

Natural Formation and Pathological Effects of *Branchiomyces* Species in Farm-grown African Catfish (*Clarias gariepinus*) in Ilorin Metropolis

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Abstract: *Branchiomyces* species are the fungi responsible for branchiomycosis in fish. Branchiomycosis in fish is manifested by necrosis of the gills, and high incidences of mortality. It is a highly feared fungal disease of fishes in fish raising farms. This study is to investigate the occurrence and histopathological effect of *Branchiomyces* species in farm raised *Clarias gariepinus* in Ilorin metropolis. Thirty-six (36) farms were selected randomly from Ilorin metropolis. A total number of 108 gills were collected from 36 fish and examined for the presence of *Branchiomyces* species. Furthermore, histological examination was carried out on infected gills by using standard methods. Eleven out of 57 samples from males representing 19.30% were infected with *Branchiomyces* species while 17 out of 51 samples from females representing 33.33% were infected. Also, females had higher load of *Branchiomyces* species (5.0×10^3 cfu/g) and males had 4.0×10^3 cfu/g. There was significant difference in the percentage of the infection between males and females ($p = 0.003$). Males had higher lamellae length and width than the females ($p > 0.05$). The gills show an observable lesion, diffuse infiltrate, sanguineous congestion dilation of marginal vascular channel and disorganization of secondary gill lamellae. The study indicated that *Branchiomyces* species showed a high presence in farm-raised African catfish. The gills of infected fishes showed early stage of alterations. The gill histomorphometry, indicated that the lamellae were affected which could lead to respiratory disorder.

Keywords: *Branchiomyces* species, *Clarias gariepinus*, gill, infection.

Ilorin Metropolünde *Branchiomyces* Türlerinin Çiftlikte Yetiştirilen Afrika Yayın Balıklarında (*Clarias gariepinus*) Doğal Oluşumu ve Patolojik Etkileri

Özet: *Branchiomyces* türleri, balıklarda branchiomycosis'ten sorumlu mantarlardır. Balıklardaki dalıyomikoz solungaçların nekrozu ve yüksek ölüm oranları ile kendini gösterir. Çiftlikte yetiştirilen balıklarda çok korkulan bir fungal hastalıktır. Bu çalışma, Ilorin metropolünde *Clarias gariepinus* yetiştiriciliği yapılan çiftliklerde *Branchiomyces* türlerinin oluşumunu ve histopatolojik etkisini araştırmak amacıyla yapılmıştır. Ilorin metropolünden rastgele otuz altı (36) çiftlik seçildi. 36 balıktan toplam 108 adet solungaç toplanmış ve *Branchiomyces* türlerinin varlığı incelenmiştir. Ayrıca standart yöntem kullanılarak enfekte solungaç üzerinde histolojik inceleme yapıldı. % 19,30'u temsil eden erkeklerden 57 örneğin 11'inde *Branchiomyces* türü bulunurken, % 33,33'ü temsil eden 51 örnekten 17'sinin enfekte olduğu belirlenmiştir. Ayrıca dişi balıklar *Branchiomyces* türlerinin daha fazlasını bulundurmaktaydı (dişilerde 5.0×10^3 cfu/g, erkeklerde 4.0×10^3 cfu/g). Erkek balıklar dişilerden daha uzun ve geniş lamellere sahiptir ($p > 0.05$). Erkek dişi lamel uzunluğuna ve genişliğine sahiptir ($p > 0.05$). Solungaçlarda, gözlemlenebilir lezyon, yaygın çoğalma, marjinal damar kanalının kanlı yoğun genişlemesi ve sekonder solungaç lamellalarının düzensizliği görülmüştür. Çalışma, çiftlikte yetiştirilen Afrika yayın balıklarında *Branchiomyces* türlerinin varlığının yüksek olduğunu göstermiştir. Enfekte balıkların solungaçları değişikliklerin erken safhalarını içermektedir. Solungaç histomorfometrisi, lamellerin etkilendiğini ve bu durumun solunum bozukluğuna yol açabileceğini göstermiştir.

Anahtar Kelimeler: *Branchiomyces* türleri, *Clarias gariepinus*, solungaç, enfeksiyon.

Introduction

African catfish, *Clarias gariepinus* is one of the most widely distributed and cultured fish species in tropical Africa (Adeshina et al., 2016) because of its high flesh quality and tolerance to wide range of water quality parameters, fast growth, and economic value (Fred and Jordan, 2011).

Branchiomycosis caused by fungi of *Branchiomyces* species, is manifested by necrosis of the gills, and high incidences of mortality (Paperna and Smirnova, 1997; Post, 1987) and mostly occur in warm climatic region (Ramaiah, 2006). The fungi have become an organism of concern because it is housed on the primary organs such as gill and skin.

Scholz (1999) has reported that *Branchiomyces* is responsible for more than 60% loss of total investment in a farm when plagued. Presence of branchiomycosis has been reported in fish from Egypt and Israel (Post, 1987), Germany, Poland, Italy, and Hungary (Fred and Jordan, 2011), Brazil and Taiwan (Scholz, 1999). Although *branchiomyces* species has not been reported in Nigeria, but its occurrence might be going on unreported, hence the need for this study.

Necrotic effect of *Branchiomyces* on the gills renders the fish to be susceptible to predation, infections from opportunistic pathogens and mortality. Fish with grossly infected gills will have difficulty in breathing, resulting to loss of appetite, body imbalance and death. Therefore, *Branchiomyces* must be avoided and attended to when noticed, to ensure profitability in aqua-business. This study investigated the occurrence and histopathological effect of *Branchiomyces* species in farm raised *Clarias gariepinus* in Ilorin metropolis.

Materials and Methods

Description of the study area

Ilorin, the capital of Kwara state, is located on the coordinates 8°30'N5°00'E, covering an area of 765Km² with sixteen (16) local government areas with population of 856 900 in 2015 (KWADEP, 2015). The four agro-ecological zones of Kwara State were adopted for this study (KWADEP). Farms in each zone were stratified into large, medium and small scales as described by Adeshina et al. (2016) and Fagbenro and Adebayo (2005). Five percent (5%) of the registered farms were randomly selected.

Collection and identification of fish: A total number of 36 adult fish of *Clarias gariepinus* were collected (3 fish x 3 scale of farming x 4 zones). The choice of this size is because it forms the most stage that have more contact with human from consumption, processing and transportation. Sex identification was carried out by considering the presence of genital papillae.

Gill collection: A total of thirty-six (36) gill was collected from the selected fish samples in replicate of three and subjected to full clinical signs, postmortem (PM) lesions and mycological examination in addition to histopathological study.

Clinical examination: The clinical examination of collected gill samples was performed according to Amlacher (1970) and Auatin (1987). Gill examination was performed by lifting the operculum and examining with naked eye. Gills from both sides kept in a petri dish containing

normal saline 9.0 % and examined under a dissecting microscope. A wet mount of gill filaments were prepared and examined under a light microscope for detection of spores and hyphae (Chien et al., 1978; Yanong, 2003).

Isolation and identification of *Branchiomyces* species

The samples were disinfected with a swab of cotton moistened with 70% ethyl alcohol (Amlacher, 1970). All positive samples examined by direct microscopy was cultured on Sabouraud's dextrose agar (SDA). The inoculated plates were incubated at 28°C for 7 days (Finegold et al., 1986) and sub-cultured on SDA plates at 38°C for 5 days (Eli et al., 2011; Ellis, 1977). Lactophenol cotton blue stain (Refai and Abd El-alim, 1987) was applied, for examination of fungal growth on microscopic slide. The shape, diameter of hyphae and spores were measured following the gross morphological examination. Percentage of infection was determined as

$$\text{Percentage of Infection} = \frac{\text{Infected samples}}{\text{Total Number of samples examined}} \times 100,$$

Mycological examination

Direct examination of the gills was done by mounting the slides under light microscope to revealed and observe the spores characteristics of *Branchiomyces* species.

Histopathological examination

Gills specimens were examined for histopathological changes. The specimen was rapidly fixed in 10% natural formalin buffered phosphate for 24 hours, washed under running tap water and dehydrated through ascending grads of ethanol. It was cleaned in chloroform and embedded in paraffin wax at 60°C. The sections of 5 µm thickness were stained by Hematoxylin and eosin stain (Adeshina et al., 2019; Eyarefe et al., 2008).

Gill morphometry and fungal density

Lamella length and breadth was measured using microscale meter (Eyarefe et al., 2008). The number of colony forming units (CFU) per gram of the weight (CFU/g) was estimated for the fungal species.

Statistical analysis

The data obtained were analyzed using descriptive statistics. Significant difference in the percentage of infection between males and females was performed using chi-square test. Mann-Whitney *U* test ($p > 0.05$) for independent samples was utilized for the comparison of means between males and females with the aid of IBM SPSS version 20.

Results

It was found that 19 of the samples used in our study were males and 17 of them were females (Table 1). There were no significant differences in the body weight, total length and standard length

of males and females ($p > 0.05$). The mean body weight of the males was 333.18 ± 13.11 g while the females had the mean of 363.00 ± 15.57 g. Males had total length of 34.64 ± 0.69 cm and standard length of 34.64 ± 0.69 cm while the females had a total length and standard length 30.33 ± 0.77 and 31.57 ± 0.53 cm, respectively (Table 1).

Table 1. Relationship between the sexes of fish.

Parameters	Males (Mean \pm SE)	Females (Mean \pm SE)	Total (Mean \pm SE)	p-value
Number of Fish	19	17	36	
Body Weight (g)	333.18 ± 13.11	363.00 ± 15.57	347.38 ± 10.39	0.073
Total Length (cm)	34.64 ± 0.69	34.73 ± 0.65	34.68 ± 0.46	0.907
Standard Length (cm)	30.33 ± 0.77	31.57 ± 0.53	30.92 ± 0.48	0.200

Note: There is no significant difference between the mean of the body weight, total length and standard length.

Occurrence of *Branchiomyces* species

The occurrence of *Branchiomyces* species in farm raised African catfish in relation to sex revealed that about 19.30% of males were infected with *Branchiomyces* species while 33.33% of females were infected (Table 2).

Also, females had higher load of *Branchiomyces* species (5.0×10^3 cfu/g) than the males (4.0×10^3 cfu/g). There was significant difference in percentage of infection between males and females ($p = 0.003$).

Table 2. Occurrence of *Branchiomyces* species in farm raised African catfish in relation to sex.

Parameters	Males	Females	Total	p-value
Number of all samples	57	51	108	
Number of infected samples	11	17	28	
Number of fungi (CFU /g)	4.0×10^3	5.0×10^3	9.0×10^3	
Percentage of Infection (%)	19.30	33.33	25.93	0.003

Gill histomorphometry of African catfish naturally infected with *Branchiomyces* species was presented in Table 3. Males had higher lamellae length 175.69 ± 9.27 mm than the females (164.32 ± 8.77). Similarly, lamellae width and area of

absorption were higher in males than the females. However, there were no significant differences in lamellae length, lamellae width and areas of absorption of infected African catfish.

Table 3. Gill morphometry of African catfish naturally infected with *Branchiomyces* species.

Parameters	Males	Females	p-value
Lamellae Length (mm)	175.69 ± 9.27	164.32 ± 8.77	0.39
Lamellae Breadth (mm)	60.22 ± 6.22	50.86 ± 6.125	0.31
Area of Absorption (mm ²)	10646.00 ± 1285.24	8704 ± 1345.92	0.32

Note: Area of absorption = Lamellae length \times Lamellae Breadth. There was no statistically significant difference between the lamellae length, lamellae breadth and area of absorption.

Histopathological examination

Histopathologically, the gills showed an observable lesion with normal primary lamellae and pillar cells. There was diffuse infiltrate in the lamellae, sanguineous congestion dilation of marginal vascular channels. Furthermore, moderate to mild lamellae hyperplasia, increasing respiratory epithelium fusion and disorganization of secondary gill lamellae were noticed (Figure 1).

Discussion and Conclusions

Presence of *Branchiomyces* species in the gill of fish have been reported to cause infection (Branchiomycosis). This disease resulted in respiratory disorder and high mortality (Khalil et al., 2015; Noga, 2010). Noga (2010) has reported that *B. sanguinis* and *B. demigrans* are the most

common species in fish. This study showed the presence of *Branchiomyces* species in farm-raised

African catfish, *Clarias gariepinus*. More so, females fishes have higher infection than the male ones.

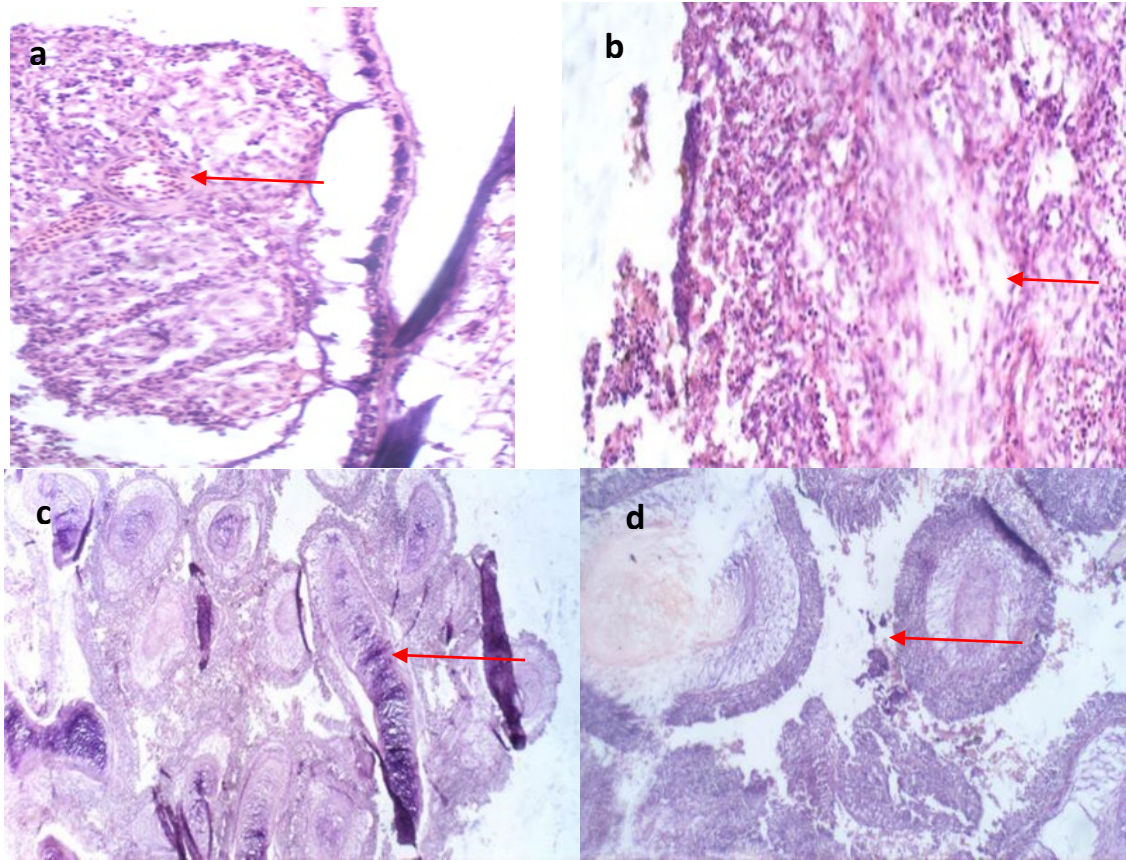


Figure 1. Histological section of gill of *Clarias gariepinus* infected with *Branchiomyces*. (a) There is observable lesion, the primary lamellae and pillar cells are normal, (b) There is diffuse infiltrate in the lamellae, sanguineous congestion dilation of marginal vascular channels, (c) There is moderate to mild lamellae hyperplasia, (d) Lifting of respiratory epithelium fusion and disorganization of secondary gill lamellae. Shortening of secondary lamellae was observed. All images were HE $\times 40, 100$.

Body weight, total length and standard length were identified as factors associated with occurrence of *Branchiomyces species* in this study ($P > 0.05$). The results of the present study were in agreement with the finding of (El-Bouhy and Heba, 2014; Riad et al., 2015) who reported the presence of *Branchiomyces* species in common carp and *Oreochromis niloticus*. Branchiomycosis, an emerging acute infection of the gills and is able to cause high mortality and respiratory discomfort in many species of freshwater fish such as *Oreochromis niloticus*, *Cyprinus carpio*, the eel, and the bass (El-Bouhy and Heba, 2014). Its occurrence has been reported majorly in different localities in Egypt (Riad et al., 2015), and also in Europe and the southeast USA.

The histological examination of infected gills show that *Branchiomyces* species created lesions on the gills of infected fish. Primary lamellae and pillar cells were normal. There was a diffuse infiltrate in the lamellae and sanguineous congestion dilation of marginal vascular channels. Furthermore, moderate to mild lamellae hyperplasia with increased

respiratory epithelium fusion and disorganization of secondary gill lamellae were noticed. Flores and Thomas (2011) have reported that there are three stages of alterations caused by Branchiomycosis. Stage 1 shows hypertrophy and hyperplasia of gill epithelium, sanguineous congestion, dilation of marginal vascular channels, lifting of respiratory epithelium, fusion and disorganization of secondary gill lamellae, shortening of secondary lamellae and leukocyte infiltration of gill epithelium. The findings of this study fall within the stage 1 as described by Flore and Thomas (2011). This further suggests *Branchiomyces* species has established itself in the sampled fish and is likely to move the next stage which could be more dangerous and devastating.

Therefore, this study indicated that there was a high incidence of *Branchiomyces* species in farm-raised African catfish in Ilorin metropolis. The gills of infected fish showed early stage of alterations. The gill histomorphometry, indicated that the lamellae were affected which could lead to respiratory disorder.

Compliance with Ethical Standard

This study was funded by Unilorin (Grant Number SDA 2015).

Conflict of Interest

Authors declare that there are no conflicts of interest.

Ethical Approval

The study was approved by animal care use and research committee of University of Ibadan (ACUREC/UI/App/2016/006).

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