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ARTICLE

A Relative Prevalence of *Oreochromis Niloticus, Clarias Gariepinus* and *Heterotis Niloticus* to *Aeromonas Hydrophila* in An Integrated Fish Farm.

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SUMMARY

A total of 120 *Clarias gariepinus*, 120 of *Heterotis niloticus* and 150 *Oreochromis niloticus* were collected from integrated fish cum chicken reservoir for *Aeromonas hydrophila* screening. Samples were collected twice monthly for one calendar year.

The physico-chemical parameters of the reservoir water were taken each sampling day. Bacterial culture involved pre-enrichment in alkaline peptone water and incubation in nutrient agar at 37°C for 24hrs. Standard biochemical assays such as Gram- staining, catalase, oxidase, hydrogen sulphide, indole, methyl red and Voges-Proskauer tests were performed to confirm the bacterium. Prevalence rates of *A. hydrophila* in O. niloticus, C. gariepinus and H. niloticus was 34%, 31.7% and 27.5% respectively. The study shows that *O. niloticus* is more prone to thebacterium followed by *C. gariepinus* and least *H. niloticus*. Although the organism was isolated all the months of the year, it was observed that there was higher prevalence of the organism during the warmer months.

KEY WORDS: *Aeromonas hydrophila, Clarias gariepinus, Heterotis niloticus, Oreochromis niloticus,* Disease, Prevalence.

INTRODUCTION

Aeromonas hydrophila is one of the most important pathogens of freshwater fishes (Hayes, 2005). It has been associated with several disease conditions in fish, including tail rot, fin rot, and haemorrahagic septicaemia. The disease condition is generally referred to as motile aeromonas septicaemia (Austin and Austin, 1985). *Aeromonas hydrophila* is a Gram-negative entero-bacterium widelydistributed in aquatic environments (Gonzalez *et al*, 2002). The bacterium can be transmitted horizontally by water-borne release and faecal contamination, as well as via reservoir hosts (Paul *et al.*, 1995; Akinwale *et al.*, 2007).

Integrated fish farming is a multiple land use approach which combines fish culture with other agricultural production, integrated fish - poultry is one of such systems. FAO (1979) enumerated the benefits of integrated fish farming, one of which is the provision of cheap feedstuffs and organic manure for pond fertilization and thus reduction of cost of inorganic fertilizers and commercial feed. In fish cum poultry integrated system, the droppings from the chicken and the spill over feed helps in fertilizing the fish pond thereby improving the productivity of the aquaculture facility. But one of the demerits of the integrated livestock cum

fish culture is the proliferation of disease causing organism in the fish culture facility occasioned by the decomposition of the faecal droppings and other waste products which ultimately lead to water contamination. Water quality is the suitability of water for the survival and growth of fish (Boyd, 1982). As a water dwelling organism, fish receive dermal exposure through whole-body immersion in the contaminated water. Bacteria are among the most encountered disease causing microorganisms in the integrated system and since *A. hydrophila* is a ubiquitous bacteria in aquatic environment, studies on its prevalence in fish species cultured in an integrated system is very essential.

Catfish (*Clarias gariepinus*), tilapia (*Oreochromis niloticus*) and heterotis (*Heterotis niloticus*) are among the important fish species cultured in Nigeria (Omeje, 2005). The objectives of the study are to ascertain the relative susceptibility of these three fish species cultured in Nigeria to *A. hydrophila* and also the seasonal prevalence of the bacteria in an integrated fish reservoir.

MATERIALS AND METHODS

The study was undertaken at the integrated fish cum poultry section of the National Institute for Freshwater Fisheries Research (NIFFR), New Bussa. The culture facility is reservoir pond of about 1.5 hectares with poultry house erected over the reservoir. Chickens defecate directly into the water which helps in fertilizing the pond (Plate I). The three main fish species cultured in Nigeria, i.e. catfish (*Clarias* sp. and *Heterobranchus* sp.), tilapia sp. (*O. niloticus* and *Sarethoridon galilaus*) and *H. niloticus* are cultured together. The fish used for the study were collected using a drag-net. The fish were collected twice monthly for one calendar year (January 2010 – December 2010). A total of one hundred and twenty (120) each of catfish and heterotis and one hundred and fifty (150) of tilapia were collected for screening for *A. hydrophila*. Relevant physico-chemical parameters such as water temperature, dissolved oxygen and pH of the reservoir water were monitored at each sampling day. Water temperature was measured with a laboratory thermometer while dissolved oxygen was determined by titremetric Winkler's method; pH was measured with pH meter (Lovibod comparator, model 3153).

Isolation and identification of *A. hydrophila*

A Portion of the intestine of each fish sample was aseptically dissected out and pulverized in sterile 0.1% peptone water using sterilized pestle and mortar. Samples were inoculated with Alkaline peptone water (enrichment) after which a loopful were streaked on nutrient agar (Biotec Laboratories Ltd, Suffolk, UK) and incubated at 37°C for 24hrs (Van Graevenltz and Bucher, 1983). The suspected colonies of *A. hydrophila* were sub-cultured on fresh nutrient agar plates and incubated at 37°C for 24 hrs to obtain a pure culture. The general methods of visual inspection of the growth, size, colour, shape, elevation, edge-characteristics, surface-presentation, consistency and transluscence 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, indole, methyl red and Voges-Proskauer tests were performed as described by Cheesebrough (2002).

RESULTS

Results of the physicochemical parameters

of the reservoir water were presented in table II. 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 conformed to the biochemical tests results such as Gram and H_2S negative, Oxidase, Catalyse, Motility, Growth on blood agar, ß- haemolysis, Methyl red and Voges proskaur positive were recorded as *A. hydrophila*. Some of the fish samples in which A. hydrophila was isolated showed clinical signs of the disease. In *O. niloticus*, darkening of the color of the skin, scales detachment, inflamed vent, exophthalmia, abdominal distension as shown in Plate II was observed while Haemorrhages and ulceration was seen in *C. gariepinus*. In *H. niloticus*, the signs observed include roughened scales, scale loss, patches of haemorrhagic ulcers and erosion of the dorsal and caudal fins.

Results of this study shows that prevalence rates of *A. hydrophila* in *O. niloticus, C. gariepinus* and *H. niloticus* was 34%, 31.7% and 27.5% respectively as shown in table 1.

DISCUSSION

The result of the relative prevalence of *A. hydrophila* in the three fish species under study shows that the bacteria were more prevalent in *O. niloticus* followed by *C. gariepinus* and least *H. niloticus*. This result are in agreement with Peter *et al.*, (1988) who opined that following exposure to *A. hydrophila*, the bacterium was recoverable with greater prevalence among the subordinate fish than from their dominant cohorts. *Oreochromis niloticus* being the subordinate of the three fish species in terms of size and position in the food chain may account for the higher prevalence rate of the bacterial isolated. In the case of *C. gariepinus* and *H. niloticus*, the fact that *C. gariepinus* are benthic in nature and lives most of the time in the mud while *H. niloticus* are more pelagic in nature and are less likely to come in contact with mud and other debris that harbor the bacterium may account for *C. gariepinus* harboring *A. hydrophila* more than *H. niloticus*.Fish takes a large number of bacteria into their gut from water, sediment, and food (Sugita *et al.* 1988). The high prevalence rate of *A. hydrophila* among the three fish species obtained in the study may be due to the fact that in integrated fish – poultry system (Plate I), droppings from the chicken and spill over feed may lead to proliferation of microorganisms. According to Ogbulie and Okpokwasili (1999), *A. hydrophila* is saprophytic in nature and thus their prevalence is consequent upon environmental deterioration.

Studies of the physico-chemical parameters of the reservoir water (Table II) indicate that the temperature, dissolved oxygen (DO) and hydrogen ion concentration (pH) obtained during the study were within the acceptable range for freshwater fish culture (Boyd, 1990). The peak temperature of the reservoir water was in May (32.3 ± 1.90^0C) while the coldest month was in December (26.8 ± 1.60^0C). No correlations could be made with the environmental temperature as the bacterium was isolated throughout the year with varying water temperature. However it was observed that there was higher prevalence of the organism during the warmer months of the year. It was observed that the highest prevalence of *A. hydrophila* isolates occurred in May which was the hottest month. The least prevalence obtained in the study occurred around December and January which are the cold period of the year. This pattern of seasonal prevalence was observed in the three fish species studied. This result was

in agreement with the work of Ibrahem *et al.*, (2008) who also reported higher prevalence rate in warmer months of the year. However some researchers (Pathak *et al*, 1988; Topic-Popovic *et al.*, 2000) though agreed on the seasonality of *A. hydrophila* infection observed higher prevalence in winter compared to summer months. The implication of such findings is that the disease may not necessarily be associated with warm or cold water but rather on extreme temperature variations. Rapid rise (Esch and Hazen, 1980; Nieto *et al.*, 1985;) or drop (Doukas *et al.*, 1998) in water temperature may be a stressful factor contributing to outbreaks of disease caused by *A. hydrophila* as fish may be immuno-compromised by such stress. Huizinga *et al.* (1979) also indicated that rising water temperatures increased metabolism, decreased overall condition, and stressed the fish. Stressed fish increased production of corticosteroids, which in turn increased their susceptibility to infection. Goldfish and koi carp are susceptible to infection with *A. hydrophila* especially during the warmer water temperature of summer (Dixon and Issvoran, 1993). They were of the opinion that increase in temperature may be favorable for the proliferation of *A. hydrophila* and hence the more prevalence of the bacteria during the warmer months.

PLATE I: Integrated poultry cum fish farm

TABLE I: Prevalence of A. hydrophila isolation from *O. niloticus, C. gariep*inus and *H. niloticus* in a reservoir culture facility integrated with poultry.

Species	No Examined	No infected	Prevalence %
O. niloticus	150	52	34.7
C. gariepinus	120	38	31.7
H. niloticus	120	33	27.5

TABLE II: Mean physico-chemical parameters (mean±SD) of the integrated fish cum poultry reservoir for the study period (January 2010 – December 2010).

Months	Mean Temp (^{0}C)	Mean DO(mg/l)	Mean pH
January	.....	5.70 ± 0.32	6.7 ± 0.50
February	30.8 ± 1.93	4.25 ± 0.24	7.0 ± 0.53
March	30.2 ± 1.90	5.10 ± 0.32	6.4 ± 0.47
April	29.9 ± 1.87	5.25 ± 0.20	7.2 ± 0.54
May	32.3 ± 1.90	4.65 ± 0.39	6.9 ± 0.51
June	28.4 ± 1.51	4.82 ± 0.26	7.2 ± 0.60
July	28.0 ± 1.75	4.76 ± 0.23	6.8 ± 0.50
August	27.3 ± 1.89	5.12 ± 0.30	7.0 ± 0.53.
September	28.5 ± 1.65	3.85 ± 0.26	7.1 ± 0.48
October	29.2 ± 1.58	4.56 ± 0.31	6.8 ± 0.45
November	28.6 ± 1.72	5.24 ± 0.27	7.0 ± 0.62
December	26.8 ± 1.60	4.68 ± 0.33	6.9 ± 0.56

PLATE II: One of the *O. niloticus* that *A. hydrophila* was isolated showing ulceration and erosion of the caudal fin.

TABLE III: Monthly and Seasonal prevalence of *A. hydrophila* isolates in fishes of integrated fish cum poultry reservoir of NIFFR, New Bussa

Month	Season	No. of fish examined	No. of fish infected	Prevalence (%)
January	Dry	30	7	23.3
February	Dry	32	11	34.4
March	Dry	35	9	25.7
April	Dry	35	11	31.4
May	Dry	32	14	43.8
June	Rainy	30	12	40.0
July	Rainy	33	10	30.3
August	Rainy	34	10	29.4
September	Rainy	35	12	34.3
October	Rainy	34	11	32.4
November	Dry	35	9	25.7
December	Dry	36	8	22.2
TOTAL		401	124	30.9

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