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HAEMATOLOGICAL AND HISTOLOGICAL RESPONSES OF *CLARIAS GARIEPINUS* AND *HETEROBRANCHUS BIDORSALIS* TO SOME BACTERIAL DISEASES IN RIVERS STATE, NIGERIA

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Abstract: Bacteriological evaluation of various diseases of *Clarias gariepinus* and *Heterobranchus bidorsalis* showed higher bacterial colonisation of the diseased than the appparently healthy fish. Identification showed the isolation of *Aeromonas hydrophila, Pseudomonas* sp., *Salmonella* sp., *Vibrio* sp., *Bacteriodes* sp., *Clostridium* sp., *Campylobacter jejuni, Yersina enterocolitica, Bacillus* sp., *Aeromonas* sp. and gram positive anaerobic cocci from apparently healthy fish while *Pseudomonas fluorescencs, Flavobacterium* sp., *Flexibacter* sp. and *Aeromonas* sp. were isolated in addition to the above genera from diseased fish samples. Haematological evaluation of diseased and apparently healthy *C. gariepinus* and *H. bidorsalis* indicated a significant difference ($p<0.5$) in the PCV, WBC, ESR and Hb of diseased and apparently healthy fish. Characteristic differences were observed in the histology of affected tissues of diseased fish. These results are indicative of these bacteria in disease aetiology.

Key words: Bacterial diseases, catfish, fish, Nigeria

INTRODUCTION

In tropical aquaculture, fish farming is vigorously pursued by utilising a minimum of land, water and labour resources to provide an economically profitable source of protein for human consumption. In an effort to achieve rapid fish growth to meet the increasing demand for fish protein, large quantities of domestic waste are generally introduced into fish ponds. Our previous report [1] revealed that the addition and subsequent decomposition of the added waste leads to an unfavourable physicochemical water quality. Other workers [2-4] have reported increased incidence of bacterial infections of fish during such stressful condition in fish culture systems. Previous studies [1,5,6] were directed towards establishing various bacterial flora of aquaculture systems and fish disease in the tropics. Thus, in-depth studies on the various biochemical responses of fish to disease are important in the search for ways of ameliorating the devastating menace of fish diseases of bacterial origin. The role of most bacterial fish pathogens such as *A. salmonicida, A.piscicida, Y.ruckeria, P.flourescens, A.hydrophila, E.tarda, Vibrios* sp., *Salmonella* sp., *Chromobacterium* sp. and others has been reported .[7-11] This study was therefore aimed at evaluating histological and haematological responses to common tropical diseases of catfish with a view to establishing novel diagnostic protocols of their management.

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METHODS AND MATERIALS

Sample Collection

Samples of *Clarias gariepinus* and *Heterobranchus bidorsalis* were collected from ponds located at Ellah Lakes, Obrikom, African Regional Aquaculture Centre (ARAC) Aluu and Fawumi Fish Farm, Rumuokoro all in Rivers State. The stocking density for the ponds was 5 fish/m^2 while the feeding rate was 5% of the body weight administered twice daily. Diseased and apparently healthy fish samples of not less than four months old measuring between 15 cm and 31 cm in length, 4.5 cm and 11 cm width and weighing between 0.42 kg and 19 kg were collected separately in oxygenated bags filled with clean fresh water up to 1/2 of the volume to ensure the survival of the fish samples during transportation to the laboratory. All the samples were processed and analyzed within three hours of collection.

Microbiological Analysis

Bacterial assays of organs and tissues of diseased and apparently healthy fish were assayed with a view to establishing the actual pathogens. Five grams of each sample were homogenised in 45ml physiological saline and the bacterial load enumerated after preparing 10-fold serial dilution of the homogenised samples. Total heterotrophic bacteria were enumerated using tryptone soya agar. *Salmonella, Vibrio* and *Pseudomonas* species were assayed using Bismuth sulphite agar, thiosulphate citrate bile salts, sucrose agar and *Pseudomonas* F agar respectively. The isolation of *Yersinia enterocolitica, Campylobacter jejuni,* total coliforms and *A. hydrophila* were as reported previously[1].

Anaerobic bacterial groups were enumerated using anaerobic blood agar as described by Dowell *et al.*[12] Except for the anaerobic bacteria which were incubated under micro-aerophilic condition at 42°C for 48h, other bacterial groups were incubated aerobically. Reported plate counts were those that had between 30-300 colony forming units (cfu).

Representative isolates were generally characterised as described by Holt[13] and this involved incubation at certain temperature condition, colony morphology, cell morphology, motility, oxidase, catalase, urease, H_2S production, citrate utilisation, oxidation and sugar fermentation tests, methyl red and Voges-Proskauer tests, growth on hippurate and sensitivity to nalidixic acid.

Histological and Haematological Assay

The histological assay was carried out as described by Baker & Sylverton [14] and Austin & Austin.[11] This involved fixing the sample with 10% formaldehyde, dehydrating to remove the formaldehyde using alcohol, dehydrating to remove

alcohol using chloroform, embedding in wax, sectioning using rotary type microtome (Keichert-Jung, London) and permanent slide preparation. The haematological assay involved the determination of red blood cell, white blood cell, packed cell volume (PCV) and erythrocyte sedimentation rate (ESR) as described by Baker & Sylverton[14] and Cruickshank.[15]

Statistical Analysis

Mean of log of colony forming units of bacteria from apparently healthy and diseased fish and white blood cell counts, red blood cell counts, packed cell volume and erythrocyte sedimentation rates of replicates from apparently healthy and diseased fish were subjected to correlation using the correlation and prediction method as described by Phillips.[16]

RESULTS

The bacterial assay of diseased and apparently healthy *C. gariepinus* and *H. bidorsalis* revealed a higher colonisation of the organs and tissues of the diseased than the apparently healthy fish. The diseased fish parts recorded high microbial load similar to the intestinal samples with more pathogenic organisms and bacteria of public health concern occurring in organs of the diseased fish such as liver, kidney and gills which harboured heavier bacterial loads than samples from the apparently healthy fish (Figures 1 and 2). This shows the extent of invasiveness of the offending bacteria. Counts of aerobic and anaerobic bacterial groups were several log cycles higher in the diseased than in the apparently healthy fish, indicative of participatory or synergistic roles of the aerobic and anaerobic bacterial groups in perpetuating the disease condition.

Identification also revealed more genera of bacterial isolates in the diseased than the apparently healthy fish. Of the 15 genera of bacteria isolated from *C. gariepinus* (Table 1), 100% of all the bacterial genera were isolated from the diseased parts (skin rot), 71.41% and 64.28% were isolated from the gill and kidney tissues respectively whereas 50% of the genera were isolated from the liver tissues. On the other hand, only nine genera of the bacteria were isolated from the apparently healthy fish (table 2) with the intestine harbouring 100% of the isolates, 90% were isolated from fish skin whereas 80%, 60% and 40% were isolated from fish gill, kidney and liver samples respectively. Identification of pathogens revealed that *Bacillus* sp., *C. jejuni, Y. enterocolitica, Vibrio* sp., *Salmonella* sp., *A. hydrophila, Escherichia coli, Klebsiella* sp., *Bacteroides* sp. and anaerobic gram positive cocci were isolated from apparently healthy *C. gariepinus* whereas *Flexibacter, Pseudomonas, Edwardsiella, Staphylococcus* and *Aeromonas* species in addition to the organisms mentioned above were isolated from the diseased fish samples.

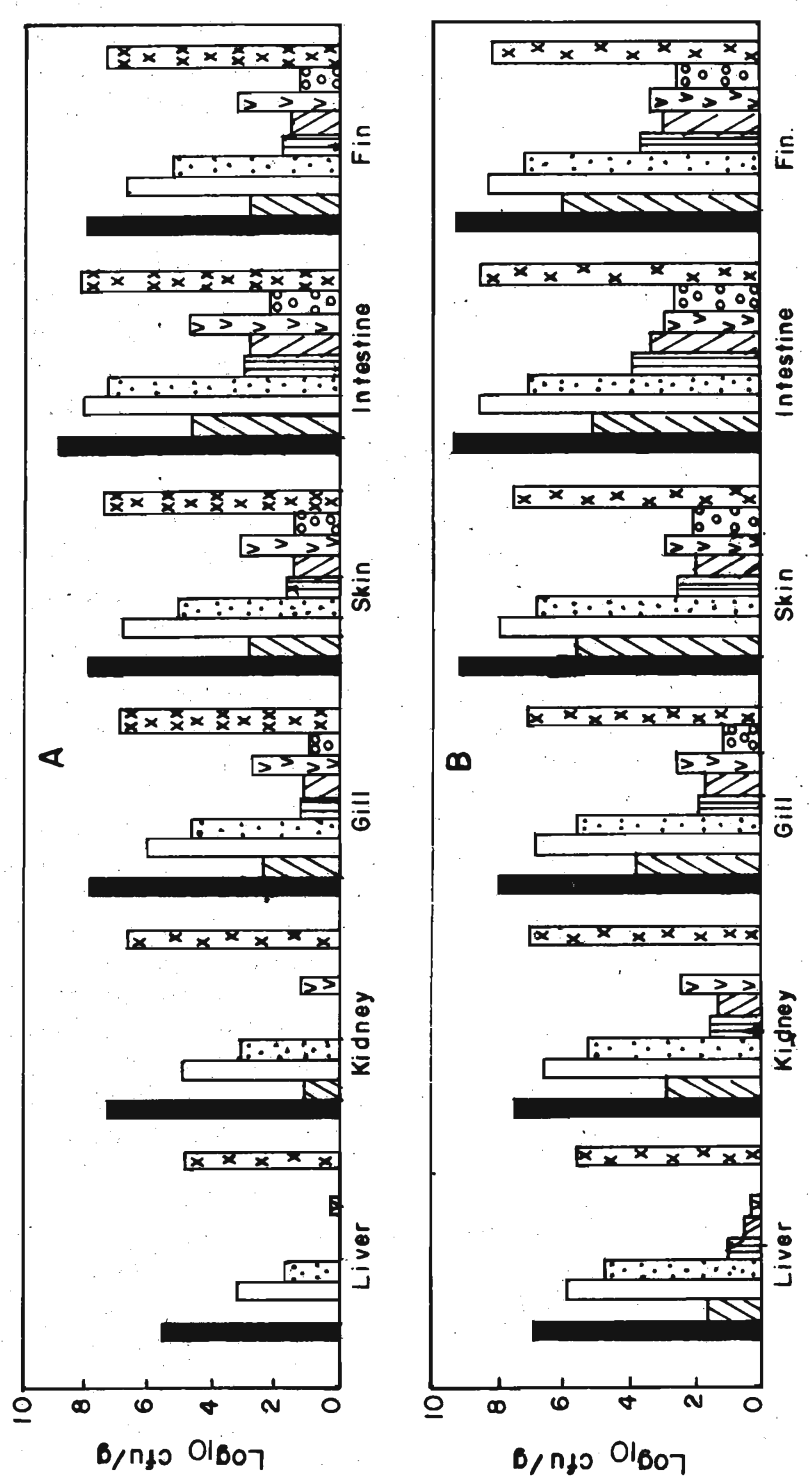


Figure 1 : Bacterial loads of organs and tissues of apparently healthy (A) and diseased (B) *Clarias gariepinus* with skin sore and advanced fin rot. Total aerobic heterotrophic counts (■) *A. hydrophila* (▨); Total coliforms (□); *Pseudomonas* sp. (▤); *Y. enterocolitica* ; (▥) *C. jejuni* (▧); *Salmonella* sp. (▩); Vibrio sp. (▦); Anaerobic heterotrophic viable counts (▩)

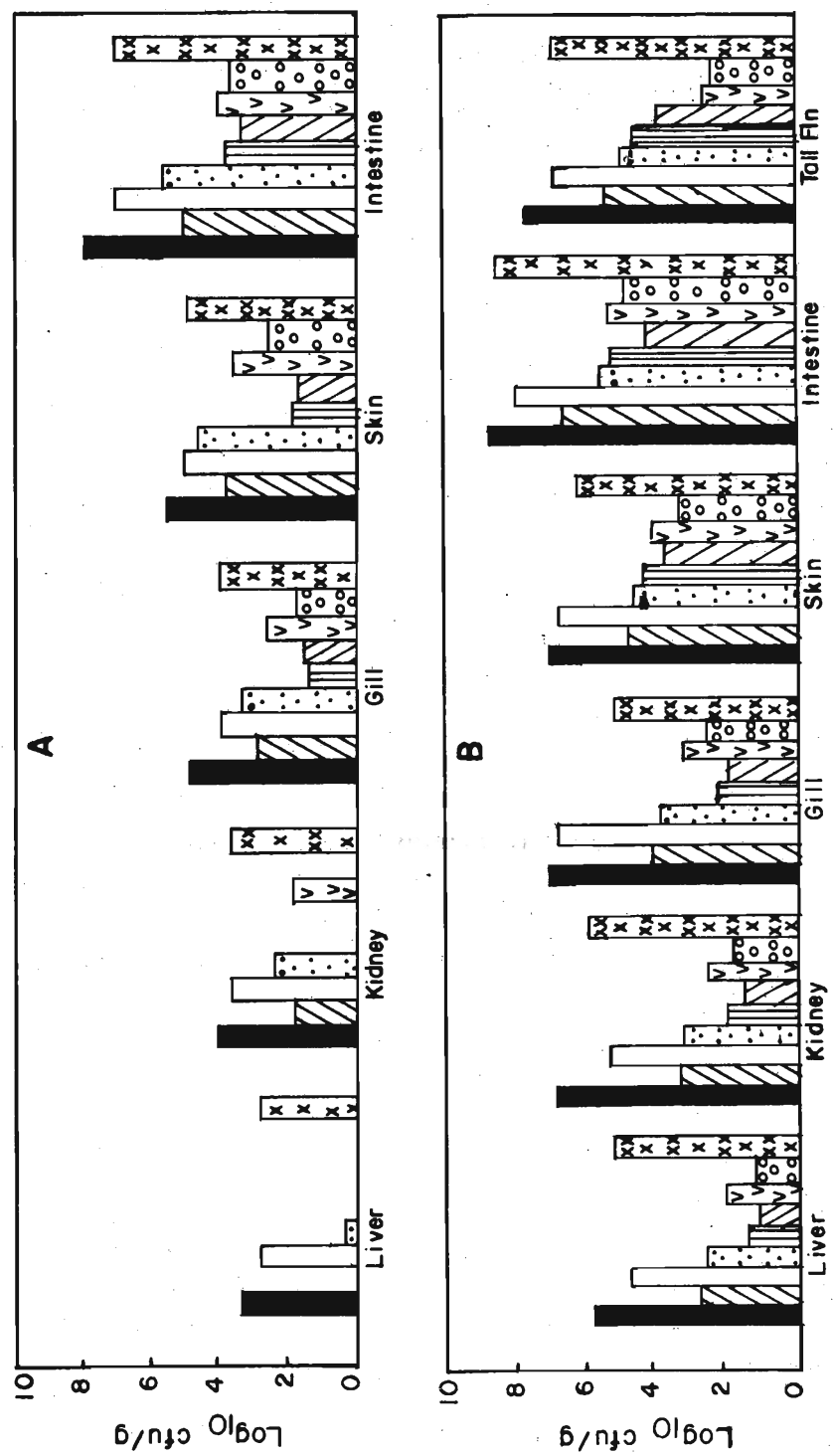


Figure 2: Bacterial loads of organs and tissues of apparently healthy (A) and diseased (B) *Heterobranchus bidorsalis* with skin sore and advanced fin rot. Total aerobic heterotrophic counts (■); *A. hydrophila* (▨); Total coliforms (□); *Pseudomonas* sp. (▦); *Y. enterocolitica* (▥); *C. jejuni* (▨); *Salmonella* sp. (▩); *Vibrio* sp. (▩); Anaerobic heterotrophic viable counts (▩).

Table 1: Bacterial isolates from *Clarias gariepinus* with advanced fin and skin rot.

Isolates	Fish Organ/Tissue					
	Liver	Kidney	Gill	Skin rot	Intestine	Fin rot
A. hydrophila	+	+	+	+	+	+
Pseudomonas sp.	+	+	+	+	+	+
Salmonella sp.	+	+	+	+	+	+
Vibrio sp.	+	+	+	+	+	+
Aeromonas sp.	-	+	+	+	+	+
C. jejuni	+	+	+	+	+	+
Escherichia coli	-	-	-	+	+	+
Edwardsiella sp.	-	+	+	+	+	+
Flexibacter sp.	-	-	-	+	+	+
P. flourescens	-	-	-	+	+	+
Staphylococcus sp.	-	-	-	+	+	+
Bacteroides sp.	-	-	-	+	+	+
Gram+ve anaerobic cocci	+	+	+	+	+	+
Nocardia sp.	-	-	+	+	-	+
% Occurrence	50	64.28	71.41	100	92.85	100

Table 2: Bacterial flora of apparently healthy *Clarias gariepinus*.

Isolates	Fish Organ/Tissue					
	Liver	Kidney	Gill	Skin	Intestine	Fin
Bacillus sp.	+	+	+	+	+	+
C.jejuni	-	-	+	+	+	+
Salmonella sp.	+	+	+	+	+	+
Y. enterocolitica	-	-	+	+	+	+
Vibrio sp.	-	-	+	+	+	+
A. hydrophila	+	+	+	+	+	+
E. coli	-	+	-	-	+	+
Klebsiella sp.	-	-	-	+	+	+
Bacteroides sp.	-	+	+	+	+	+
Gram+ve anaerobic cocci	+	+	+	+	+	+
% Occurrence	40	60	80	90	100	90

Table 3: Bacterial flora of *Heterobranchus bidorsalis* with severe skin and fin rot.

Isolates	Fish Organ/Tissue					
	Liver	Kidney	Gill	Intestine	Skin rot	Fin rot
Aeromonas sp.	+	+	+	+	+	+
Enterobacter sp.	+	+	+	+	+	+
Pseudomonas sp.	+	+	+	+	+	+
Flavobacterium sp.	-	-	+	-	+	+
Salmonella sp.	+	+	-	+	+	+
C. jejuni	+	+	+	+	+	+
Y. enterocolitica	+	+	+	+	+	+
Bacteroides sp.	+	+	+	+	+	+
Clostridium sp.	-	-	-	+	+	+
Nocardia sp.	-	-	+	-	+	+
A. hydrophila	+	+	+	+	+	-
P. fluorescens	-	-	-	+	+	+
Proteus sp.	-	-	+	-	+	+
Edwardsiella sp.	-	-	-	+	+	-
Vibrio sp.	+	+	+	+	+	+
No. of Genera	9	9	11	12	15	13
% Occurrence	60	60	73.33	80	100	86.67

The haematological examination of the diseased and the apparently healthy *C. gariepinus* (Table 5) and *H. bidorsalis* (Table 6) revealed higher haemoglobin counts in the control (apparently healthy fish) than in the diseased fish samples. Whereas high WBC counts and erythrocyte sedimentation rates were associated with diseased fish. Lower PCV was associated with apparently healthy fish samples than the diseased fish. Statistical analysis revealed that there was a significant difference in the haemoglobin (rbc) count, white blood cell (WBC) count, erythrocyte sedimentation rate (ESR) and packed cell volume (PCV) of the diseased and apparently healthy (control) fish samples. The histological examination (Figure 3) elucidated the impact of the offending pathogen(s) on the tissue and organs of the diseased fish. Tissue examination revealed an invasive action of the bacterial pathogens on the organs and tissues of the diseased fish, whereas no observable effect was noticed in the respective tissues of the apparently healthy fish. Photomicrographs (Figure 3) show vacuolar degeneration, cell necrosis and tissue erosion in the diseased as against the healthy fish muscle tissues. Clumps of necrotizing bacteria in the degraded tissues are also evident.

Table 4: Bacterial flora of apparently healthy *Heterobranchus bidorsalis*.

Isolates	Fish Organ/Tissue					
	Liver	Kidney	Gill	Intestine	Skin	Fin
A. hydriphila	+	+	+	+	+	+
Pseudomonas sp.	+	+	+	+	+	+
Salmonella sp.	-	+	+	+	+	+
Vibrio sp.	-	-	+	+	+	+
Bacteroides sp.	-	+	+	+	+	+
Clostridium sp.	-	-	-	+	-	+
C.jejuni	-	+	+	+	+	
Y.enterocolitica	-	-	+	+	+	-
Bacillus sp.	-	-	+	+	-	+
Aeromonas sp.	-	-	+	+	-	-
Gram+ve anaerobic cocci	+	+	+	+	+	+
No. of Genera	3	5	10	11	9	10
% Occurrence	27.27	45.45	90.91	100	81.82	90.91

Table 5: Haematological characteristics of apparently healthy and diseased *Clarias gariepinus*

S/No.	Haemoglobin (g/100cm³)		WBC (cell/mm³)		PCV (%)		ESR (mm/h)	
	DF.	HF.	DF.	HF.	DF.	HF.	DF.	HF.
1	8.5	10.0	58,080	48,140	25	30	9.8	6.1
2	8.3	9.4	67,120	51,880	27	31	9.3	6.0
3	8.0	10.4	64,840	46,890	26	30	9.5	7.0
4	8.6	9.4	54,810	50,730	25	29	8.0	5.1
5	8.4	9.4	49,840	43,420	30	30	8.3	6.2
6	8.8	8.8	52,630	47,840	30	39	8.8	5.0
7	8.9	9.6	51,230	48,480	27	28	9.2	7.0
8	9.0	9.0	57,130	49,843	26	30	9.0	4.8
9	8.5	9.3	54,310	48,380	28	30	8.7	4.9
10	9.1	9.4	52,160	49,840	27	30	8.0	4.1
Mean	8.6	9.5	56,215	48,543	25.1	30.7	8.9	5.6
SD	0.3	0.4	5754	2331	2.8	3.0	6.6	1.0

DF, diseased fish; HF, healthy fish, WBC, white blood cell;PCV, packed cell volume; ESR, erythrocyte sedimentation rate; SD, standard deviation.

Table 6: Haematological characteristics of apparently and diseased *Heterobranchus bidorsalis*.

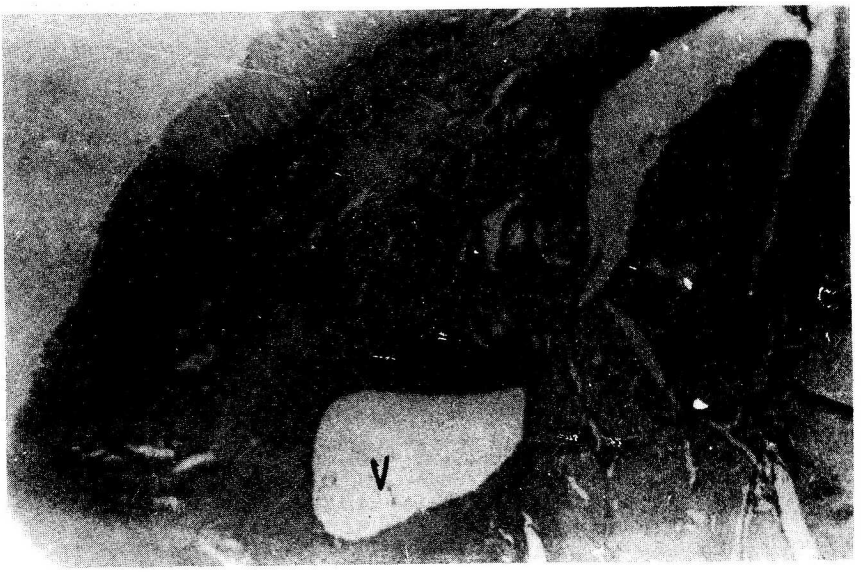
S/No.	Haemoglobin (g/100cm^3)		WBC (cell/mm^3)		PCV (%)		ESR (mm/h)	
	DF	HF	DF	HF	DF	HF	DF	HF
1	8.0	8.9	57,480	47,540	26	30	8.7	6.4
2	8.5	10.1	60,010	49,220	23	30	8.4	6.8
3	8.2	9.8	59,860	46,440	28	31	9.0	7.2
4	8.6	9.6	56,630	43,280	28	29	9.0	7.0
5	8.7	9.4	58,420	48,870	26	30	8.6	7.4
6	8.4	10.0	50,281	50,120	29	32	7.9	6.9
7	8.4	9.8	63,260	54,180	24	29	9.1	6.8
8	8.0	9.6	49,340	44,670	30	30	9.3	7.0
9	8.2	9.7	53,970	49,110	26	30	8.8	7.0
10	8.3	10.1	60,340	48,320	25	31	9.4	6.6
Mean	8.3	9.7	56,961.1	48,175	26.5	30.2	8.9	6.9
SD	0.2	0.34	4508.3	2863.1	2.90	0.87	0.4	0.87

DF, diseased fish; HF, healthy fish, WBC, white blood cell, PCV, packed cell volume; ESR, erythrocyte sedimentation rate; SD, standard deviation.

DISCUSSION

Bacterial diseases of fish have remained a source of concern to fish farmers in Nigeria. Amongst the major fish commonly reared in Nigerian culture systems are *Clarias gariepinus* and *Oreochromis nilotica* (tilapia), *Heterobranchus bidorsalis* and ornamental fish.[17] These fish species suffer chequered breeding process because of the inevitable influence of the physico-chemical conditions of the environment which culminated in various bacterial diseases. An evaluation of various bacterial diseases of fish revealed that while skin sore was occasional, most fish had one form of fin rot or the other .[17] Our previous reports [1,6] have attributed this to degenerated pond water quality. This corroborates the report of Lipton & Lakshmanan (1986) who associated fin rot to overcrowding, dirty and muddy water and other parameters engendered by poor water quality.

Evaluation of *C. gariepinus* with multiple cases of fin rot, sore head, skin and anal pore diseases revealed an assemblage of bacterial genera in the diseased parts and some organs of the fish. The bacteriological assay showed a greater colonisation of the diseased fish than the apparently healthy (control) fish by both aerobic and anaerobic bacterial genera. The high bacterial loads of diseased fish



(a)

(b)

Figure 3: Histological section through the muscle of (a) apparently *C. gariepinus* with intact vacuoles (V) and tissues and (b) diseased *C. gariepinus* showing vacuolar degeneration, cell necrosis, structural collapse and erosion of the tissues as well as clumps of necrotizing bacteria (B) in the degraded tissues.

samples may be as a result of depressed immune system and the magnitude of infection. Furthermore, the high population of microbial genera isolated justified the possibility of overwhelmed immune system. Higher counts of all bacteria occurred in the intestine than other organs except for *Pseudomonas* sp., *Flavobacterium* sp. and *Aeromonas* sp. which occurred more in only diseased parts than in healthy parts of the fish. The high recovery of *Pseudomonas, Flavobacterium* and *Aeromonas* species in the diseased parts suggested their role in the infection. This corroborates their high pathogenicity amongs farmed fish as earlier reported .[18,19] Furthermore, the evidence of stressful culture condition notwithstanding, *C. gariepinus* has been reported to be rendered moribund under natural culture conditions by species of *Pseudomonas* especially *P. flourescens* and *P. aeruginosa.*[4] The implication of *Aeromonas* species as the causative agents of fin rot and skin sore of fish by other researchers[4] and their being found amongst the normal flora of Nigerian fish culture systems [1-6] makes the possibility of their being the principal offending pathogens in this study very obvious.

The present report of bacterial invasion of diseased tissues and internal organs is noteworthy. Previous report[4] has documented a possibility of bacterial invasive attack of fish internal organs such as kidney and liver which is responsible for the higher bacterial counts of such organs in the diseased fish as compared with the apparently healthy fish (control) sample. The present result corroborates the report of Wang & Xu[20] who recorded similarly high counts in the liver, kidney and diseased parts of tilapia with skin rot. Microbial invasion from diseased parts into internal organs upon infection as reported by Cruickshank *et al.*[21] and not as a result of depressed immune system, could account for such an extensive infection. Fish treated with varying concentrations of copper sulphate as recommended by Dupree & Hurner[3] on earlier infection had their rot or sore healed. The diseased fish had higher counts of organisms of public health concern than the apparently healthy fish. The isolation of these organisms of public health concern from kidney and liver of fish indicates the extent of the bacterial invasion. The isolation of organisms of public health concern in tropical fish culture systems had been reported.[1] Studies [22-24] have revealed that these organisms to abound in domestic and poultry wastes which are normally used for pond water enrichment in Nigerian culture system. Moreover, these bacteria of public health concern which may be secondary invaders apart from the fin rot and sore causing organisms, may have their peculiar disease condition which they may be causing to the fish.

Generally, the isolation of *Flavobacterium, Staphylococcus, Pseudomonas* and *Aeromonas* species from diseased fish in addition to *Bacillus* sp., *Micrococcus* sp., *Vibrio* sp., *A. hydrophila, Escherichia coli, Klebsiella* sp., *Bacteroides* sp. and anaerobic gram positive cocci supports earlier reports [25, 26] which incriminated *Flavobacterium, Pseudomonas* and *Aeromonas* as the bacterial species associated with this disease. Investigations [27-29] have associated the genus *Aeromonas* with a

number of fish diseases. The ability of *Aeromonas* sp. to play a prominent role as a fish pathogen is apparent in view of its inevitable presence in water receiving heavy organic load with its concomitant stress on fish in culture. In addition, previous studies[1,5,6] have consistently shown *A. hydrophila* as an authocthonous inhabitant of Nigerian culture system. Apart from *Aeromonas, Flavobacterium* and *Pseudomonas,* other bacterial genera were observed to be present in undamaged skin and also constitute the normal flora of the intestine. These bacteria are saprophytic in nature[30,31] and thus their ability to contribute to fish disease is consequent upon environmental deterioration. Under such circumstances, bacteria may then invade internal organs where they could be found in large numbers. Other workers[32] have confirmed the association of bacterial skin sore disease of fish to careless handling during fish selection for either breeding or sales. This, if unavoidable, can be reduced by adequate treatment of fish with antibiotics such as amikacin or kanamycin as recommended in our recent report[33] or other antimicrobial agents.[3,34]

The diseased fish samples had haemoglobin, WBC, PCV and ESR records that were statistically different from the healthy samples. High haemoglobin counts and packed cell volumes were associated with apparently healthy fish while low counts were obtained in the diseased fish samples. This corroborates the report of Larsen & Snieszko[35] who associated high haemoglobin counts with healthy rainbow trout. The association of high haemoglobin counts with healthy fish is not surprising as the method estimates the sum of oxygen carrying blood cells in fish. However, both the diseased and the apparently healthy fish had haemoglobin counts that fell within the range recorded by Blaxhall & Daisley[36] for healthy fish. Furthermore, since there is no recorded or existing standard for *C. gariepinus* and *H. bidorsalis* haemoglobin level in the tropics, one may assume the low haemoglobin obtained from diseased fish to be as a result of the infection. On the other hand, the consistently high PCV recorded in the apparently healthy *C. gariepinus* and *H. bidorsalis* samples against low counts obtained in the diseased fish samples corroborates the report of Cushing[37], thus establishing the fact that fish respond haematologicaly to pathological conditions as reported by Klantz.[38] Moreover, the mean PCV of 30.7 and 30.2/100cm^3 obtained respectively from healthy *C. gariepinus* and *H. bidorsalis* are comparable to that (32.0/100cm^3) reported as the mean PCV for healthy brown trouts.[36]

The association of high ESR and WBC with the diseased than the apparently healthy fish samples corroborates the report of previous workers.[36,39] White blood cells in living organisms are known to increase in response to bacterial infections.[15] Thus the association of high WBC in diseased fish could be as a result of the fish response to bacterial infections. Furthermore, since ESR in the fish is reported to increase with increased cell distribution in rainbow trout[36], it is possible that the high ESR obtained for diseased fish in this study was triggered by the infection which had overwhelmed the fish immune system. Generally, statistical

evaluation of the haemoglobin, ESR, PCV and WBC of the diseased and the apparently health *C. gariepinus* and *H. bidorsalis* revealed a significant difference which confirmed the influence of bacterial diseases of fish on the haematology.

The histological studies revealed necrosis resulting from the invasive activities of the offending bacteria leading to structural collapse and erosion of the tissues of diseased *C. gariepinus*. This peculiar histological result which was similar to those of other diseased tissues of fish and other animals, especially mammals[14] was observed only in the tissue of diseased and not in the apparently healthy fish, thus demonstrating the histological response of the fish to the bacterial pathogens and underscoring the pathological process that had taken place in diseased fish tissue.

In conclusion this study demonstrates the invasive nature of the bacterial pathogens of fish not only in the affected area but also in the different internal organs, thereby affecting the haematology and histology of the diseased fish. The haematological and histological evidence which are important diagnostice features are recommended in the diagnosis of fish diseases. Generally, the significant differences in the haematology and histology of the diseased and apparently healthy fish samples are indications of the impact of the offending pathogens on fish tissues and organs. Such clear diagnostic evidence should be landmarks in establishing the role of bacteria in emergent tropical fish disease syndromes.

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