



Occurrence and risk factors associated with *Eimeria* species infections in *Clarias gariepinus* and *Heteroclaris* species

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Abstract

Eimeria infection can cause a serious health risk to fish in the wild and especially to the fish production enterprise. *Eimeria* species are a group of protozoan parasites common in many fish farming systems with little information available on the occurrence, impact, and risks associated with the infection in fish populations. This study was conducted to ascertain the prevalence and risk factors associated with *Eimeria* species infections in three hundred and seventy-six *Clarias gariepinus* and *Heteroclaris* species from some selected fish farms in Kaduna state, Nigeria. Using direct smear techniques, oocysts were recovered from the mucous and intestinal content of the fish. Data on the risk factors were obtained by administering a well-structured questionnaire to the fish farmers. An overall prevalence of 30.3 % was obtained for the apicomplexan parasite of the genus *Eimeria*. The monthly prevalence of *Eimeria* species infection among fish was highest in October and the lowest in July. Some risk factors were significantly ($p < 0.05$) associated with fishes in the univariate model. Sex of fish, level of formal education of farmers, feeding of fish with dead poultry and fish, and the number of ponds in a fish farm were the significant risk factors in the multivariate model. This finding indicates that *Eimeria* species infection is endemic and of great concern among the fish population, thereby posing significant health problems. There is a need for improved fish management practices and biosecurity protocols.

Keywords: Aquaculture, *Coccidia*, management practice, risk factors, Nigeria

Introduction

Clarias gariepinus and *Heteroclaris* species are among the most cultured freshwater fishes in tropical Africa. They can be reared in different holding facilities, such as earthen ponds concrete, and plastic tanks. They are a good source of cheap high-quality

protein especially in developing countries [1].

Clarias gariepinus sourced from the wild has drastically reduced, mainly due to overfishing, pollution, predation, and climate change necessitating an intensification of fish production through pisciculture which is usually associated with

overstocking, and increased susceptibility to diseases. The increased susceptibility to diseases has become the major impediment to fish farming globally [2].

Coccidia parasites infect aquatic species globally and cause intestinal or extraintestinal infections. Several studies have been conducted on coccidia infection of marine fishes in various parts of the world [3, 4]. Most studies conducted on *Eimeria* infection of *Clarias gariepinus* and *Heteroclaris* species is limited to experimental studies [5].

There is a paucity of information in the literature on *Eimeria* species affecting *C. gariepinus* and *Heteroclaris* species in cultured mixed farming in the tropics and very little is known about their epidemiology and the associated risk factors in this species [2, 6]. This study was performed to ascertain the presence, the prevalence of *Eimeria* species and the risk factors associated with its infections in *C. gariepinus* and *Heteroclaris* species in different holding facilities in the study area.

Materials and Methods

Study Area

The study was carried out in Kaduna State in which 4 Local Government Areas (LGAs) comprising Sabo-Gari, Kaduna-South, Kaduna North, and Zaria LGAs were randomly selected. Kaduna State is geographically located in the Northwest geopolitical zone of Nigeria. The global location of the State is on Latitude 10°31'35"N and Longitude 7°26'19" E. The State occupies an area of 48,473.2 square kilometres and with an estimated population of six million people [7].

Study design and collection of samples

A stratified random sampling method was employed, in which four Local Government Areas (LGAs) were selected and samples were collected from forty-seven fish farms. The fish were reared in concrete ponds, earthen ponds, and plastic tanks. A total of three hundred and seventy-six fish samples were collected between June to November 2018. *Clarias gariepinus* and its hybrid species (*Heteroclaris*), which is a cross between female *Clarias gariepinus* and male *Heterobranchius bidorsalis* of different weights and lengths were collected from the farms using a fishnet between the hours of 06.00 and 08.00 h; and were transported in a plastic receptacle with a perforated cover containing pond water to the Parasitology Laboratory of the Faculty of Veterinary

Medicine, Ahmadu Bello University Zaria for further processing. Data on the potential risk factors were obtained during fish collection by using a properly structured questionnaire to gather information on the date the fish was hatched in the hatcheries, age of the fish, the holding facilities, and fertilization and its type, water source, source of stock, feed type, feeding, water and general management practices on their farms.

Examination of the Fish

The age of fish was obtained from the questionnaires administered to the fish farmers. Physical and postmortem examination was carried out on each live fish after euthanizing by brain spiking to minimize suffering. The fishes were sexed by examining the anal opening for the presence or absence of papilla just before the anal fin. Sex was further confirmed posteriorly through the presence or absence of testes or ovaries [8]. The weight of the fish was determined to the nearest grams and the total length of each specimen was obtained in centimetres from the snout to the extended tip of the caudal fin. Both measures were used in determining Fulton's condition factor using the formula of Ikomi and Odum, [8] as shown below:

$$K = 100 (\text{Weight (gms)} / \text{Length (cm)}^3)$$

Parasitological Analysis

The fish was placed dorsally to expose the ventral area of the fish and an incision was made from the operculum to the anal opening and two more cuts were made on the lateral side to expose the internal organs using scissors. The gastrointestinal tract of each fish was examined for the presence of coccidian oocyst. Impression of faeces, mucous and intestinal scrapings as pooled samples were prepared from each fish on a glass slide with a coverslip and examined under a light microscope under x 10 and x 40 objectives lens and the presence of oocyst was recorded accordingly [2,10]

Identification of *Eimeria* oocyst

The *Eimeria* oocysts were identified to genus level considering the morphometric measurements, the features of the sporulated oocysts, the counts of sporocysts contained therein and the sporozoites per sporocyst. Furthermore, identification of the genus was carried out using the most current edition of The Illustrated Guide to the Protozoa [11].

Data Analysis

The prevalence of *Eimeria* species was determined,

Chi-square test (univariate analysis) for discrete variables at a 95% confidence interval at $p < 0.05$ was used to obtain the associations of potential risk factors with the isolation of coccidia oocyst. Multivariable unconditional logistic regression was used to determine predictors for *Eimeria* infection controlling for other covariates at $p < 0.20$ and biologically plausible factors. We tested for collinearity among predictors using the Chi-square test for binomial variables. We also assessed the interactions between selected variables. The variables that were significant in the univariate analysis and those that are biologically plausible were included in the multivariate analysis. A forward stepwise method was used. The Statistical Package for the Social Sciences (SPSS, Chicago, Illinois, USA) for windows version 22.0 was used for all analyses, and values of $p < 0.05$ were considered significant.

Results

The oocysts of *Eimeria* species were found in the faeces, mucous material, and gastrointestinal tract of *C. gariepinus* and *Heteroclarus* species. Out of the 376 fishes sampled in this study from the different holding facilities, 114 were infected with *Eimeria* species oocyst representing 30.3% (95% CI = 25.8 – 35.1). The monthly prevalence of *Eimeria* species infection among fishes was highest in October (39.3; 95% CI = 27.2 – 52.5) and lowest in July (21.2%; 95% CI = 12.6 – 32.3) (Figure 1).

Infected fish with *Eimeria* species oocyst had pale gills, reduced or absence of abdominal fat, and haemorrhages in the gastrointestinal tract. The age, gender, and Fulton condition score of the fish were significantly ($p < 0.05$) associated with the prevalence of *Eimeria* species infection among fish in Kaduna State Nigeria. *Clarias gariepinus* and *Heteroclarus* species older than 12 months were 6.7 and 2.6 times more likely to be infected with *Eimeria* species compared to those less than 6 months and those between 7 to 12 months respectively. Female fishes were 1.7 times more likely to be infected with the protozoan compared to males. The prevalence of *Eimeria* species infection decreases with the increase of the Fulton condition score of fish (Table 1).

The age and level of formal education of fish farmers were the owner's epidemiological factors associated with *Eimeria* species infection among fishes. The prevalence of *Eimeria* species infection increased as the age of fish farmers increased, while the

prevalence almost perfectly decreased as the level of formal education of farmers increased (Table 2).

The univariate association between management epidemiological factors and *Eimeria* species infection among fishes in Kaduna State is presented in Table 3. Farm types, what the fish were fed with, holding facilities, and sources of fertilization were the factors associated with the infection in this category. *Clarias gariepinus* and *Heteroclarus* species raised in integrated farms with poultry were about 17 times more vulnerable to *Eimeria* infection compared to *C. gariepinus* raised alone. Fish fed with dead poultry were about 7 times more likely to be infected with the protozoan compared to fishes not fed with dead poultry. There was a higher likelihood of infection among fishes raised in concrete tanks and earthen ponds compared to those raised in plastic tanks. There was a noticeable higher prevalence of infection among fishes reared in ponds fertilized with poultry litter compared to those raised in other pond fertilization options. Fishes fed with dead fishes were 36.7 times more likely to be infected with *Eimeria* species compare to fishes not fed with dead fishes. Local Government Areas and the number of ponds on a farm were the environmental risk factors associated with the infection among fishes in Kaduna State (Table 4). Gender of fishes, level of formal education of farmers, feeding fishes with dead poultry and dead fishes, and the number of ponds in a fish farm were the significant factors in the multivariate model (Table 5)

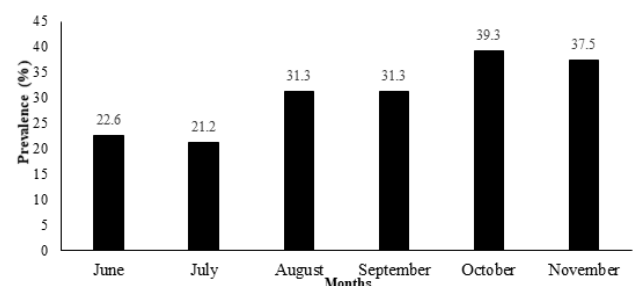


Figure 1. Monthly prevalence (%) of *Eimeria* species infections among fishes in fish farms from Kaduna State Nigeria.

Table 1. Univariate association between host epidemiological factors and *Eimeria* species infection among fishes in fish farms from Kaduna State, Nigeria.

Risk factors	n	<i>Eimeria</i> species (+)	OR (95%CI)	p-Value
Age				
<6 months	138	24 (17.39)	0.15 (0.07, 0.34)	<0.01 ^{††}
7-12 months	200	68 (34.00)	0.38 (0.18, 0.76)	0.01 ^{††}
>12 months [‡]	38	22 (57.89)	1.00	
Gender				
Female	176	64 (36.36)	1.714 (1.10, 2.67)	0.02 ^{††}
Male [‡]	200	50 (25.00)	1.00	
Species of fishes				
<i>Heteroclarus</i> (hybrid)*	128	42 (32.81)	1.19 (0.75, 1.89)	0.48
<i>C. gariepinus</i> [‡]	248	72 (29.03)	1.00	
Fulton Condition Score				
<1	234	88 (37.61)	10.19 (2.79, 64.40)	<0.01 ^{††}
1-2	106	24 (22.64)	4.93 (1.27, 32.50)	0.02 ^{††}
>2 [‡]	36	2 (5.56)	1.00	

+ = **positive**[‡] Reference category, n = number of fishes, OR = odds ratio, CI = confidence interval, ^{††} significant at $p < 0.05$ * = a cross between female *Clarias gariepinus* and male *Heterobranchus bidorsalis*.

Table 2. Univariate association between farmer's epidemiological factors and *Eimeria* species infection among fishes from fish farms in Kaduna State, Nigeria.

Risk factors	N	Nf	<i>Eimeria</i> species (+)	OR (95%CI)	p-Value
Age of Owners					
<39 years	11	88	18 (20.45)	0.45 (0.23, 0.86)	0.02 ^{††}
40-50 years	23	184	58 (31.52)	0.80 (0.48, 1.33)	0.39
>51 years [‡]	13	104	38 (36.54)	1.00	
Gender					
Female	8	64	24 (37.50)	1.48 (0.84, 2.60)	0.18
Male [‡]	39	312	90 (28.85)	1.00	
Level of formal education					
None	4	32	18 (56.25)	3.51 (1.63, 7.68)	<0.01 ^{††}
Primary	6	46	20 (43.48)	2.11 (1.08, 4.09)	0.03 ^{††}
Secondary	11	88	20 (22.73)	0.81 (0.44, 1.44)	0.48
Tertiary [‡]	26	210	56 (26.67)	1.00	

+ = **positive**[‡] Reference category, n = number of farmers in each category, n = number of fishes, OR = odds ratio, CI = confidence interval, ^{††} significant at $p < 0.05$.

Table 3. Univariate association between management epidemiological factors and *Eimeria* species infection among fishes in fish farms from Kaduna State, Nigeria.

Risk factors	n	<i>Eimeria</i> species (+)	OR (95%CI)	<i>p</i> -Value
Age of farms				
<1 – 3 years	232	64 (27.59)	0.64 (0.29, 1.41)	0.26
4 – 6 years	112	38 (33.93)	0.86 (0.38, 1.99)	0.71
7 – 9 years [¥]	32	12 (37.50)	1.00	
Farm types				
Fish raised with poultry	264	112 (42.42)	16.85 (4.72, 105.2)	<0.01 ^{††}
Fish raised with other animals	64	0 (0.00)	X	X
Fish raised alone [¥]	48	2 (4.17)	1.00	
Are fishes fed with dead poultry?				
Yes	94	58 (61.70)	6.46 (3.90, 10.83)	<0.01 ^{††}
No [¥]	282	56 (19.86)	1.00	
Holding facilities				
Concrete	176	68 (38.64)	3.55 (1.82, 7.31)	<0.01 ^{††}
Earthen	120	34 (28.33)	2.23 (1.09, 4.79)	0.03 ^{††}
Plastic [¥]	80	12 (15.00)	1.00	
Management systems				
Semi-intensive	88	22 (25.00)	0.71 (0.41, 1.22)	0.24
Intensive [¥]	288	92 (31.94)	1.00	
Types of feed				
SP	88	18 (20.45)	1.03 (0.41, 2.74)	0.97
FP	248	88 (35.48)	2.20 (0.99, 5.28)	0.05
KLO [¥]	40	8 (20.00)	1.00	
Water sources				
Borehole	272	88 (32.35)	1.26 (0.73, 2.22)	0.42
Well	16	4 (25.00)	0.88 (0.22, 2.95)	0.87
Pipe-borne	8	0 (0.00)	X	X
Stream [¥]	80	22 (27.50)	1.00	
Sources of fertilization				
Poultry litter	168	90 (53.57)	6.87 (3.38, 15.01)	<0.01 ^{††}
Cow dung	80	14 (17.50)	1.27 (0.52, 3.17)	0.60
Organic fertilizer	58	0 (0.00)	X	X
None [¥]	70	10 (14.29)	1.00	
Are fishes fed with dead fishes?				
Yes	270	112 (41.48)	36.65 (10.53, 225.40)	<0.01 ^{††}
No [¥]	106	2 (1.89)	1.00	

+ = **positive**[¥] Reference category, n = number of fishes, OR = odds ratio, CI = confidence interval,^{††} significant at $p < 0.05$, SP Sinking pellets; FP floating pellets, KLO, kitchen leftover.

Table 4. Univariate association between environmental epidemiological factors and *Eimeria* species infection among fishes in fish farms from Kaduna State, Nigeria.

Risk factors	n	<i>Eimeria</i> species (+)	OR (95%CI)	p-Value
Local Government Areas				
Sabon-Gari	80	28 (35.00)	1.82 (0.93, 3.64)	0.08
Kaduna North	120	34 (28.33)	1.34 (0.71, 2.57)	0.37
Zaria	88	32 (36.36)	1.94 (1.00, 3.80)	0.04 ^{††}
Kaduna South [‡]	88	20 (22.73)	1.00	
Number of ponds				
1 – 4	104	16 (15.38)	0.08 (0.03, 0.16)	<0.01 ^{††}
5 – 7	210	54 (25.71)	0.14 (0.07, 0.27)	<0.01 ^{††}
>7 [‡]	62	44 (71.00)	1.00	
Presence of neighbouring farms				
Yes	206	70 (33.98)	1.47 (0.94, 2.32)	0.09
No [‡]	170	44 (25.88)		

+ = **positive**[‡] Reference category, n = number of fishes, OR = odds ratio, CI = confidence interval, ^{††} significant at $p < 0.05$.

Table 5. Multivariate association between epidemiological factors and *Eimeria* species infection among fishes in fish farms from Kaduna State, Nigeria.

Risk factors		SE	OR	95% CI		p-Value
				Lower	Upper	
Gender of fishes						
Female	0.952	0.326	2.591	1.368	4.905	<0.01 ^{††}
Male [¥]			1.000			
Level of formal education of farmers						
None	2.474	0.925	11.875	1.936	72.844	0.01 ^{††}
Primary	0.557	1.052	1.745	0.222	13.719	0.60
Secondary	-1.013	0.608	0.363	0.110	1.196	0.10
Tertiary [¥]			1.000			
Are fishes fed with dead poultry?						
Yes	1.038	0.462	2.823	1.141	6.986	0.03 ^{††}
No [¥]			1.000			
Are fishes fed with dead fishes?						
Yes	3.956	1.159	52.252	5.388	506.742	<0.01 ^{††}
No [¥]			1.000			
Number of ponds						
1 – 4	-1.670	1.096	0.188	0.022	1.615	0.13
5 – 7	-1.825	0.738	0.161	0.038	0.685	0.01 ^{††}
>7 [¥]			1.000			

[‡] Reference category, β = Standardized beta coefficient, SE = standard error, OR = odds ratio, CI = confidence interval, ^{††} significant at $p < 0.05$

Discussion

The overall prevalence of *Eimeria* infection 30.3% observed in *C. gariepinus* and *Heteroclaris* species from the fish farms revealed the importance of *Eimeria* oocyst which could result in reduced productivity, morbidity, and mortality in the fish population [5,12]. Furthermore, the prevalence rate obtained in this study was lower than the reported prevalence of 40.15% of *Eimeria* species in *Clarias gariepinus* from Benin Republic [2] and higher than the prevalence of 15% in *Oreochromis niloticus* in Tanzania [13]. The differences in the prevalence of *Eimeria* oocysts may be due to the difference in fish species, sample size, management systems, the season of sampling, geographic location, and differences in the thermal environmental conditions of the study areas.

The monthly prevalence of *Eimeria* infection showed that it was highest in October and least in June; this result reveals the association between *Eimeria* species and the season of the year. It showed that the infection increased as the rainy season of the year progressed. Moreso, piscine coccidiosis was reported to have a seasonal pattern where the prevalence peaks during the rainy season in which there is an increase in the adaptation to thermal environmental factors that enhance sporulation or survival of oocysts in the environment [14]. Our observation is similar to the findings of El-Mansy, [15] and Matsche *et al.* [14]

The prevalence of *Eimeria* infection in this study was higher in adult *C. gariepinus* and *Heteroclaris* species compared to young ones. However, this is in contrast to what was observed in an experimental study with *Clarias gariepinus* in Benin republic in which the prevalence and infection were lower in the older fish [2]. A possible explanation of the rise in prevalence in older fish in this study may be due to parasite build up during the lifetime of the fish and the continuous exposure to water contaminated with oocysts or sporocysts originating from surface water runoff into the different holding facilities [16].

It was observed that females had a higher prevalence compared to males, but this was different from the findings of Siko *et al.* [2]. This could be due to the enormous spawning stress and osmoregulatory changes making them more susceptible to the infection.

The Fulton condition factor of the fish was altered by the presence of the *Eimeria* oocysts; the condition

factor indicates the well-being or health status of the fish [17]. *Eimeria* oocysts were seen more in the fish whose Fulton condition factor was less than one. They were likely stressed by the infection of *Eimeria* species that are known to impair ingestion of feed and uptake of nutrients leading to disease outbreak manifesting as decreased appetite, and may subsequently lead to weight loss, lethargy, death, and economic losses [3, 6]. Furthermore, infections caused by coccidia species have been linked to poor body conditions in wild blue whiting in Northern Portugal [4].

It was observed that fish reared in integrated fish and poultry farms were much more vulnerable to *Eimeria* infections compared to those reared in the farms where fish alone is raised. However, there is a paucity of information on the host specificity of fish apicomplexans, but it seems fish coccidia is less host-specific than the mammalian coccidia [18]. One of the dangers involved in mixed livestock farming is the role of other livestock in the possible transmission of disease-causing pathogens to cultured fish that may also affect the health of the fish, reduce fish growth rate, disease resistance, and cause fish mortalities [19].

This study has further established a high rate of infection occurring in fish reared in concrete and earthen ponds compared to fish raised in plastic tanks. This may be due to the use of fertilizers of animal origin (poultry and cow) for pond preparation in concrete and earthen ponds and that may have exposed the fish more to protozoans compared to plastic tanks where these fertilizers are not used [20].

There was a significant relationship between the knowledge level of fish farmers and the infection of *Eimeria* oocysts. We observed that there was a higher prevalence of *Eimeria* infection in fish farmed by fish farmers with no formal education. The high prevalence recorded among fish farmed by farmers with no formal education may be attributed to their low educational status and their unwillingness to acquire skills from formal educational institutions or training programs. The lack of knowledge may have probably led to lapses in management practices [21] which could have increased the susceptibility of the fish to infections since the level of knowledge, experience, wisdom in operation, and management practice will influence the success of fish production [22].

In conclusion, *Eimeria* species were detected in *C. gariepinus* and *Heteroclaris* species from selected fish farms and there was a positive relationship between age, gender, and Fulton condition score of the fish and the infection of *Eimeria* species. Also, age, level of formal education of fish farm owners, the gender of fishes, what the fish were fed with and the number of ponds in a fish farm was the epidemiological factors associated with *Eimeria* species infection. Therefore, care should be taken on the feed provided for the fish. Furthermore, farmers should acquire knowledge on best management practices on fish farming such as biosecurity measures in order to mitigate the occurrence, losses and impact on the fish and fish farms.

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Authors' contributions

Adah, A. D., Lawal, S. and Oniye, S. J. participated in the study design of the research. Adah, A. S. and Adah, A. D. were involved in the fish collection from the fish farms and dissection of the fish. The parasitological analysis was carried out by Okunbanjo, O. O., Ola-Fadunsin, S. D. analysed the data generated from the study. The first draft of the manuscript was prepared by Adah, A. D. and edited by all authors. The final draft of the manuscript was read and approved by all the authors.

Competing interests

The authors declare that they have no competing interests.

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