Journal of Biology and Nature

7(4): 169-176, 2017
ISSN: 2395-5376 (P), ISSN: 2395-5384 (O), (NLM ID: 101679666)

PREVALENCE OF BACTERIA AND NEMATODE PARASITES IN AFRICAN CATFISH *Clarias gariepinus* CULTURED IN SMALLHOLDER CONCRETE PONDS IN NIGERIA

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author UDE designed the work and provided the materials. Moreover author UDE wrote the protocol and interpreted the data. Author CJM anchored the field study, gathered the initial data and performed preliminary data analysis. Authors UDE and CJM managed the literature searches and produced the initial draft. Both authors read and approved the final manuscript.

Received: 14th September 2016
Accepted: 4th December 2016
Published: 10th July 2017

Original Research Article

ABSTRACT

Nigeria is leader in sub-Saharan aquaculture production but over 80% of the farms are smallholder fish farms. Culture systems and poor management practice of farmers encourages pathogen infections, mainly parasites. We therefore surveyed prevalence of bacteria and nematode parasites of African catfish (*Clarias gariepinus*) cultured in smallholder fish farms located in Enugu of south eastern Nigeria. A total of eighty (80) African catfish of weight range 200-280 g (average wt 240 g) were randomly collected from four randomly selected smallholder concrete pond farms within Enugu metropolis. The catfish were examined for prevalence of nematode parasites and bacterial pathogens. Examination covered the ecto and endoparasites. The catfish were dissected from the mouth to the esophagus down to anus and the entrails were individually separated. Endoparasites and bacteria were examined by swabbing and incubating the organisms in agar for 24 h-48 h. Gram staining and biochemical tests were carried out for identification of the bacteria. A total of 15 catfish were infested with nematode parasites *Procamallanus laevioncus*, 10 catfish were infested with protozoan parasites *Ichthyophthirius multifiliis*. The rest of the catfish harbored either of three types of bacteria species *Flavobacterium, Streptococcus* and *Pseudomonas* or together with *Ichthyopthirius multifiliis*. The prevalent rates of the bacteria were *Streptococcus* 33%, *Flavobacterium* 34% and *Pseudomonas* 33%. Protozoan parasite *Ichthyopthirius multifiliis* was endemic in the skin, nematode parasites were more endemic in the intestine while the bacteria were endemic in the fins, skin, internal organs and the gills.

Keywords: Fish disease; *Ichthyopthirius multifilis*; *Streptoccocosis*; *Pseudomonas* and animal welfare.

1. INTRODUCTION

Nigeria is leader in sub-Saharan aquaculture production but more than 80% of the farms are smallholder fish farms. By international standard farms below 10 hectares are smallholder [1,2]. There is increase in the number of smallholder fish farming in Nigeria. This is as a result of increased incentives,

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trainings, awareness and ready market of farmed fish. Smallholder fish farming is less than 10 hectares; low investment based and has been estimated to have capacity of raising fish production up to more than 500,000 metric tons in Nigeria [3]. Indeed, as at 2014, aquaculture production in Nigeria stood at 790,000 metric tons [4], and over 80% of this is from smallholder fish farms. Nevertheless, some of the fish farms in Nigeria are attacked by parasitic infections [5-7]. Hatcheries and juvenile fish culture are most affected probably because the fish have not developed enough immunity. In previous researches Elezuo et al. [5] working at Etiosa LGA in Lagos state Nigeria found digenean trematode parasite identified as *Sanguinicola* and a nematode they did not specify as parasite of *Clarias gariepinus*. Similarly Omeji et al. [6] studied parasites of catfish cultured in Makurdi Nigeria and noted higher infestation for earthen pond fishes (60%) than concrete pond fish (20%). The major parasites the authors identified were (*Eustrongylides* and *Camallanus*), and two species of protozoa (*Hexamita* and *Trypanosoma*). Parasitic infections of cultured fish adversely affect normal body functions and the daily feed intake [8]. The impact of parasites on host food intake has been highlighted by Milinski [9], Candolin and Voigt [10] and Enyidi and Eneje [7]. Some parasites like *Aphanomyces invadans* infects most fresh water and brackish water fish causing reduced feeding, swimming with head out of water and deep ulcer, epizootic ulcerative syndrome (EUS) [11]. This causes a trade-off between the allocations of limited resources used in reproduction, parasitic infestations, and parasite resistance [10]. Parasites result in heavy loose in hatcheries and have capacity of eliminating mass fish production. Some bacterial outbreaks are periodic and cause serious mortality in tilapia cultured in earthen pond example *Flavobacterium. columnare*, a long gram negative bacterium together with *M. tilapiae* (myxosporean spore) [12]. Parasites of fish can influence the mating and reproduction of fish. In populations of mosquitoes fish (*Gambusia affinis*), males prefer to mate with female mosquito fish that are not affected by parasitic larval nematodes, *Eustrongylides ignotus* [13]. The parasitic larval nematode causes a decrease on the female host fish body mass and fecundity and also reduces the fish embryos during reproduction [13].

Some fish parasites are zoonotic and potentially harmful to fish gourmets, for example *Clonorchis sinensis* [14]. Specifically unlike fish ectoparasites, the endoparasites can and do infect humans if the filet biomass is not well cooked [15]. Parasitic infections can be triggered by environmental factors like low dissolved oxygen and temperature. Increasing temperature from above 25°C may reduce DO_2 and at same time reduce fish immunity making them vulnerable to pathogens [16,17].

Parasitic infections can cause serious economic loss in fish farming. African catfish grows well under high stocking density [18,19]. High stocking density of fish can create enabling environment for multiplying parasites of African catfish. *Clarias gariepinus* has been noted to be susceptible to both nematode and bacteria [7,20]. Microbial pathogens like *Flavobacterium, Pseudomonas* and *Aeromonas hydrophila* have been noted as prevalent in cultured African catfish [7,21,22]. Cultured fish are more susceptible to outbreaks because they are in captivity in culture systems. Juveniles of African catfish *C. gariepinus* reared in tanks had pathological signs of ulcers from where *Aeromonas*, dominant sp, *Pseudomonas, Flavobacterium* sp, *Micrococcus* sp, and *Staphylococcus sp* were isolated from ulcer and kidney of the fish [22]. In nature African catfish has also been known to be infested with Piscine haemoparasite belonging to the genus *Trypanosoma*, which is a haemoflagellate and *Dactylosoma* a haemosporidia [23]. Proper welfare of the catfish in homestead ponds will reduce parasitism.

There is paucity of researches on fish parasite in Africa [24]. The most cultured fish in Africa is the African catfish *C. gariepinus* [4]. African fish farming and Nigerian fish farming in particular are mostly done in smallholder farms. It is therefore imperative to study the microbial and nematode parasites of African catfish *Clarias gariepinus* cultured in smallholder fish farms. This research is meant to study the prevalence bacteria and nematode parasites of African catfish cultured in smallholder fish farms located within Enugu metropolis capital city of Eastern Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in smallholder farms located within Enugu metropolis Nigeria. Enugu is the capital city of Enugu state Nigeria and is a hub of over 300 smallholder homestead fish farms and about 10 industrial size fish farms. Within Nigerian map Enugu is located at longitude of 6°20 N and 7o 29''OE.

2.2 Physico Chemical Parameters

Water samples from the randomly selected fish farms were collected in a brown but 2 liters opaque plastic container and taken to the laboratory on Godfrey Okoye University Enugu. The water parameters

analyzed were for pH, Temperature, turbidity and salinity and dissolved oxygen. The Water parameter results are recorded in Table 1. The pH was measured using a pH meter (Combo pH & EC meter, Hanna Instruments, Arizona, USA), turbidity was measured with a secchi disc calibrated in meter and reported in Nephelometric turbidity unit (NTU). Water dissolved oxygen was measured using oxygen meter at the recorded water temperature using (YSI oxygen meter model 550A, YSI Inc., Yellow Springs, USA). Temperature was measured with glass thermometer and reported in Celsius scale.

Table 1. Physico chemical parameters of water collected from randomly selected smallholder fish farms located at Enugu senatorial district. The farms were labeled as farm 1 to farm 4 for easy identification

Parameters	Farm 1	Farm 2	Farm 3	Farms 4
pH	5.02	5.94	5.86	5.82
DO_2	2.96	3.45	3.44	3.6
Salinity	0.057	0.067	0.054	0.072
Turbidity	3.2	4.8	5.7	4.5
Temp.	29.4	30	28.4	30.7

Where DO_2 were measured in mg/l, turbidity in NTU, temp in $^{\circ}$C,(temperature measurements were taken in the afternoon)

2.3 Experimental Fish

Samples of African catfish *Clarias gariepinus* were collected from four randomly selected smallholder fish farms located within Enugu senatorial district in Nigeria. The fish were transported in a 25 cm x 32 cm plastic aquarium to the wet laboratories of Godfrey Okoye University Emene Enugu Nigeria. The length, weight and sex of the catfish were taken and recorded. The Sex of the fish was determined based on the shape and nature of the external genital papillae. The male catfish have pointed and erect papillae while the female have rounded papillae. The fish were weighed using an electronic balance (model AE 166).

2.3.1 Probing for parasites

The bodies of the fish were examined for possible presence of ectoparasites. The fins were carefully examined and the tail regions also. The gills of the fish were opened and examined for parasites. Parasites were extracted and placed in 90% alcohol solution till identified. The guts of the catfish were cut open with a dissecting set knife for examination, by making incisions from the mouth to the anus. The gut entrails were carefully separated in a petri dish containing normal saline water. The dissected gut contents were viewed under the microscope at x10x40 magnification. The identified parasites were identified and recorded and stored in ethanol.

2.3.2 Microbiological examination

For microbiological examination sterile swab sticks were used in swabbing skin, gills and stomach regions of dissected fish. The samples were culture in nutrient agar. The plates were duly labeled and incubated at 37°C for 24 h. Isolates grew in the plates and were sub cultured in fresh nutrient agar plates. The cultures were subjected gram staining and biochemical tests.

2.3.3 Gram staining

Using a sterile inoculation loop a drop of suspended culture that is to be examined was put in a slide. The loop was used in making a circle of the drop to a diameter of approximately 1.5 cm. The smear was dried in the air and heat fixed by passing it over a Bunsen flame for some second. Crystal violet drops were added to the fixed smear for 10-60 seconds and then iodine solution were added on top the smear. After the iodine the stains were washed off with copious amount of water. A decolorizer was added to the smear and rinsed off after 5 seconds. The smear was counter stained and the slides were viewed under the microscope at 10 x 40 magnification.

2.4 Catalase Test

For the catalase test, sterile wire loop were used in collecting a colony of organisms and placed in a test tube. Five drops of 3% hydrogen peroxide were dropped into the test tubes. The test tubes were placed on a dark background and care were taken to identify if immediate bubble formation were noticed. The presence of effervescence indicated positive catalase test while the absence of effervescence indicates negative catalase.

2.5 Oxidase Test

In performing the oxidase test tryptic soy agar plates were prepared and inoculated with inoculums from the isolates. The plates were incubated at 37°C for 4 h. About 3 drops of aminodimethlamine were added to the plates. Care was taken to observe change in color within 30 seconds. Careful observation of color change was pivotal for result interpretation. Color changes from pink to purple indicates positive oxidase test while absence of color change indicates the negative test.

2.6 Indole Test

The tests for indole were carried out to determine if suspected organisms would split tryptophan to form the compound indole. In this reaction tryptophan would be hydrolyzed to tryptophanase producing

three end products including indole. The production of indole were detected by kovacs or ehrlichs reagents containing 4(p)-dimethylamino benzaldehyde. This chemical reacts with indole to produce red coloration. Isolates of suspected organisms were used in inoculating tubes of peptone water. The tubes were incubated at 37°C for 48 hs. Four drops of kovac reagent were added to 1ml of each of the culture tubes. Positive indole test was verified by red color immediately the additions were made at the upper part of the test tube.

2.7 Statistical Analysis and Calculations

The differences in the prevalence rate were analyzed using one way analysis of variance. Fishers least significant difference ($P<0.05$) was used in means separation. PASW statistical package was used in statistical analysis.

$$\text{Prevalence (\%)} = \frac{\text{No. of fish host infested x 100}}{\text{Total no. of fish host examined}}$$

3. RESULTS

The water samples collected from smallholder fish farms were mainly acidic. The pH of the water from different ponds ranged from pH 5.2 to 5.94 (Table 1). The dissolved oxygen contents of the water were low ranging from 2.96 mg/l of farm 1 to 3.6 mg/l of farm 4. The farms had turbid water and turbidity ranged from 3.2 to 5.7 (Table 1). The analysis of the ectoparasites of African catfish showed that thirty seven ectoparasites were extracted from the fish. There were no ectoparasites extracted from the first farm, but farms 2-4 had 37 ectoparasites as total ecto parasites seen (Table 2). We noted that bigger fish seems to harbor more parasites (Table 2). The parasites were belonging to nematode *Procamallanus cyathopharynx* and *Procamallanus laevionchus*. The red worms were noticed protruding from the anus of the fish. Nematode parasites *Procamallanus* Spp. protrude from the anus of the fish and at that stage could appear as ecto parasite as they are alive and hanging from the fish. Protozoan parasite *Ichthyopthirius multifilis* (Ich) were extracted from numerous white spots on the body of the catfish. Ich was obtained from the tail, gills and the general body surface infected.

The pathogenic microbes extracted and identified from the African catfish were *Flavobacterium columnnare*, *Pseudomonas* spp and *Streptococcus* spp. The most prevalent bacteria were the *Flavobacterium* spp. with 34% prevalent rate followed by the *Streptococcus* spp and *Pseudomonas* spp. with 33% prevalent rate respectively (Fig 1). There were no significant relationship found between the weight of fish and prevalence of bacteria. The pathological features exhibited by the fish according to the parasitic infections are tabulated in Table 3. Body sore were noted in some of the catfish and these were those infested with *Pseudomonas* spp and *Flavobacterium* spp, but were not seen in those infested with *Streptococcus* spp. Fin erosion were noted on the catfish infested with *Streptococcus* spp and *Flavobacterium* spp but not on those with *Pseudomonas* spp. Eroded tail cases were noticed on catfish infested with *Streptococcus* spp and *Flavobacterium* spp but not on those with *Pseudomonas* spp (Table 3). The catfish that had eroded barbells were those infested with *Streptococcus* spp but not those of *Flavobacterium* spp nor the *Pseudomonas* spp. Eye sore were noticed in the catfish infested with *Streptococcus* spp but not the *Pseudomonas* nor the *Flavobacterium* infected catfish (Table 3). All the fishes showed signs of lethargy and high mortality were recorded within days after sampling for pathogens.

Table 2. Prevalence of ectoparasite and endoparasites of African catfish cultured in Small holder fish ponds within Enugu metropolis Eastern Nigeria

Farms	No ectoparasites	No endoparasites	Weight of fish	Control
Farm 1	0	0	2.38^{ns}	4
Farm 2	4^{b}	9^{c}	3.26^{ns}	4
Farm 3	18^{a}	21^{a}	3.25^{ns}	4
Farm 4	15^{a}	17^{b}	3.50^{ns}	4

Table 3. Pathological features noticed in the body of African catfish *Clarias gariepinus* cultured in smallholder homestead ponds and infested with three bacterial pathogens *Streptococcus* spp, *Pseudomonas* spp and *Flavobacterium* spp.

Pathogens	Pathological signs						
	Body ulcer	Eroded fins	Eye exophthalmia	Eroded barbells	Eroded tail	Lethargic	Mortality
Streptococcus spp	-	+	+	+	+	+	+
Pseudomonas spp	+	-	-	-	-	+	+
Flavobacterium spp	+	+	-	-	+	+	+

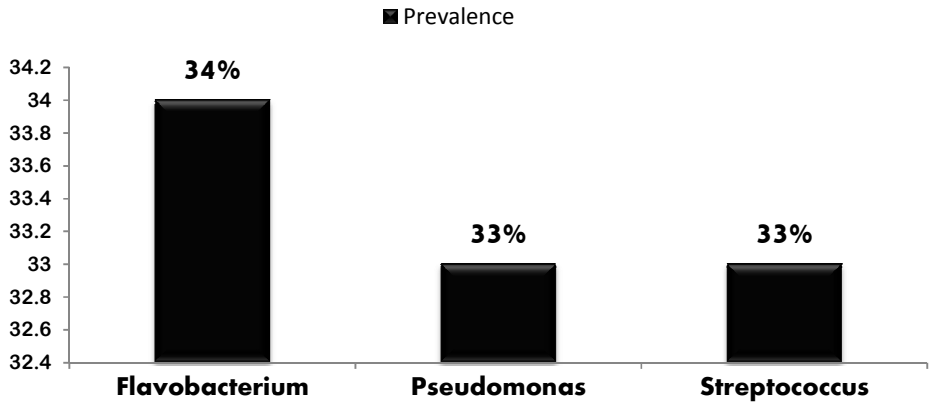

Fig. 1. Prevalence rate of bacterial pathogens of African catfish *Clarias gariepinus* cultured in smallholder homestead farms located within Enugu metropolis Eastern Nigeria

4. DISCUSSION

Poorly managed smallholder fish farms encourage growth of both obligatory and facultative bacteria in African catfish *Clarias gariepinus*. Acidic water condition in culture systems as we noted in this experiment is inimical to growth of fish and causes stress. Stress factors weaken immunity of fish making them vulnerable to pathogen infections. Nevertheless up till date there have not been major cases of nationwide smallholder farm disease outbreaks in Nigeria as previously reported by Hecht and Endemann [24]. There had been isolated cases of diseases in farms [5-7]. However the infections noted in this experiment seem opportunistic. Stress factors in the sampled smallholder farms like poor water conditions, low pH and low dissolved oxygen content, high temperature and overcrowding could have reduced the immune system of the fish making them prone to infections. Ponds with very low dissolved oxygen ≤ 3mg/l have been known to trigger infections of *Flavobacterium columnare* [12]. The cases of ichthyophthiriosis caused by infestation of protozoan ecto parasite *Ichtyopthirius multifilis* could be due to elevated water temperature (this experiment was done during hot periods months of January to early May 2016). Moreso poor fish welfare and overcrowding and feeding noted in the farms may have polluted the water creating enabling environment for proliferation of opportunistic parasites. High infestation of *I. multifilis* have also been noted in *Clarias gariepinus* cultured in farms in Egypt [25] and in *C. gariepinus* cultured in homestead ponds of Enugu eastern Nigeria [7]. Infestation with *ich* renders the fish listless, reducing feeding rate and leads to altered growth. Altered growth due to parasitism is easily noticeable in laboratory situations [26,27] contrary to the wild where poorly growing catfish may get predated.

The *Flavobacterium* infection leads to erosion of fish tails. There were dermal lesions on the skin of the catfish. The poor water conditions and high temperature must have stressed the fish enabling the infection with *Flavobacterium.* Poor water condition and other environmental stress factors have been noted to trigger infections with *Flavobacterium* [28,29]. The fish infected with *Flavobacterium* feed sparingly and had body ulcer, lethargic and impaired swimming. Impaired swimming and reduced appetite has also been noted for *Labeo bata* infected with *Aphanomyces invadans* with manifestation of epizootic ulcerative syndrome (EUS) [30]. High mortality of catfish infested with *Flavobacterium* must have resulted from the pathological manifestation and feeding impairment. This is in line with Barthel et al. [31] and Huntingford et al. [32] who noted that fin erosion impairs or reduces range of fin movement, cause poor maneuvering and generally poor swimming, making fish to be susceptible to predation and feed poorly.

The *Streptococcus* infections of the catfish were diagnosed with eroded barbells, exophthalmia, dermal lesion and erratic swimming and swimming in circles. The swimming movement seems to be due to malfunctioning of the fins, these were also noted by Eldar et al. [33]. Nile tilapia infected with *Streptococcus* developed spiral swimming, loss of balance, impaired feeding and death [34]. Environmental stressors like high temperature >25°C and salinity of 30 ppt had been noted to increase mortality of fish infected *Streptococcus* [34]. In our research salinity was not a problem but pH, dissolved oxygen and poor water management could have contributed to susceptibility of the African catfish to these bacteria.

Pseudomonas infected catfish was characterized by skin ulceration, lethargic movement and eventual death. The feeding of the catfish was reduced. *Pseudomonas* sp has been isolated from body of healthy African catfish *Clarias gariepinus* [22]. Similarly the liver and kidney of healthy turbot have been found to be populated by mostly *Pseudomonas* and *Vibrio*, including *V. fischeri*, *V. harveyi*, *V. pelagius*, and *V. splendidus* [35]. It seems *Pesudomonas* is opportunistic bacteria that becomes pathogenic when favorable condition arises otherwise it can exist on fish host without manifestation. This suggests that *Pseudomonas* infection in the African catfish could be triggered by adverse conditions like stress and poor rearing water condition. *P. flourescens*, which is ubiquitous in fresh water and is generally regarded as secondary invader of damaged tissue, has also been associated with outbreaks of septicemia [36,37]. In our study *Pseudomonas* spp. was only associated with body ulcer mortality. Some *Pseudomonas* spp. have been implicated as producing histamine that is involved in fish spoilage [38,39 and 40].

5. CONCLUSIONS

The prevalence of African catfish parasites and bacteria in smallholder ponds is dependent upon management system of the farm. Parasites can exist in an environment without attacking the fish but environmental stressors can act as triggers. Protozoan and nematode parasites are prevalent in homesteads ponds and can reach high infestation thereby compromising welfare of the African catfish *C. gariepinus*. African catfish has resistance to some pathogens but compromising the catfish welfare adversely affect the immune system making fish vulnerable to pathogens like *Flavobacterium, Pseudomonads* and *Streptococcus* spp. There can be high mortality recoded from these pathogens but adoption of hygiene and integrated management will reduce their prevalence.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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