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ARTICLE

Prevalence of *Aeromonas hydrophila* Isolates in cultured and Feral *Clarias gariepinus* of the Kainji Lake Area, Nigeria.

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SUMMARY

Bacterial diseases especially those caused by Gram- negative organisms are responsible for mass mortality in both wild and cultured fish species. Aeromonas hydrophila is one of the Gram- negative bacteria commonly encountered in an aquatic environment. The prevalence of A. hydrophila the causative agent of aeromonad septicaemia of fish was investigated among the wild C. gariepinus caught from different sampling sites in Kainji Lake and cultured fish samples from homestead fish farms within the Kainji Lake area. Morphometric measurements were taken for each fish sample collected, isolation and identification of the bacteria using morphological characteristics and biochemical tests was carried out. Morphometric characteristics of the fish species shows that the mean condition factor (K) of the infected C. gariepinus was 1.09±0.15 while the uninfected was 1.81±0.14. The difference in K of infected and uninfected was statistically significant ($p<0.05$). Hemorrhages on the body surface, ulcers of various sizes on the skin, fin erosions, inflamed vent, abdominal distension and bulging eyes were some of the sign observed in some of the fish samples that A. hydrophila was isolated. The bacteria was isolated from 19.2% of the wild C. gariepinus sampled while 30.5% from the cultured samples.

KEY WORDS: Prevalence, Aeromonas hydrophila, morphometric Clarias gariepinus

INTRODUCTION

Fish cultured in an aquarium, ponds and even those in the wild are prone to many communicable and non-communicable diseases (Pillay, 1996). According to Kumar (1992), about one-third of economically important fish perish every year through disease and about 60% of these losses are due to microbial pathogens; bacteria, virus and fungi. Representatives from more than 20 genera of bacteria have been isolated from fish of which at least 15 species are recognized as actual or potential pathogen (Frerichs, 1984). A. hydrophila and other motile aeromonads are among the most common bacteria in freshwater habitat throughout the world and these bacteria frequently cause disease among cultured and feral fishes (Cipriano, 2001). A variety

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of freshwater and brackish water fishes such as Clarias gariepinus (Ogbulie and Okpokwsili, 1999; Austin and Austin, 1999) tilapia (Amin et al, 1985), Cyprinus carpio (common carp) (Newman, 1983) and Heterotis niloticus (Obiajuru and Ogbulie, 2006) have been reported to be susceptible to the aeromonad infections. Important fact about A. hydrophila is that it is a zoonotic disease and one of the fish diseases that can be easily transmitted to man (Francis-Floyd, 2002). It has been implicated as a potential agent of gastroenteritis (Aslani and Alikhani, 2004) and is frequently isolated from wound infections sustained in aquatic environments (Krovacek et al., 1992). A. hydrophila is a ubiquitous Gram negative, motile, rod shaped bacterium which can be commonly isolated from freshwater ponds and is also a normal inhabitant of the gastrointestinal tract of fish. In tropical aquaculture, it is considered to be a major economic problem (Amin et al, 1985).

The occurrence of disease outbreak in fish farming is usually associated with bad husbandry practices, since the disease causing organisms especially bacteria are often ubiquitous and cause few problems until the fish are stressed through inadequate dietary or environmental conditions (Shepherd, 1978). The prevention and control of the introduction and multiplication of these disease agents are very important for profitable fish culture and as a measure to reduce production losses.

Kainji Lake the largest man- made lake in Nigeria apart from its primary function of hydro-electricity generation support artisanal fisheries activities. Lake Kainji receives organic waste through livestock husbandry operations in which cattle defecate into and around the bank of the lake. Due to the influence of the Kainji Lake and possibly the existence of National Institute for Freshwater fisheries Research, New Bussa, aquaculture activities within the Kainji Lake area are thriving. There are a large number of fish farms within the area some of whom source their fingerling which they use in stocking their ponds from the Kainji Lake. This practice makes it possible for easy transfer of disease agents from the fish in the wild (Kainji Lake) to those in the culture facilities. Since A. hydrophila is ubiquitous in the aquatic environment, this study of its prevalence in both wild and cultured C. gariepinus is aimed at understanding its epidemiology with a view to controlling the spread of the pathogen.

MATERIALS and METHOD

Study Area:

Lake Kainji which is the largest man- made lake in Nigeria was created in 1968 after the damming of river Niger for hydro-electricity generation. The Lake also offers opportunities for developmental projects like fisheries and irrigation farming. The lake lies between latitude 9° 50′ and 10° 55′ N and longitude 4° 25′ – 4° 45′ E and between the borders of sub- Saharan and Northern Guinea savanna zones (Obot, 1989). Six stations (S1 – S6) on the lake Kainji were sampled for the isolation of A. hydrophila in the fish species caught in those sites. The sites were selected following stratified random sampling. The sampling stations include; Zamare (S1) located at the Northern basin, Musawa (S2), Shagunu (S3) and Wara (S4) which are located at the central basin of the lake. The fifth sampling station yuna (S5) falls within the southern basin while the sixth sampling station Faku (S6) is located south of the dam site (out flow from the Kainji dam to the River Niger) as shown in figure I.

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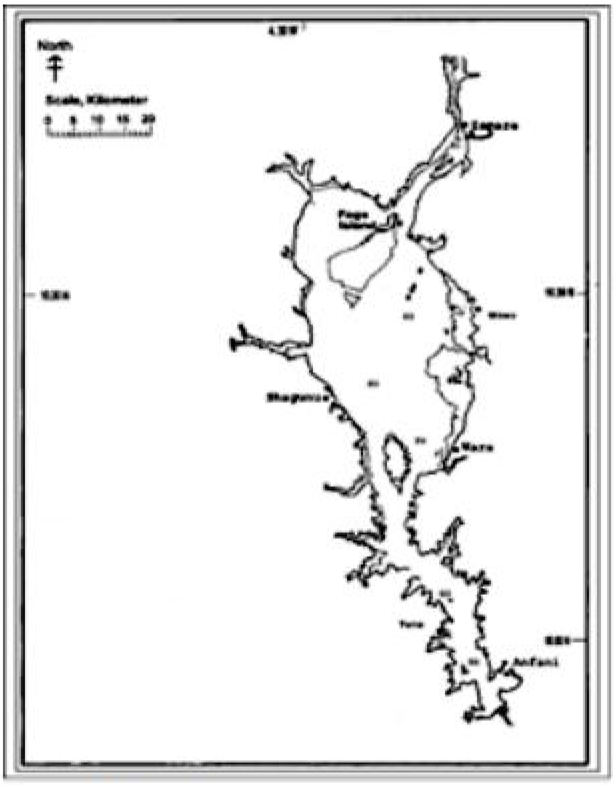

FIGURE I: Map of Kainji lake showing the sampling stations.
Station 1: Zamare
Station 2: Shagunu
Station 3: Musawa
Station 4: Wara
Station 5: Yuna
Station 6: Faku

Also selected homestead fish farms within Kainji lake area were sampled for A. hydrophila. A total of eight homestead fish farms selected following stratified random sampling were sampled, they include; F1 located in Monai, F2 in New Bussa, F3 in Monai, F4, F5 and F6 also located in New Bussa, while F7 and F8 were located in Monai and Dogongari respectively. None of the fish farms sourced their water from the Kainji lake.

Experimental fish:

The fish samples were purchased from fisher men and fish mongers at different landing sites along the kainji lake area (the six sampling stations). Collection of sample was done twice monthly for one calendar year (June 2009 to May 2010). After collection, the samples were transported in ice box while live samples were transported live to the laboratory of the National Institute for Freshwater Fisheries Research, New Bussa where the primary isolation of the A. hydrophila was carried out. A total of 120 C. gariepinus were collected from the six sampling sites in the Kainji Lake fish landing sites. Also 16 adult C. gariepinus were collected from each of the 8 homestead fish farms making a total of 128 samples from cultured facilities for screening for A. hydrophila.

The total and standard lengths of the fish samples were measured using a measuring board graduated in centimeters while top loading balance (Satarius) was used to measure their body weights to the nearest grams. Condition factor (K) or coefficient is widely used to express the relative robustness of a fish sample. The K was calculated using the formula adopted by (Ricker, 1975)

$$K = \frac{100W}{L3}$$

Where L = standard length (cm); W = Body weight (g)

Isolation and identification of A. hydrophila

Each fish were aseptically dissected and a swab from the intestine were inoculated with Alkaline peptone water (enrichment) after which a loopful were streaked on nutrient agar (Biotec Laboratories Ltd, Suffolk, UK) plate and incubated at 37oC for 24hrs (Van Graevenltz and Bucher, 1983). The suspected colonies of A. hydrophila were sub-cultured on fresh nutrient agar plates and incubated at 37oC for 24 hrs to obtain a pure culture. The general methods of visual inspection of the growth, size, colour, shape, elevation, edge-characteristics, and surface-presentation of the colonies followed those of Holt (1982). To confirm the identity of the isolated bacterium, standard biochemical assays such as Gram- staining, catalase, oxidase, hydrogen sulphide, methyl red and Voges-Proskauer tests were performed as described by Cheesebrough (2002).

Data Analysis

Results are presented as means with standard deviations. All data collected were subjected to analysis of variance (ANOVA). Variant means were separated using Duncan Multiple range test. Mean differences with p<0.05 were considered statistically significant.

PLATES I and II: C. gariepinus that are infected with A. hydrophila. Haemorrhages and ulceration are pronounced.

PLATE III: C. gariepinus with severe erosion of the anal and caudal fins caused by A. hydrophila. Skin at the fin bases were swollen and bright red from hemorrhag

RESULTS

Clinical findings

While most of the fish samples that tested positive to A. hydrophila infection showed no clinical manifestation of the disease, some showed signs according to the stage of disease; these include hemorrhages on the body surface, ulcers of various sizes on the skin, fin erosions, inflamed vent, abdominal distension and protrusion of the eye ball are the sign observed as shown in Plates I, II, and III.

Morphological and Biochemical features of isolates

The morphological presentations of the isolates that were presumptively identified as A. hydrophila include smooth, circular and yellowish growth on agar media. All identified isolates that conform to the biochemical tests results such as Gram and H2S negative, Oxidase, Catalyse, Motility, Methyl red and Voges proskaur positive were recorded as A. hydrophila.

Morphometric characteristics of the infected and uninfected fish samples

The mean K for uninfected C. gariepinus was 1.81 ± 0.14 (range; 0.94 – 1.52) while K for infected was 1.09 ± 0.15 (range; 0.9 – 1.41). There is a significant difference (p < 0.05) in the condition factor of infected and uninfected C. gariepinus as shown in Table I.

TABLE I: **The morphometric characteristics of the infected and non -infected *C. gariepinus***

	Mean weight(g)	**Mean Total length (cm)**	**Mean Standard length (cm)**	**Mean Condition factor (K)**
Uninfected	589.23 ± 233.9^{a} (140.0–1150.0)	39.07 ± 5.52^{a} (26.1 – 49.6)	36.27 ± 5.23^{a} (22.5 – 47.2)	1.18 ± 0.14^{a} (0.94 – 1.52)
Infected	658.94 ± 174.2^{b} (353.2–1020.5)	41.29 ± 3.44^{a} (35.0 – 48.2)	39.0 ± 3.45^{a} (33.3 – 46.0)	1.09 ± 0.15^{b} (0.9 – 1.41)

Mean with the same superscripts along the vertical row are not significantly different (P > 0.05) while those of different superscript are significantly different (p<0.05).

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TABLE II: **Prevalence of *A. hydrophila* isolates in *C. gariepinus* specimen among the sampling sites within the Kainji Lake**.

Sampling site	Number sampled	Number infected	Prevalence (%)
Zamare	20	4	20
Musawa	20	7	35
Shagunu	20	5	25
Wara	20	2	10
Yuna	20	2	10
Faku	20	3	15
Total	**120**	**23**	**19.2**

Prevalence of A. hydrophila from feral and cultured fish samples

Results of the A. hydrophila isolation from C. gariepinus from the different sampling sites shows the highest prevalence rate (35%) in Musawa fish landing site followed by Shagunu (25%) while the least (10%) was obtained in Yuna and Wara [Table II]

Results of the A. hydrophila isolation from C. gariepinus from the different homestead fish farms within Kainji Lake area shows the highest prevalence rate (43.75%) in fish farm, F4 located in New Bussa followed by fish farm, F2 located also in New Bussa and fish farm, F7 located in Monai while the least (18.26%) was recorded in fish farm, F6 located in New Bussa. [Table III]. Out of 120 C. gariepinus from the wild, 23 (19.2%) yielded A. hydrophila while out of 128 samples of cultured C. gariepinus, 39 (30.5%) were positive (Table IV).

TABLE III: Prevalence of *A. hydrophila* isolates in *C. gariepinus* specimen among the selected fish farms within the Kainji Lake area.

Fish farm	№ sampled	№ infected	Prevalence (%)
F1	16	3	18.75
F2	16	6	37.5
F3	16	5	31.25
F4	16	7	43.75
F5	16	5	31.25
F6	16	3	18.26
F7	16	6	37.5
F8	16	4	25
Total	**128**	**39**	**30.5**

TABLE IV: Comparative prevalence of *A. hydrophila* isolates among the feral and cultured *C. gariepinus* in the Kainji Lake area.

	Feral fish	Cultured fish
Sample size	120	128
№ uninfected	97	89
№ infected	23	39
Prevalence (%)	19.2	30.5

DISCUSSION

Some of the fish samples showed no obvious signs of disease but harboured A. hydrophila. Some of the clinical signs observed were similar to those of Dilek and Hulya (2007) who observed haemorrhagic skin lesions, brown or red spotted skin changes of varying degrees along the bodies of carp infected with A. hydrophila. In the present study, although most of the fish samples in which A. hydrophila were isolated showed no outward clinical signs of the disease, some of the samples manifested varying degrees of signs. Hemorrhages on the body surface, ulcers of various sizes on the skin, fin erosions, inflamed vent, abdominal distension and protrusion of the eye ball are the sign observed in some of the C. gariepinus. While these signs are not pathognomonic for A. hydrophila infection, they were assumed to have been as a result of the bacterium since no other pathogen was isolated. Tentative diagnosis of A. hydrophila infection may be based on the past disease status of the fish and the presence of clinical manifestations of disease. However, isolation and biochemical identification of the bacteria is required to provide confirmatory diagnosis (Cipriano, 2001).

All isolates in the present study were morphologically and biochemically similar. The colonies of A. hydrophila which were isolated appeared yellowish, round, convex and flattened in solid agar media. The results of the biochemical characterization of the isolates were in agreement with those reported by Gosling (1986). The morphometric characteristics of infected and uninfected C. gariepinus obtained from the study indicates that there is a significant difference ($p < 0.05$) in the condition factor (K) of the two groups. The uninfected C. gariepinus had a mean K value of 1.81 ± 0.14 while the infected had 1.09 ± 0.15 as shown in Table I. The fact that uninfected C. gariepinus had a higher K value is not surprising since condition factor is a measure of wellbeing of a fish. Neff and Cargnelli (2004.) reported lowered condition factor in blue-gill sunfish infected with parasites. Cone (1989) indicated that the relationship between fish weight and length (condition factor) is frequently used to compare the effect of biotic and abiotic factors on the health or well-being of a fish population. Plump fish may be indicators of favorable environmental conditions (e.g., quality water, adequate nutrition and absence of disease condition), whereas thin fish may indicate less favorable environmental conditions. This is based on the hypothesis that heavier fish of a particular length are in a better physiological condition (Bagenal, 1978).

The isolation study revealed presence of A. hydrophila from fish collected from different sampling sites, however the highest prevalence of the bacterium was obtained in Musawa followed by Shagunu and the least in Yuna and Wara respectively as shown in table II. The differences in the relative prevalence of A. hydrophila in the different sampling sites within Lake Kainji could be due to the pollution levels at the different sampling sites. Musawa fishing village has a cattle ranch and other agents of pollution within the shoreline. Cattle drink and defecate in and around the lake at Musawa also people living in the fishing village dump their refuse on the shore which is invariably washed into the lake by run-off water. The same scenario was also obtainable in Shagunu, the fishing village hosts a big fish market. Garbage and other wastes from the market and adjoining fishing village empty into the lake. This development is favourable for propagation of a number of micro organisms including A. hydrophila which may account for the high prevalence of the bacterium in the two sampling stations. A. hydrophila is saprophytic in nature and thus their prevalence is consequent upon environmental deterioration (Ogbulie and Okpokwasili, 1999). Results of the A. hydrophila isolation from the different homestead fish farms within Kainji Lake area shows the highest prevalence rate was recorded in Fish farm F4, New Bussa while the least was recorded in fish farm; F6 in Monai as shown in table III. The prevalence rate of isolation of A. hydrophila in each of the fish farms depends on the hygiene levels practiced by the farm and the water quality of the fish ponds. Most of the farms are rain fed and also from the domestic water supply.

The result of this study shows that pathogenic A. hydrophila occurs in confined and feral fishes. The high prevalence rate of A. hydrophila in both cultured and in the wild as recorded in this study is in agreement with Trust et al. (1974) who opined that the bacteria are common in the intestinal flora of apparently healthy fish. The high prevalence of A. hydrophila in diseased and apparently healthy fishes from various sampling sites of the Kainji Lake and different fish farms within the Kainji Lake area strongly suggested the ubiquitous nature of the bacterium (Topic- Popovic et al., 2000). It was observed that none of the fish farms sampled is free of A. hydrophila.

When the percentage prevalence of A. hydrophila isolates in the wild (Lake Kainji) was compared with that of the culture facilities (fish farms) (Table IV), it was observed that there are higher prevalence of the bacterium in the culture than in the wild. It can be deduced that A. hydrophila is more likely to be an important pathogen of cultured rather than of feral fish. This finding is in agreement with the work of Eisa et al. (1994). The higher prevalence of A. hydrophila in culture facilities than in the wild are probably due to density dependent factors such as higher number of the bacteria per unit of water volume, increased contact times between the fish and the bacteria.

In conclusion, it can be deduced from the study that cultured fishes are more susceptible to A. hydrophila than their feral counterparts. The organism was isolated most on fish samples from high stocking density and low water volume fish farms and also in parts of the lake that are polluted with organic wastes.

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