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Prevalence of metazoan parasites in Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) raised in a community dam and a private farm, Eastern Province, Rwanda

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Abstract
In Rwanda, efforts exist to intensify aquaculture production. In intensive systems, fish coexists with pathogens, hence higher risks of parasitism that might affect growth and lead to diseases. Furthermore, literature on aquaculture parasitology and epidemiology is limited, hence the need for this study. We investigated the parasite burden and the relationship between husbandry and parasitism in dam and pond reared fish in the eastern province. A cross-sectional study was adopted using a questionnaire to collect management data, and fish samples collected for parasite analysis. Parasitology data was analysed using Microsoft Office Excel 2010, and management data summarised into a table. Findings revealed higher parasitism in dam reared fish than in pond fish; with *Goezia sinamora* (48.3%) as the most prevalent parasite. Poor husbandry influenced fish parasitism among dam-reared fish. This is the first study to elaborate parasite burden in Rwanda's farmed fish, and sets the scene for further epidemiological studies.

Keywords: epidemiology, metazoan parasites, Nile tilapia, pond/dam reared fish

1. Introduction
The Rwandan aquaculture sector received a boost in 2011 from the Inland Lakes Integrated Development and Management Support (PAIGELAC) project helping to transform the industry from subsistence to a commercial scale to increase fisheries productivity [1]. The PAIGELAC project projected to improve fishery production from 7,000 to 25,000 metric tonnes by organising fishers and farmers into cooperatives, provide fish seed, trainings and financial support. By 2013, a total of 5,600,000 Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) fingerlings had been distributed to cage and pond farmers [1].
However, studies show that in intensive aquaculture systems: fish coexist with parasites under ideal conditions, but disequilibrium can set in due to changes in the water quality [2-5]; improper management [3, 6]; resulting into infections by bacterial infections [7]. Consequently, parasites can be a major setback for aquaculture development since they reduce growth, lead to diseases, which negatively impacts farm profitability as they increase production costs due to treatments, making epidemiological studies in fish farms important [7-9]. In fish, common metazoan parasites that can infest different organs of the fish include Cestodes, Trematodes, Crustacae, Isopoda, Copepoda, Nematoda, Bivalvia, Acanthocephala, Hirudinea and Myxosporean [10].
Additionally, freshwater fish are intermediate hosts of some parasites, which presents a risk of transmission to humans following consumption of raw or undercooked fishery products [11]. In Rwanda, literature on prevalence of parasites in farmed fish is rare and epidemiological studies on the subject are non-existent, setting a strong need for this study. Since some parasites are zoonotic, they pose a public health concern in the country. The only existing literature on fish parasites is contained in a review [12] who noted lack of involvement in fish health management at the existing facility at Kigembe fish station and the Rwasave demonstration site in Butare.
In the district of Nyagatare, community irrigation dams exist along River Muvumba and some of these were stocked through the PAIGELAC project support with *O. niloticus* to boost fish production and supply high quality protein to the community. Thus, a private fish farm, Rafiki and a Kyabayaga based community dam along River Muvumba served as study sites in this

study. We determined and quantified the types of metazoan parasites in pond and dam reared *O. niloticus*, and analysed the relationship between pond/dam husbandry and fish parasitism. The study provides information that could be exploited to provide guidelines for safe and quality fish farming for human consumption and sets the scene for further research on farmed fish parasite epidemiology.

2. Materials and Methods

2.1 Project design

The study adopted a cross sectional design that targeted to analyse fish samples from the dry August season of the year from the community dam and the private farm. A sample questionnaire was used to collect information on the management procedures. Fish samples were obtained from the pond and the dam in August 2015 and handled following recommended procedures described by Hoffman [13] for transport to the lab at the University of Rwanda (UR) Nyagatare campus.

2.2 Diagnosis for the parasites

2.2.1 Parasite diagnosis and fish measurements

Fish samples collected were examined for parasites using the procedure described by Aloo, Anam [14] and [13]. The length-weight data was taken with a meter rule and a lab scale and used to compute for Fulton's condition factor (K): $K= (100W/L^3)$. Where K: conditional factor, W: weight of fish in gram (g), L: total length of fish in centimeters (cm).

2.2.2 Ectoparasites (Monogeneans & Crustacean) diagnosis

Fish were examined around the fins, nostril, operculum & buccal cavity, using a hand lense, whole gills were examined under a trinocular dissecting microscope (Konus Crystal 7x - 45x- 5426) and gill pieces were placed in 4% formalin in vials, shaken and the sediment examined under the trinocular dissecting microscope.

2.2.3 Endoparasite diagnosis

Fish were opened dorso-ventrally, the entire gut removed and placed in a Petri dish with physiological saline. The intestine, gonads, liver, heart, gall bladder and muscle tissue were examined for Nematodes, Cestodes and Trematodes following standard procedures [14].

2.2.4 Collection of farm and dam husbandry data

A qualitative open-ended questionnaire was administered to the respective managers to generate information on fish seed source, feed type and feed source, manure application, manure sources and types, possibility of integration with livestock, water source, water quality management and monitoring, pond conditioning & neighborhood activities.

2.3 Statistical analysis

Parasitological data was analyzed for Parasite mean intensity, relative abundance and prevalence following the formula described by Margolis, Esch [15] using Microsoft Office Excel 2010. Data for farm husbandry practices was summarized and presented into tables.

3. Results and Discussion

3.1 Prevalence of metazoan parasites in dam and pond farmed *O. niloticus*

Overall, almost 83% (24/29) of the fish examined from Kyabayaga dam and 3.3% (1/30) of the samples studied from Rafiki farm in Kageyo were infested by at least one parasite. Similar findings in Morogoro, Tanzania reported that 16.3% of *O. niloticus* reared under small scale fish pond farms were infested with intestinal parasites [16].

The total number of parasite species identified was four, including two nematodes, a trematode and a crustacean from 25 of the 59 samples examined from the two sites (Table.1). For samples from Kyabayaga dam, the most prevalent parasite species was a Nematode, *Goezia sinamora* (48.3%), followed by a trematode gill fluke Dactylogyrus sp. Generally, endoparasites need not to be underestimated as they may supress fish reproductive potential [17], increase susceptibility to predation [18, 19] and damages host tissues [20].

Table 1: Parasite prevalence, mean intensity and relative abundance in pond farmed and dam raised *O. niloticus*

Parasites	Infested hosts	Prevalence (%)	Parasites Isolated	Mean intensity	Relative abundance	Body part found
Kyabayaga community Dam raised *O. niloticus*						
Nematodes						
G. sinamora	14.0	48.3	18.0	1.3	62.1	Intestine
Hysterothylacium sp.	4.0	13.8	6.0	1.5	20.7	Intestine
Trematodes						
Gill flukes (*Dactylogyrus sp.*)	6.0	20.7	29.0	4.8	100.0	Gills
Crustacean (Acanthocephala)						
Neoechinorhynchus sp.	0.0	0.0	0.0	0.0	0.0	Liver
Kageyo-Rafiki Farmed *O. niloticus*						
Nematodes						
G. sinamora	0.0	0.0	0.0	0.0	0.0	Intestine
Hysterothylacium sp.	0.0	0.0	0.0	0.0	0.0	Intestine
Trematodes						
Gill flukes (*Dactylogyrus sp.*)	0.0	0.0	0.0	0.0	0.0	Gills
Crustacean (Acanthocephala)						
Neoechinorhynchus sp.	1.0	3.3	2.0	2.0	6.7	Liver

On the other hand, the mean parasite abundance and intensity was highest with the ectoparasite trematode Dactylogyrus sp (Table. 1), which was previously reported on gills with *O. niloticus* elsewhere [21]. It must be noted that since gills are delicate, respiratory, ion balance and feeding structures in *O. niloticus*, the presence of parasites ultimately interferes general physiology, with the possibility of causing mortality [22, 23], and also increase fish susceptibility to secondary infections [24-26] due to weakened integuments. Dactylogyrus sp is commonly reported in *O. niloticus* among Latin American producing countries of Costa Rica, Uruguay, Mexico and Brazil [7, 27-32]. In the current study, dominant

ectoparasite species included Dactylogyrus sp. while endoparasites were *Goezia sinamora*, Hysterothylacium sp.
For samples from Rafiki farm in Kageyo, only one parasite species a crustacean, Neoechinorhynchus sp. was found (3.3%), and presented with a mean intensity of two parasites in the only one infested individual sample observed (Table. 1). The difference in endoparasites parasite prevalence between the two sites could be associated with differences in management practices at the two sites. Specifically, fish reared in Kyabayaga dam mostly depend on foraging aquatic phytoplankton, plants, zooplankton, benthos, detritus and added mixtures of uncontrolled organic manures (Table.3). This would expose the fish more to parasites compared to the private farm in Kageyo where fish were fed using on-farm made feed.

3.1.1 Effect of parasitism on fish condition

Regarding an assessment of parasite effect on fish condition or wellbeing, there was no serious damage at the two sampling sites as the mean condition factor (K) between infected and uninfected fish was similar (Table. 2).

Table 2: Effect of parasites on fish condition factor (K) of *O. niloticus*

Fish status	Mean K±SD	Mean length (cm) ± SD	Mean weight (g) ± SD
Kyabayaga Dam			
Infested fish	1.710±0.229	21.426±2.912	182.263±83.295
Un-infested fish	1.686±0.335	17.660±4.206	110.100±79.303
Rafiki fish farm, Kageyo			
Infested fish	1.684±0.000	17.800±0.00	95.000±0.000
Un-infested fish	1.675±0.177	20.310±1.660	141.660±32.790

Despite the higher parasitism in fish from Kyabayaga dam than in Rafiki farm in Kageyo, findings from this study suggest that the infestation rates were not high enough to affect growth and general physiological state of *O. niloticus*. Additionally, the low stock densities, and continuous water outflows for rice irrigation from Kyabayaga dam could have contributed to the observed results. Similar findings have been reported for *O. niloticus* reared in Agua Vermelha reservoir in Brazil [33].
In addition, the observed trend of parasitism by the nematodes and trematode revealed a change from a monoxenous parasite population in young fish to a heteroxenous community in adults (Fig. 1). These findings have been previously reported in *O. niloticus* [21]. This change in parasite population composition is associated with an ontogenetic feeding shift, with a prolonged exposure to intermediate hosts/infectious stages in older (larger) fish as has been reported [34, 35]. Further, literature indicates that despite the slight changes in *O. niloticus* prey size with host age [36], the changes increase exposure of adult fish to trophically transmitted infections [21].

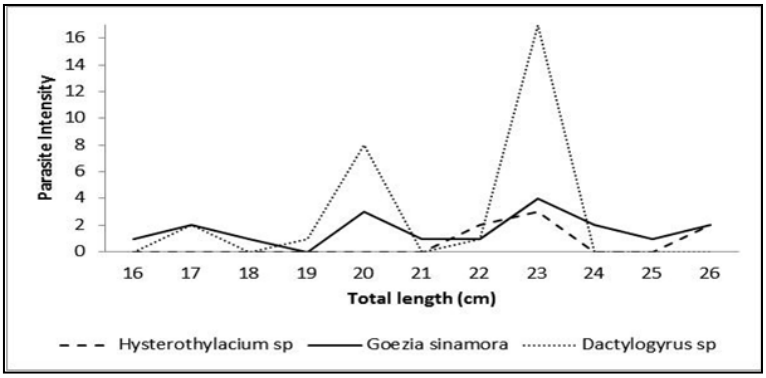

Fig 1: The relationship between parasite intensity and size of *O. niloticus* from Kyabayaga dam, Nyagatare district, Rwanda

3.2 Influence of management practices on fish parasitism

With respect to the effect of management practices and their influence on fish parasitism, serious consequences were noted for *O. niloticus* from Kyabayaga dam compared to Rafiki farm in Kageyo (Table.3). Kyabayaga dam samples were identified with the highest parasite prevalence and diversity. This was probably due to a lapse in management practices, as indicated by the use of mixed and uncontrolled source of animal fertiliser, including its exposure to a diversity of animals as a drinking source and the diversity of wildlife (Table. 3) compared to Rafiki fish farm in Kageyo. Similar findings have been noted in Morogoro [16], where fish farming in rural areas is always integrated with poultry production, which reportedly contributes to parasite infestation [37]. Kyabayaga dam is also exposed to high community pollution through direct runoff from Kyabayaga town and contamination with agro-chemicals from horticultural activities around its shoreline.

Table 3: Management practices at Kyabayaga dam and Rafiki fish farm in Kageyo, eastern province Rwanda

Management practices	Rafiki farm	Kyabayaga dam
Seed source	First seed from PAIGELAC in 2013	First seed from Lake Muhazi fry producers cooperative
	Currently produce own seed	Then PAIGELAC
Feed type	Rice bran, own formulated (rice bran, Sambaza, *Limnothrissa miodon* & premixes)	Own formulated (Rice bran, Soy & *L. miodon*)
Feed source	Rice bran from Kalangazi, *L. miodon* from Kigali	Soy and rice bran sourced locally

		L. miodon from Kigali
Manure use	Manure used commonly	Added twice in a year
Manure sources	Own pig stable	Abattoir waste, homes
Manure types	Pig waste only	Cow dung and pig waste
Livestock integration	Piggery only	Piggery
Water source	Canal	Muvumba river
Conditioning	Ponds never been conditioned since commencement in 2012	Dried once during repairs in 2011
Neighbourhood activities	Agriculture (banana, sweet potatoes, cabbage, tomatoes), livestock farming, urbanisation, brick making and wild birds.	Agriculture (tomatoes, cabbages), Forestry nurseries, Harding (cows, goats), Urban pollution, Wildlife (wild geese, herons & marabou storks)
Water quality monitoring	No monitoring	No monitoring

Moreover, findings from Kyabayaga dam agree with literature which suggest that poor fish environment, pond management, malnutrition and other stress factors accelerate parasitic infection [38]. Additionally, both study sites were reported to source culture water from river Muvumba in Kyabayaga and a local canal in Kageyo, which can potentially be a source of contamination by parasites from neighbouring communities, as was reported in Morogoro [16]. In addition, both sites were found with accumulated silt since their establishment due to lack of periodical maintenance and conditioning, which affects pond chemistry. Too much silt provides a conducive environment for growth of benthic macro invertebrates, which serve as parasite hosts, that can be transmitted to fish via the trophic systems [38, 39].

3.2.1 Water quality parameters

In terms of water quality parameters, mean temperature, dissolved oxygen and pH values were relatively higher at Rafiki farm: 25.383°C, 5.733 mg/L and 8.550, and lower at Kyabayaga: 22.233°C, 4.920 mg/L and 7.860 (Table. 4) respectively.

Table 4: Water quality at the two sampled sites (values are averages of seven observations)

Parameter	Rafiki farm-Kageyo	Kyabayaga dam
Temperature (°C)	25.383±0.504	22.233±0.367
Dissolved Oxygen (mg/L)	5.733±1.118	4.920±0.356
pH	8.550±0.695	7.860±0.195

Overall, dissolved oxygen and pH values observed in the current study were within the recommended range for culture of *O. niloticus* except temperature, which was lower than the recommended value of 28°C for *O. niloticus*. Similar findings have been reported for the same species [33] reared in cages in a hydroelectric reservoir in Brazil. Generally, temperatures lower than 28°C can negatively affect feeding and growth, suppress fish immunity with consequences for increased susceptibility to attack by pathogens [27]. Other parasitism and disease predisposing aspects are stress factors related to nutrition, handling, transport, water quality and organic matter loading.

4. Conclusion

This study was designed with the objective to quantify the types of metazoan parasites in pond and dam reared *O. niloticus*, and analyse the relationship between pond/dam management procedures and parasitism. A cross sectional design targeting the dry season of the year was employed to collect fish samples and husbandry data. Generally, findings revealed that dam reared *O. niloticus* was highly parasitized than pond reared fish. The most prevalent parasite was a nematode *G. sinamora* (48.3%), and this was followed by a trematode gill fluke Dactylogyrus sp. (20.7%) for fish from Kyabayaga, while fish from Rafiki farm had only a crustacean parasite found in one (3.3%) of the samples examined. Furthermore, lack of a proper management plans has a huge influence on fish parasitism as was indicated by high parasite prevalence and intensity at Kyabayaga dam than in fish samples from Rafiki farm in Kageyo. This is the first attempt in Rwanda to elaborate the parasite burden in farmed fish and lays the foundation for further studies on parasite epidemiological studies.

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