Mathenge.C.M, 2010)

Class Cestoda

These worms are generally called tapeworms and their strobila is divided into proglottids each containing a simple reproductive system apart from order Caryophyllaeidea that is not segmented (Schmidt, 1986). They also contain a scolex at the anterior end and adults are parasitic in the intestines while he larvae encyst the viscera and muscle (Mbuthia et al, 1993)

Examples include *Diphylobothrium spp, Proteocephalus Glanduliger, Polyonchobothrium clarias* and *Bothriocephalus acheilognathi*

2.3.2 Phylum Aschelminthes/Nemathelminthes

Class Nematoda

Also called roundworms and they are bilaterally symmetrical elongated worms with a body tapering at both ends. The common nematodes affecting fish are: -*Camillanus,Capillaria, Anisakis* and *Eustrongiloides*

Camillanus is thread-like and normally protrudes from the fish anus while *Capillaria*is a largeroundworm common in the gut of angelfish. *Eustrongiloides* uses fish as an intermediate host with the definitive host being the wading bird. It normally encysts in the peritoneum or muscles of fish causing little or no harm.

According to the Animal Health and Production Compendium(2011), Adult *Anisakis* spp occur in whales and seals whereas their third-stage larvae are found in fish muscles.*Anguillicola, philometra, skrjabillanus* and *Anisakis* are all zoonitic causing varying degrees of pathology in humans. In fish, nematodes cause emaciation and anaemia as the two most common clinical signs (AHPC, 2011)

2.3.4 Phylum Acanthocephala

These are sometimes known as spiny or thorny headed worms and have elongated cylindrical bodies with hooks on their proboscis(Mathenge C.M.2010).they have no gut and sexes are separate and they require at least one intermediate host(FAO,1996b;Paperna,1964). The adult worms are all gut parasites as illustrated by George & Nadakal (1973)

Their most pathology occurs due to adult attachment to the digestive tract or larval encysting hence causing granulomas,fibrosis and occasionally peritonitis in severe infections, mostly in farmed fish (Bullock, 1963; Bauer, 1959). Blockage of the gastrointestinal tract has also been noted

The main species of hookworms are *Neoechinornhynchus* spp, *Heterotis niloticus, Citharinus citharus, Acanthocephalus* spp, *Pomphornchynchus* spp and *Acanthogyrus tilapiae*

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The field study was carried out at various fish farms owned by private farmers in Nyeri County. In total, 50 fish farms were sampled using stratified random sampling technique across Tetu constituency

This county is located 152 kilometres North East of Nairobi with an altitude of 1,768 M. The mean annual rainfall received ranges from 1200mm To 1600mm with tea farming, coffee farming,horticulture,livestock and aquaculture as the main economic activities(Jacob et al,2007)

3.2 Sampling

50 samples were collected with the following representations:-25 *Oreochromis,* 16 *Clarias* and 9 *Cyprinus*. The systematic random sampling ensured that the even distribution was adhered to hence reducing sampling errors

Evisceration was done at the site and the organs of interest (gastrointestinal tract) collected, labelled, recorded and put in 70% alcohol and transported to the department of veterinary pathology microbiology and parasitology laboratory

During harvesting of the gastrointestinal tract, the fish were stunned with a blow at the back of the head and then pitted (Theodore, 2000). The fish were each placed on the dissection tray and a midline incision made to expose the internal organs. The body wall was deflected to have a better exposure of the viscera. The stomach and the intestines were then detached and placed in the 70% alcohol

3.3 Examination of the gastrointestinal tract

Each single piece of the tract was picked and placed on the dissecting microscope and with a dissecting needle; worms were identified, isolated and harvested under different magnifications. Depending on the content of the fish gut, the whole portion was taken and examined but if the contents were a lot, an aliquot was taken after thorough mixing and examined. The counting of the worms was done and then multiplied by the original volume of the contents (Robert, 1989)

3.4 Processing of helminths

For the nematodes, three worms were picked, put on a slide. Drop of lactic acid was added to clear the fat and debris. The acid digest the debris for 30 minutes then examine under a microscope starting with x5 magnification

Platyhelminthes were stained with aceto carmine stain before a histological staining procedure was done starting that was shortened to a slow differentiation in 0.5% hydrochloric acid in 70% alcohol for 1-12hours depending on the worm size (Vet Pathology, 2015). They were then stained using heidenhain's azan stain (Roberts, 1989)

3.5 Identification of helminths

Worms that were isolated were counted, recorded and stored in 70% alcohol. identification of nematodes was through use of morphological features as described by Anderson et al(1974) and FAO(1996b) while that of acanthocephalans was through identification of the features, especially that of hooks(Kabata,1985) . Platyhelminthes were identified using sizes and shapes of scolex of suckers among others (Schmidt, 1986)

3.6 Data analysis

The data collected was recorded and analyzed for interpretation using Microsoft Excel to come up with tables, pie charts, and graphs. These then formed the basis for the discussion and conclusions

CHAPTER FOUR

4.0 RESULTS

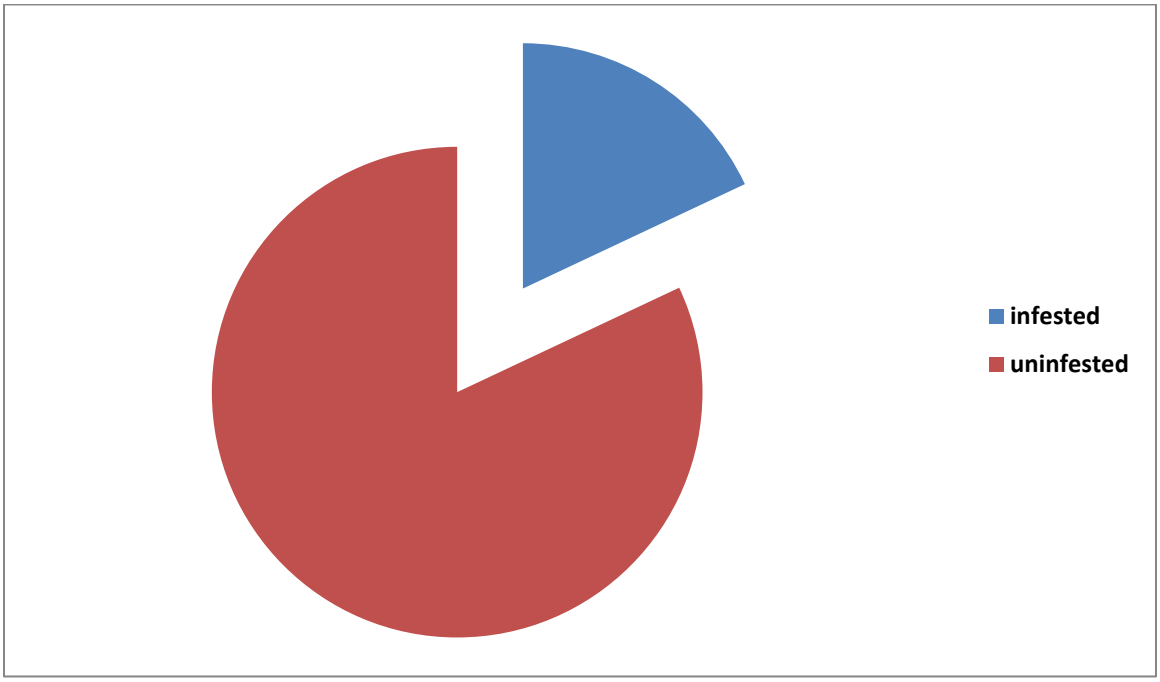
A total of 50 samples were examined and this comprised of 50% *Oreochromis spp*, 32% *Clarias spp* and 18% *Cyprinus spp*. Of the 50 samples, 9 tested positive for helminths. Of the 9 helminths, 4 were found in *Oreochromis spp*, 3 in *Clarias spp* and 2 in *Cyprinus spp*. The five helminths affecting the tilapine species were *Heterophylus spp* (2), and *Clinostomum spp* (2).

Those found in the fish from the *Clarias spp* included *Heterophylus spp* (1), and *Clinostomum* (2). *Cyprinus spp was* infested with *Heterophylus spp* (1) and *Clinostomum spp* (1)

Table 1: Prevalence of different helminths in the three sampled fish species

Fish species	**Sample size**	**Total worms**	**Helminth species/number**		**Percentage of total helminths (%)**
Oreochromis spp	25	4	*Heterophylus spp*	2	22.22
			Clinostomum spp	2	22.22
Clarias spp	16	3	*Heterophylus spp*	1	11.11
			Clinostomum spp	2	22.22
Cyprinus spp	9	2	*Heterophylus spp*	1	11.11
			Clinostomum spp	1	11.11
Total	**50**	**9**	**2**	**11**	**100**

Figure 1: Helminth infestation in the sample population



This pie chart shows clearly that helminth infestation in farmed fish farmed in Nyeri county stands at 18% and this corroborates what Khalil(1997) said about helminth infections in Africa

Figure 2: Sample sizes taken

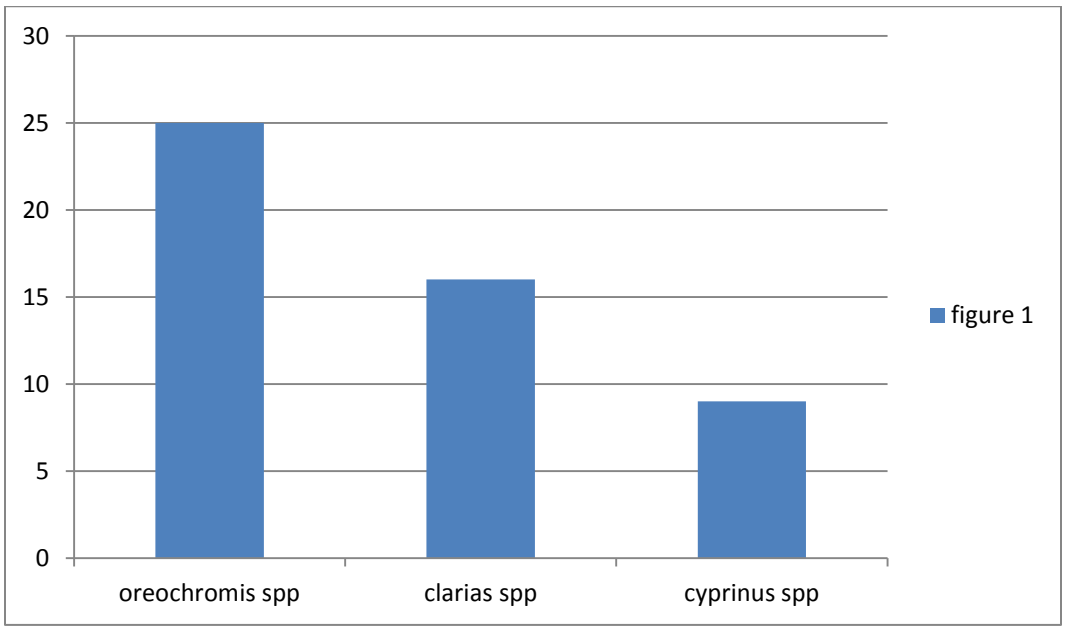
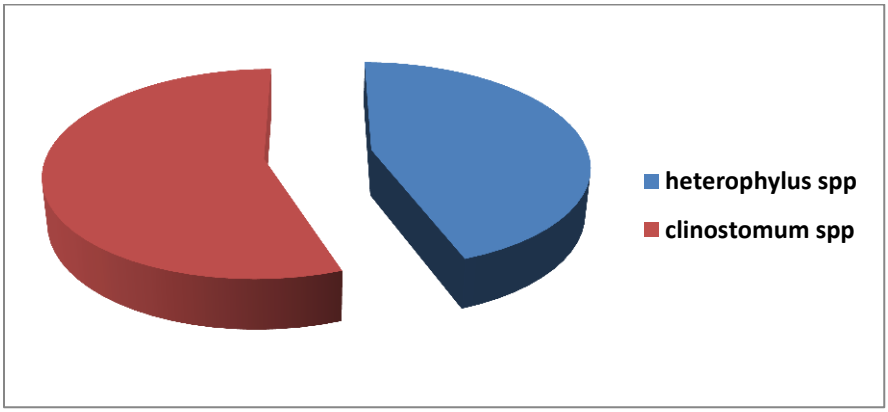
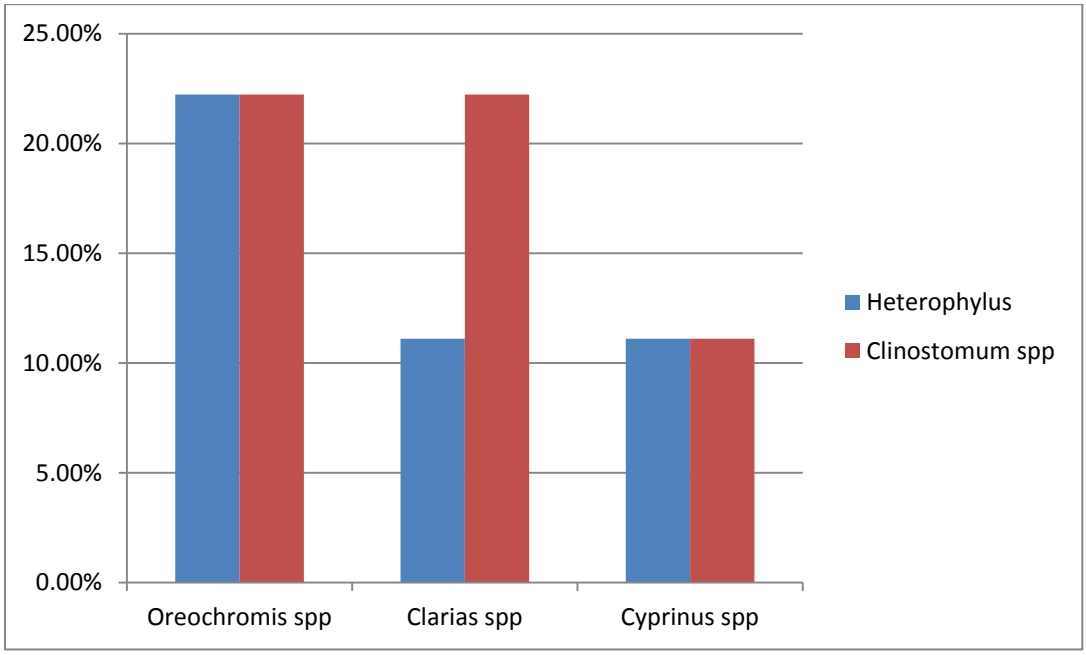


Figure 3: Individual helminth prevalence rates



Clinostomum spp lead the way in the infestations with their effects and encystations in the muscles previously more researched on than their presence in the gastrointestinal tract.

Figure 3: Prevalence rates of each helminth species per fish species



The prevalence rate of *Clinostomum spp* (metecercariae) has a higher prevalence rate in all the three fish species apart from *Cyprinus spp* where its prevalence is similar to that of *Heterophylus spp*

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

According to this study, farmed fish are mildly infested with gastrointestinal helminths that pose a major challenge to aquaculture. The helminths recovered were digeneans (trematodes- *Heterophylus spp* and *Clinostomum spp*) with a prevalence rate 18 %(n=50). Looking at the negligible difference in the prevalences of *Heterophylus spp* (8%; n=50) and *Clinostomum spp* (10%; n=50) clearly shows that trematodes are the most common gastrointestinal helminth class in farmed fish in Nyeri county.

According to previous studies in Africa, digeneans were more prevalent in farmed fish (Douellou, 1992.a.b.Barson and Avenant-Oldewage, 2006). This may be attributable to the good water quality in aquaculture that favours trematodes and their intermediate hosts (Mathenge C.M, 2010)

The trematodes (*Heterophylus spp* and *Clinostomum spp*) had a prevalence rate of 18 %(n=50) overall but 16 %(n=25) in *Oreochromis spp*, 18.75 %(n=16) in *Clarias spp* and 22.22% in *Cyprinus spp* (n=9). This was similar to works done by Violante-Gonzalez et al (2009) in Mexico that showed a higher prevalence of trematodes in tilapia fish than the other fish. Considering the fact that fish digeneans are intermediate host specific, their occurrence is dependent on availability of particular snail species (Mackenzie, 1983) hence the presence of snails in fish farms in relatively high numbers explains the higher prevalence of digeneans

This study also corroborates Dayhoum (2003) who noted *Clinostomum tilapiae* in high numbers in Egyptian farmed fish

In addition to the intermediate hosts, digenean distribution also mirrors that of their definitive hosts-the piscivirous birds hence migration of these birds may lead to redistribution of these worms

5.2 CONCLUSIONS

1. Gastrointestinal helminths are prevalent in farmed fish in Nyeri County, Kenya.
2. Evidence also points to trematodes from the order digenea being the most prevalent with *Heterophylus spp* and *Clinostomum spp*, the worms recovered

5.3 RECOMMENDATIONS

From this and previous limited studies, it's clear that more research needs to be dedicated to clearly mapping out the prevalences, intensities and distribution of gastrointestinal helminths in fish in Africa

Intervention strategies should also be formulated to cut down on the losses by farmers and avoid a crisis in this important part of the agriculture sector

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