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Helminth parasites of farmed fish and water birds in Kirinyaga County, Kenya

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Abstract
Aquaculture products demand has increased due to population growth, declining natural fish stocks and government aquaculture promotion. Intensification alters natural fish environment and new infections emerge. Parasite infested fish are unaesthetic. Public health concerns arise with zoonotic fish parasites. Piscivorous birds influence aquaculture profitability due to predation and transmission of fish parasites. 289 fish from 15 private farms and one government farm were sampled in Kirinyaga County, Kenya. Earthen pond fish had higher (52%) parasite infestation than those from liner (7%) and concrete ponds. Fish parasites recovered were *Diplostomum* spp. (tilapia 21.7%; catfish 10.5%), *Acanthocephala* spp. (tilapia 11.3%; catfish 3.5%), *Dactylogyrus* spp. (tilapia 3%; catfish 4.7%) *Contracaecum* (catfish 24.4%), *Paracamallanus* spp. (catfish 16.3%) *Gyrodactylus* spp. (catfish 5.8%) and *Clinostomum* spp. (tilapia 4.9%). Fifty water birds were captured using mist nets. On necropsy, 46% were infected with parasites like *Acanthocephala* spp. (16%), Pseudophyllid and Protecephallid cestodes (36%) *Contracaecum* spp. (2%) and *Clinostomum* spp. (2%). *Clinostomum* spp., *Contracaecum* spp. and *Acanthocephala* spp. are zoonotic. Farmers, traders and consumers should be advised on handling and cooking fish to avoid infestation with zoonotic parasites. Control of piscivorous birds and other predators should be effected to maximize the profitability of fish farming.

Keywords: Fish parasites, piscivorous birds, aquaculture, *Clinostomum*, *Contracaecum*

1. Introduction

The increased trends in fish farming over recent years in Kenya has brought about the need to monitor the health of fish stock to produce fish that is safe for human consumption. Intensification in aquaculture alters the natural environment for fish hence new infections are cropping up due to increased stress levels and reduced immunity [1]. Kenya in its vision 2030 aims to be a food secure country with fish farming forming one of the strategic pillars. The demand for aquaculture products is high due to a rapidly growing population, declining natural fish stocks and active fish farming promotion by the government [2].

Fish are known to be hosts to various ecto- and endo-parasites. Fish infested with parasites can be unsightly and their market value greatly decreased [3]. There are also public health concerns as some fish parasites are zoonotic [4, 5]. Intensive fish farming encourages propagation of parasites and can result to serious outbreaks. The number of parasites necessary to cause harm to fish varies considerably with fish species, size, and health status [6]. Tilapia and catfish are the common species of fish farmed in many regions in Kenya. Helminths affecting fish include Platyhelminthes (flatworms) and Nemathelminthes (round worms). Flatworms of the class monogenea are ectoparasites infesting skin and gills of fish while digeneans and cestodes are endoparasites. Intermediate stages of digenean parasites are found in fish as metacercariae while adult stages are in predatory birds and mammals. Most helminth parasites have indirect life cycles involving one or more intermediate hosts. Studies on fish parasites have been done in some regions in Kenya [7–10]. Information is still lacking on the nomenclature of some fish parasites, their life cycles and those of public health concern in farmed fish in Kenya.

In aquaculture, water birds influence profitability of fish farming due to predation [11] and being involved in the life cycle of fish parasites [12]. Fish eating birds are spread throughout the fish farming regions of Kenya with their numbers differing between seasons. They include: Ciconiiformes (storks, egrets, hamerkops and herons), Plecaniformes (cormorants and darters), and Halcyniformes (kingfishers).

Predatory birds have been observed in many regions preying on fish from ponds. They are definitive hosts of fish parasites like, *Clinostomum* spp. [13], *Diplostomum* spp. [14], *Contracaecum* spp. [15] and Gryporhynchidae cestodes [16] among others. Studies on type and extent of fish predation in Kenya are few with only Ogoma (2012) [17] undertaking a baseline survey on water birds and fish farmers conflict in coastal Kenya. There is no adequate documentation of the specific role of water birds in the life cycles of various fish parasites in Kenyan aquaculture other than the study of Gustinelli *et al.* (2010) [13] on herons. Thus, this study was conducted to determine occurrence of fish parasites and link them to those found in piscivorous birds in Kenya.

2. Materials and Methods

2.1 Study area

This study was done in Kirinyaga County, Kenya, which is found 100 km North East of Nairobi, at an altitude of 1230 m above sea level, latitude 0°39'S and longitude 37°12'E. Annual temperatures range between 12 °C and 26 °C and annual precipitation averages about 1250mm. Three sub counties were purposively selected based on the availability of active fish ponds and presence of fish predatory birds. These were Kirinyaga Central, Kirinyaga West and Mwea East.

2.2 Sampling

The sample size (n) was determined using the formula of Naing *et al.* (2006) [18]: $n = \frac{z^2 p(1-p)}{d^2}$ Where, *z* is the *z* value for the corresponding confidence level (1.96 for 95% confidence); *d* is the precision (5%) and *p* is the estimated proportion of a sample that have the condition of interest {15% parasitic prevalence (Otachi *et al.,* 2011) [19]}. This gave a minimum sample size of 200 fish. A list of fish farmers was obtained from the county Fisheries Department. Those with active fish ponds were identified and their verbal consent sought before sampling. Fifteen private farms and one government farm were selected where ten fish per pond were purchased. More fish were purchased in one government farm and one private farm where higher presence of fish predatory birds was noted during the study period. During the study period, 50 tilapia from Kirinyaga Central, 51 tilapia and 40 catfish from Mwea East and 102 tilapia and 46 catfish from Kirinyaga West sub-counties were acquired. Fish were transported live to the laboratory where they were sacrificed and post mortem examination done using standard procedures [3, 20]. Direct microscopic examination at X10 and X40 was done on samples from the skin, gills, eyes and intestine for any parasites. The gastrointestinal tract from the fish was collected and preserved in 70% ethanol for parasitological analysis.

Capture of water birds was done with permission from Kenya Wildlife Service. Mist nets were set up in Sagana Aquaculture Center and Mwea Aqua fish farm to capture the birds. Fifty water birds of different species were captured during the study period. The birds were transported to the laboratory in cages where they were euthanized by cervical dislocation and post mortem examination done as described by Brown (2012) [21]. The gastrointestinal tract from the birds was collected and preserved in 70% ethanol for parasitological analysis.

2.3 Isolation and identification of parasites

Fish and bird intestines were opened longitudinally using scissors and dissecting pins. The contents were expressed, washed with water and scanned for parasites using a dissecting microscope. Parasites isolated were counted and observed on a compound microscope at X10 and X40 magnification. Parasite identification guides by Chubb *et al.* (1987), Hoffman (1999), Woo (2006) and Roberts (2012) [3, 14, 22, 23] were used in the morphologic identification of parasites isolated.

2.4 Data analysis

Data were entered into the computer, cleaned and sorted using Microsoft Excel 2016 and analyzed using the Statistical Package for Social Sciences (SPSS version 16.0) and Epi Info version 7. Descriptive statistics consisting of frequencies were computed for different data categories to facilitate comparisons of parasitic infestations between fish farms, fish species and water birds. Chi square test was used to compare proportions and prevalence of parasite infestations. Student's t-test and p values were used to determine statistical significance of the results.

3. Results

3.1 Helminth parasites of fish

Out of the total 289 fish, 203 were tilapia with a mean weight of 130.5±79.5 g while 86 were catfish with a mean weight of 392±2.88 g. Of these, 20% (10/50) from Kirinyaga Central, 34% (31/90) from Mwea East and 52% (77/148) from Kirinyaga West sub-counties were infested with at least one species of parasites. Fish from earthen ponds (52%) (114/219) were found to be more infested with parasites than liner (7%) (4/60) and concrete ponds (0/10). Piscivorous birds and snails of the genus *Melanoides* were noted in many areas.

3.2 Prevalence of fish helminth parasites

The prevalence of parasites recovered from the sub-counties are shown in Fig 1. The highest prevalence of *Diplostomum* spp. was in Mwea East sub-county. *Contracaecum* spp. and *Clinostomum* spp. were isolated from Kirinyaga West sub-county only.

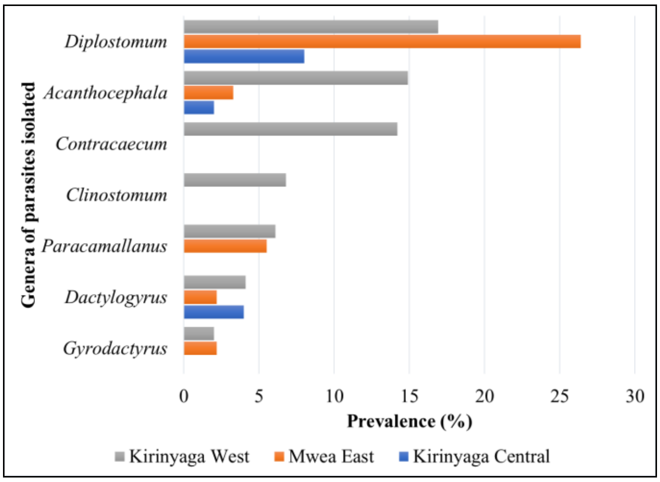

Fig 1: Prevalence of fish helminth parasites in Kirinyaga County

Among the farms visited during the study period, two farms namely Sagana Aquaculture Center in Kirinyaga West sub-county and Mwea Aqua fish farm in Mwea East sub-county had significantly higher parasitic infestations ($p<0.05$) than the rest of the farms with an infestation rate of 69% and 68% respectively while the rest had a below 50% level of infestation. Mwea Aqua Fish Farm had a higher infestation with *Diplostomum* spp. (59%) than Sagana Aquaculture Center (23%). *Contracaecum* spp., *Acanthocephala* spp. and *Clinostomum* spp. were isolated from Sagana Acuaculture Center and were not found in Mwea Aqua Fish Farm (Fig 2). However, there was no significant difference between overall parasitic infestation rates of the two farms (p>0.05).

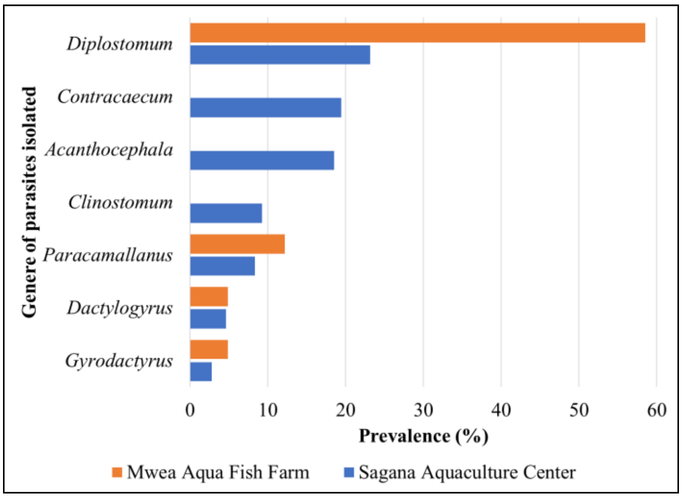

Fig 2: Prevalence of parasites from the major breeding farms

In all, 39% (79/203) of tilapia and 45% (39/86) of catfish examined were infested with at least one species of helminth parasite. There was no significant difference between the infestation rates of the two species (p=0.45, risk ratio=0.86). There was also no significant difference of infestation between fish sexes as 39% (82/208) of males and 44% (36/81) of females had at least one species of parasite (p=0.57, risk ratio=0.89). However, fish recovered from earthen ponds (52%) (114/163) had significantly higher parasitic infestations relative to those from liner ponds (7%) (4/60) (p<0.05, risk ratio=7.43). The overall prevalence of various helminth parasites recovered from the fish are shown in Table 1. *Contracaecum* spp., *Gyrodactylus* spp., and *Paracamallanus* spp. were recovered from catfish only while *Clinostomum* spp. was recovered only from tilapia fish.

Table 1: Prevalence of helminth parasites recovered from different fish organs in Kirinyaga County

Parasite (Genus)	Organ	Prevalence (%)	
		Tilapia	Catfish
Acanthocephala	Intestines	11.3	3.5
Clinostomum	Muscles, skin	4.9	0.0
Contracaecum	Abdominal cavity	0.0	24.4
Dactylogyrus	Gills	3.0	4.7
Diplostomum	Eyes	21.7	10.5
Gyrodactylus	Skin, gills	0.0	5.8
Paracamallanus	Intestines	0.0	16.3

3.3 Intensities of fish helminths
3.3.1 Monogeneans
The monogeneans isolated were *Dactylogyrus* spp. and *Gyrodactylus* spp. (Fig 3). The mean intensity of *Dactylogyrus* spp. in tilapia was 1.2±0.4 while, in catfish it was 1.3±0.5 with an abundance range of 0-2 parasites in both species. There was no statistical difference between the intensities in the two species (t=-0.29; df=8). The intensities in male and female fish were 1.0 and 1.3±0.5 respectively which was not significantly different (t=-0.98; df=8).

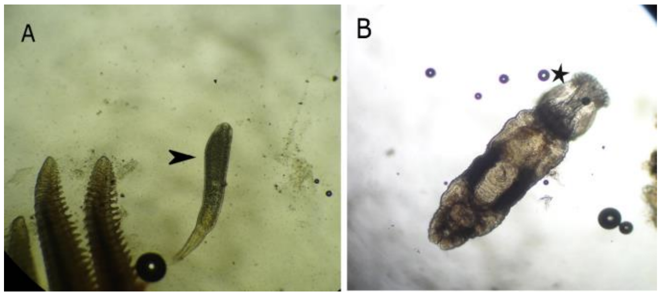

Fig 3: A). *Dactylogyrus* spp. from the gills of tilapia (arrow head) and B). *Gyrodactylus* spp. from the skin of catfish showing the haptor (star)

3.3.2 Digeneans
The mean intensity of *Diplostomum* spp. (Fig 4) in tilapia was 2.5±2.2 with an abundance range of 0-10 while, in catfish it was 1.9±1.4 worms per eye with an abundance range of 0-5 in the sampled fish. There was no significant difference in the intensities of *Diplostomum* spp. in the two species (t=0.783; df=51). The mean intensity in the males was 2.6±2.3 while, in females was 1.4±0.7 showing no statistical difference (t=1.675; df=51).

Clinostomum spp. was isolated from tilapia only with a mean intensity of 9.6±14.7 (std. error=4.7) with a range of 1-48 metacercarial cysts per fish in the infested fish. The mean intensity of *Clinostomum* in the male fish was 5.2±7.1 while in female fish was 19.7±24.8 which was not statistically different (t=-1.507; df=8).

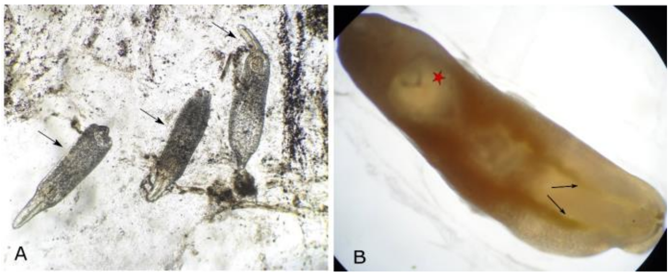

Fig 4: *Diplostomum* spp (arrows) recovered from the vitreous humor of examined fish (A) and *Clinostomum* spp from the skin of tilapia showing the anterior sucker (red star) and intestinal caeca (arrows) (B)

3.3.4 Cestodes
Two catfish from Sagana National Aquaculture Center were infested with a Pseudophyllidean and a Proteocephallid tapeworm, respectively (Fig 5).

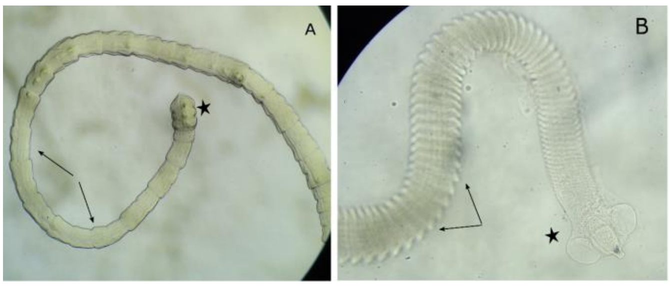

Fig 5: A). Pseudophyllidean and B). Proteocephallid cestodes from the intestines of catfish showing the scolex (star) and proglottids (arrows)

3.4 Intensity of nematode parasites

3.4.1 *Contracaecum* species

Contracaecum spp. (Family Anisakidae) was isolated from the peritoneal cavity of 20 catfish from Sagana National Aquaculture Center with a range of 2-56 worms per fish (Fig 6). The overall mean intensity was 15±13.3 (std. error=3) worms per fish. The mean intensity in males was 15.1±9.5 while, in females was 14.8±16.2 and was not significantly different (t=0.048; df=18).

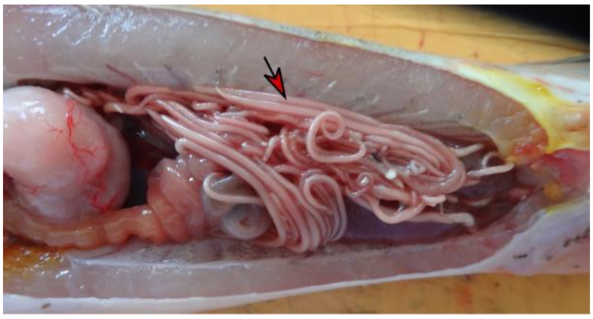

Fig 6: *Contracaecum* worms (arrow) in the peritoneal cavity of catfish from Sagana Aquaculture Center

3.4.2 *Paracamallanus* species

These worms were isolated from catfish with an abundance of 0-12 and a mean intensity of 3.8±3.1 worms in the infested fish (Fig 7). The mean intensity in males was 3.7±2.1 while in females was 4±5.3 showing no statistical difference (t=0.033; df=12).

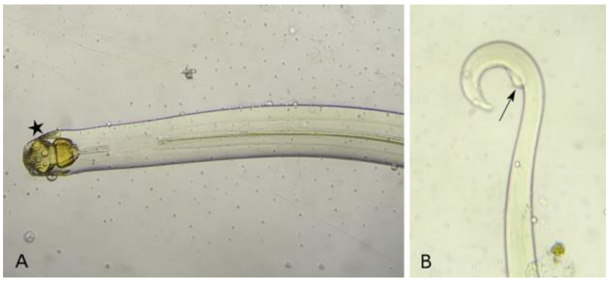

Fig 7: Anterior segment (A) of *Paracamallanus* spp. from the intestines of catfish showing the buccal capsule (star) and Posterior segment (B) with the genital flap (arrow)

3.5 Intensity of Acanthocephala species

Acanthocephalan worms were common in both catfish and tilapia (Fig 8). The mean intensity in tilapia was 1.5±0.8 with an abundance range of 0-4 and while, in catfish it was 1. The mean intensity was not significantly different between tilapia and catfish (t=1.05; df=24). The mean intensity in males was 1.5±0.8 while, in females it was 1.4±0.9 showing no statistical difference (t=0.185; df=24).

Fig 8: *Acanthocephala* spp. showing proboscis with hooks (arrow) (A) and with retracted proboscis (B)

3.6 Parasitic infestations of water birds

Piscivorous birds of different species were captured from Sagana Aquaculture Center and Mwea Aqua Fish Farm. Of the 50 water birds of different species captured, 46% (23/50) were infested with at least one species of helminth parasite on necropsy examination. Infested birds whose diets mainly consists of fish include the herons, kingfisher, cormorant, spoonbill and hamerkop.

3.7 Prevalence of helminths in piscivorous birds

Of the captured birds, 34% (11/32) form Sagana Aquaculture Center and 67% (12/18) from Mwea Aqua Fish Farm were infested with at least one species of helminth parasite.

3.7.1 Acanthocephalans

One pied kingfisher (*Ceryle rudis*), two giant kingfishers (*Megaceryle maxima*), one wood sandpiper (*Tringa glareola*), one great painted snipe (*Rostratula benghalensis*), two hamerkops (*Scopus umbretta*) and one Temminck's stilt (*Calidris temminckii*) had acanthocephalan worms on necropsy examination. The overall prevalence of acanthocephalan worms was 16% and were only recovered in birds from Mwea Aqua Fish Farm.

3.7.2 Cestodes

The overall prevalence of cestode infestation in water birds from the two farms was 36%. These were isolated from one black winged stilt (*Himantopus himantopus*), one striated heron (*Butorides striata*), two giant kingfishers, two great painted snipes, three (3) three banded plovers (*Charadrius tricollaris*), two temmink's stilts, one common snipe (*Gallinago gallinago*), one grey heron (*Ardea cinerea*), one long toed plover (*Calidris subminuta*), one reed cormorant (*Microcarbo africanus*), one African spoonbill (*Platalea alba*), one spur winged plover (*Vanellus miles*) and one yellow billed duck (*Anas undulata*). Dilepidid cestodes (Cyclophyllidea) were identified in the cormorant and Proteocephalid cestodes from the African spoonbill (Fig 9, Fig 10).

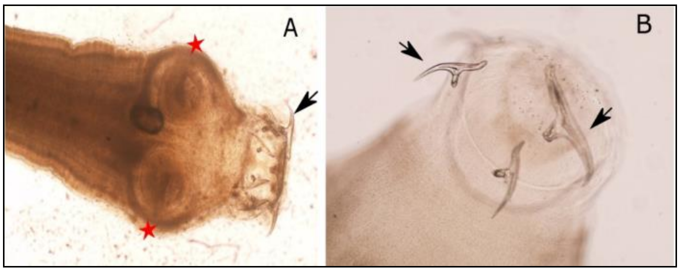

Fig 9: Armed scolexes of Dilepidid cestodes (A and B) from the reed cormorant with hooks (arrows)

Fig 10: Cestode scolexes isolated from the African spoonbill (A and B) showing suckers (red stars) and hooks (arrows)

3.7.3 Nematodes
Adult *Contracaecum* spp. (Family Anisakidae) were only isolated from one grey heron from Sagana Aquaculture Center (Fig 11) with a 2% prevalence.

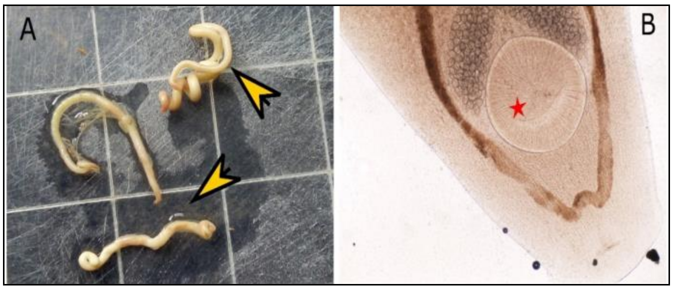

Fig 11: Adult *Contracaecum* spp. (arrows) (A) and *Clinostomum* spp. (B) from the grey heron showing the anterior sucker (red star)

3.7.4 Digenean trematodes
Clinostomum spp. was recovered from one grey heron from Sagana Aquaculture Center with an overall prevalence of 2% (Fig 11).

3.8 Comparison of parasites of fish and birds
Parasites were common to both fish and water birds. The prevalence of *Acanthocephala* spp. in fish was 11.3% in tilapia and 3.5% in catfish while in birds it was 16%. *Contracaecum* spp. was present in catfish and grey heron while *Clinostomum* spp. was present in tilapia and grey heron. Cestode parasites were recovered from catfish and birds including the reed cormorant and African spoonbill.

4. Discussion
Kirinyaga West Sub-county had the highest parasite infestation levels due to presence of Sagana Aquaculture Center which was frequented by many species of birds. The same scenario was replicated in Mwea Aqua Fish Farm in Mwea East sub-county which had the highest *Diplostomum* spp. infestations. Migration of birds poses a risk of spreading parasites and other disease causing pathogens from one region to another. At times, it is difficult to completely keep birds off fish farms without incurring costs. During this study, it was established that parasites are equally common to fish (tilapia and catfish) as well as water birds. Of the water birds sampled, the fish-eaters were infested with more than one species of parasites. Piscivorous birds play a role in the life cycles fish parasites and also cause losses when they feed on fish. Earthen ponds had the highest levels of parasitic infestations due to the interplay of vegetation growth, presence of snails and piscivorous birds in these ponds.

Monogenean trematodes reported in this study were *Dactylogyrus* spp.and *Gyrodactylus* spp. Poor management practices like overstocking of fish ponds and poor environmental promote heavy infestations with monogeneans and can result to fish losses [24]. Though monogeneans have a direct life cycle, it is possible for other hosts like birds to be infested as accidental hosts and spread the parasites [25]. Two Digenean parasites, *Diplostomum* spp. and *Clinostomum* spp. were recovered in this study. The metacercariae of Diplostomatid eye flukes are found in the vitreous humor of the eyes in fish without major pathological effects. However, cases of cataract are seen with *D. spathecum* which is found non-encysted in the lens. This causes blindness and fish are more prone to predation [26]. This was however not recovered in this study. The adult forms of digenean parasites are found in piscivorous birds [27]. Snails of the genus *Melanoides*, known intermediate hosts of digenean trematodes were recovered in various sampling sites. *Clinostomum* species of the *'cutaneum'* group were isolated in both fish and piscivorous birds (grey heron) in agreement with a study by Gustinelli *et al.* (2010) [13], that was done in Sagana Aquaculture Center. This parasite causes 'yellow grubs' that make fish unsightly when present in large numbers. This causes economic losses due to rejection by consumers [3, 5]. Human cases of *Clinostomum* spp. infestation have been reported in Korea [28] and Japan [29] hence a public health concern. Human infection has not been reported in Kenya.

Cestodes of the families Dilepididae, Pseudophyllidae and Proteocephallidae were recovered in birds more than fish. Only two catfish had a tapeworm each in the intestines. Water birds, mainly piscivorous species have been reported to harbor tapeworms which can spread to fishes and humans. Cyclophyllidean cestodes of the family Dilepididae have been described in piscivorous birds in Mexico [30]. Proteocepalidae cestodes [31, 32] also affect fish. *Diphyllobothrium latum*, *Proteocephalus* spp. and *Caryophyllaeidea* spp have been reported in Kenya by Khamis *et al.* (2017). *Diphyllobothrium* spp have been reported to be zoonotic [33]. This species was however not recovered during this study.

Fish nematodes recovered in this study were *Contracaecum* and *Paracamallanus* spp. in catfish only. *Contracaecum* in catfish has been reported in other regions in Kenya [9, 10] and Zimbabwe [34]. This parasite has been reported in tilapia and carp in other studies [5, 35, 36]. Sagana Aquaculture Center had a 19% prevalence of *Contracaecum* spp. Although this farm kept both tilapia and catfish, some even in the same ponds, tilapia species were not infested with *Contracaecum* spp. This parasite was also recorded in piscivorous birds by Barson and Marshall, (2004) [15] in Zimbabwe by genetic and electric microscopy comparisons of the larvae from fish and adults from birds. Parasites of the Anisakidae family are reported to be zoonotic. Allergic reactions have been reported where sensitization occurs on ingesting live parasites in raw fish or those killed by cooking or pasteurization [37]. Experimental infestation of *Contracaecum* spp. in the domestic cat resulted to successful development to adults causing hemorrhages in the intestine [38]. *Contracaecum* spp. is thus an important parasite with zoonotic potential. Camallid nematodes were recovered in catfish. These are common parasite to this species of fish without major pathological effects [39].

Acanthocephalans, or thorny-headed worms commonly parasitize fish, amphibians, birds and mammals. Low and moderate infestations result to localized changes but heavy infestations have been reported to cause granulomas and fibrosis in the intestines [40]. Florio *et al.* (2009) [5] described infestations of tilapia fish with *Acanthosentis tilapia* in East Africa. Infestations of humans with acanthocephalans after eating raw or undercooked fish has been documented [41].

Piscivorous birds, mainly herons, cormorants and ibis which had more parasitic infestations were difficult to capture using mist nets in the fairly open landscape of Sagana Aquaculture Center and Mwea Aqua Fish Farm. During this study, mists nets were the available capture method as the capture permit restricted use of shooting to catch specific birds. Bird capture methods can be fairly expensive hence restricting our options due to financial and time constraints.

5. Conclusions
- Water birds and fish were infested with helminth parasites

- Earthen ponds were more predisposed to parasite infestations than liner and concrete ponds.
- Parasites of zoonotic importance like *Clinostomum* spp, *Contracaecum* spp and *Acanthocephala* spp. were identified in farmed fish in Kirinyaga County.

6. Recommendations
- Farmers should be made aware of risks of parasitic infestations and other diseases of fish and the need to consult qualified experts in such cases
- Farmers, traders and consumers should be advised on handling and cooking fish to avoid infestation with zoonotic parasites
- Proper predator control methods should be undertaken in fish farms

7. Acknowledgements
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