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Experimental *Pseudomonas aeruginosa* Infection in Adult and Juvenile African Catfish (*Clarias gariepinus*): Pathogenicity and Pathology

***Monsuru Oladunjoye Tijani, Gbolahanmi Akinola Oladosu,**

Department of Veterinary Pathology, University of Ibadan. Nigeria. *Corresponding aurthors email; adeolatok@gmail.com Phone: 08029972709

SUMMARY

The pathology and pathogenesis of *Pseudomonas aeruginosa*, a Gram-negative bacterium often isolated from diseased *Clarias gariepinus*, remain unclear. This study seeks to elucidate its pathology and pathogenesis in this species through experimental infection. An overnight broth culture of *Pseudomonas aeruginosa* ($1.7x10^9$ CFU/ml) originally isolated from a *Clarias gariepinus* carcass during a previous outbreak was used. The culture was administered via intraperitoneal injection to 50 adult and 50 juveniles *Clarias gariepinus*, using doses of 1 ml and 0.2 ml, respectively, while a control group of 10 adults and 10 juveniles each received equivalent saline doses. Mortality was 70% in adults within 6 days and 16% in juveniles within 6 hours, with no subsequent deaths. Gross lesions, including enlarged pale yellow to brown enlarged livers, ascites and widespread haemorrhages were frequently observed in adults but absent in the juveniles. Histopathology revealed degenerative and inflammatory lesions mainly in the liver, gills and arborescent organs of infected adults. The haematology showed that infected adults developed anaemia (PCV 31.42±7.62% vs. control 39.60±5.62%), leukocytosis (WBC 18085.42±1810.75/µL vs. control 17060.00±936.84/µL), thrombocytosis (PLT 157083.33±32521.45/µL vs. control 103100.00±19941.30/µL) and hypoproteinaemia (TP 6.08±0.97g/dl vs. control 7.08±0.74g/dl) 48 hours post-infection. At day 35, there was also anaemia and thrombocytosis, however, there was a leukopaenia (WBC 15,795.33±1,050.09/µl vs. Control 17,060.00±936.84 /µl) and a hypoglobulinaemia (GLB 4.58±0.31g/dl vs. control 4.90±0.22). We conclude that the *Pseudomonas aeruginosa* isolate is highly pathogenic to *Clarias gariepinus*, causing acute fatal septicaemia with severe organ pathology. Adults were more susceptible, and survivors had suppressed immunity.

Key words: African catfish, Experimental infection, Pathology, *Pseudomonas aeruginosa*

Running title: Experimental *P. aeruginosa* septicaemia in *Clarias gariepinus*

INTRODUCTION

Pseudomonas aeruginosa is highly adaptable and thrives in diverse habitats such as land, water, flora, fauna, and commercial aquaculture systems (Chevalier *et al.,* 2017). Various researchers have associated these bacteria with septicaemia, skin and fin rot in various species of fish including the Nile tilapia, striped mullet, common carp, silver carp and the banded grunt often causing significant morbidity, mortality and heavy financial losses (El-Hady and Samy 2011; El-Barbary and Hal, 2016; Qorany and Mansour,2023;). *P. aeruginosa* is a resource-efficient pathogen that requires minimal nutrition and is resilient on a wide range of surfaces including hospital furniture and equipment. *P.aeruginosa* has also been reported to remain viable in dry conditions in healthcare facilities for up to 180 days (Diggle and Whiteley, 2020). Although there are published reports of *Pseudomonas* septicaemia outbreaks in several species of fish**,** the pathology of this bacterial disease has not been thoroughly elucidated in the African catfish (*Clarias gariepinus*). This study therefore aims to throw more light on the pathogenesis, pathology, haematology and serum protein changes associated with experimental infection in adult and juvenile African catfish.

MATERIALS AND METHODS

The study animals

A total of 120 *Clarias gariepinus* were allocated to the study: fifty adults (average weight 270g) and fifty juveniles (average weight 35g) constituted the treatment group, while a control group of ten adults and ten juveniles was also established. The fish were acclimatized for two weeks in the Department of Veterinary Medicine, University of Ibadan aquarium facility in 50L plastic tanks provisioned with a continuous daily supply of fresh borehole water. During acclimatization, they were fed a commercial catfish diet twice daily. Ethical approval for this study was sought and approved by University of Ibadan animal care and use research ethics committee (UI-ACUREC). The assigned approval number is UI-ACUREC/18/0014.

Preparation of Pseudomonas aeruginosa broth culture

The pour plate method was used to determine the concentration of the inoculum for experimental infection. A pure isolate of *Pseudomonas aeruginosa* was cultured from the dorsal kidney of a dead African catfish sampled during a disease outbreak in a fish farm located in Ibadan. The isolate was inoculated into tryptone soy broth and incubated overnight at 25°C. 0.1 ml of the 10^{-4} dilution of the overnight broth culture was streaked on tryptone soy agar plate and incubated at 25°C for 24 hours. The bacteria colonies were counted and the colony forming units per milliliter (CFU/ml) were calculated using the formula: CFU/ml = (Number of colonies x dilution factor) ÷ Volume of culture plated (ml).

Experimental infection via intraperitoneal route

1ml of 1.7x 10^9 CFU/ml and 0.2ml of 1.7x 10^9 CFU/ml *Pseudomonas aeruginosa* overnight broth culture was injected into the peritoneum of 50 adult and 50 juvenile

Clarias gariepinus respectively. 1ml and 0.2ml of sterile saline were injected into the peritoneum of 10 adult and 10 juvenile *Clarias gariepinus* respectively (control group).

Haematology and serum protein chemistry

At 48 hours and 35 days post-infection, 5 mls of blood was drawn from 24 and 15 adult fish respectively in the *Pseudomonas aeruginosa* treatment group and from 10 fish (48 hours) in the control group by venipuncture of the caudal vein and dispensed into EDTA sample bottles for haematology and serum protein chemistry (total protein, albumin and globulin). Giemsa-stained blood smears were examined under a light microscope at x100 (oil immersion) for the differential count. RBC count was done using a Neubauer haemocytometer (Jain, 1986). Packed cell volume (PCV) and haemoglobin (Hb) concentration were determined using the microhaematocrit method (Stephen *et al.,* 2017) and cyanmethaemoglobin method (Bhaskaram *et al.,* 2003) respectively. Total protein and albumin were quantified using Randox® total protein and albumin kits according to the manufacturer's instructions. Globulin values were calculated from the difference between total protein and albumin values.

Gross pathology and histopathology

Each freshly dead fish was thoroughly examined systematically at postmortem and the observed gross lesions were documented. Fish that were still alive at day 35 were euthanized by cervical transection and pithing prior to necropsy. Samples of the eye, skin, muscle, brain, heart, gills, arborescent organ, stomach, intestines, liver, spleen and gonads were trimmed using a clean scalpel blade and scissors, fixed in 10% neutral buffered formalin for 24 hours, routinely processed for histology and stained with haematoxylin and eosin. Subsequently, the prepared slides were examined for lesions with an Olympus® light microscope.

Bacteria isolation and identification

Aseptically collected swabs from the dorsal kidneys of three freshly dead adult and juvenile African catfish (*Pseudomonas aeruginosa* treatment group) were streaked on tryptone soy agar and kept in an incubator at 25°C for 24 hours. Pure bacteria cultures obtained were then spread on MacConkey agar, Gram stained and identified biochemically to species level using Microbact-24E Oxoid® bacterial identification system and conventional methods, while adhering to the manufacturer's protocol.

Statistical analysis

Data collected was analyzed by descriptive statistics, and independent samples t-test using the IBM SPSS V26 software.

RESULTS

Clinical signs and mortality rate

Anorexia, sluggish swimming and loss of balance were observed in adults, three days post infection. Morbidity rates of over 70% were recorded. By day six, 70% of the adult *Clarias gariepinus* (n=35) were dead.

On the other hand, the juveniles exhibited a lower morbidity rate of over 20%. Furthermore, similar clinical signs observed

in the adults were recorded much later at day five following intraperitoneal injection with *Pseudomonas aeruginosa*. Eight juveniles (16%) died in the first six hours following experimental infection and mortality ceased afterwards. The results of clinical signs and mortality rate in adult and juvenile *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* is summarized in table 1 and Fig 1.

Table 1: Clinical signs/mortality pattern of *Clarias gariepinus* infected with *Pseudomonas aeruginosa*

	ADULT	**JUVENILE**
MORBIDITY	Over 70%	Over 20%
ANOREXIA	3 days pi	5 days pi
SLUGGISH SWIMMING	++++	++++
LOSS OF BALANCE	++++	++++
EVENTUAL DEATH	35 (70%) dead by day 6 days	8 (16%) dead by day 1 T+6h

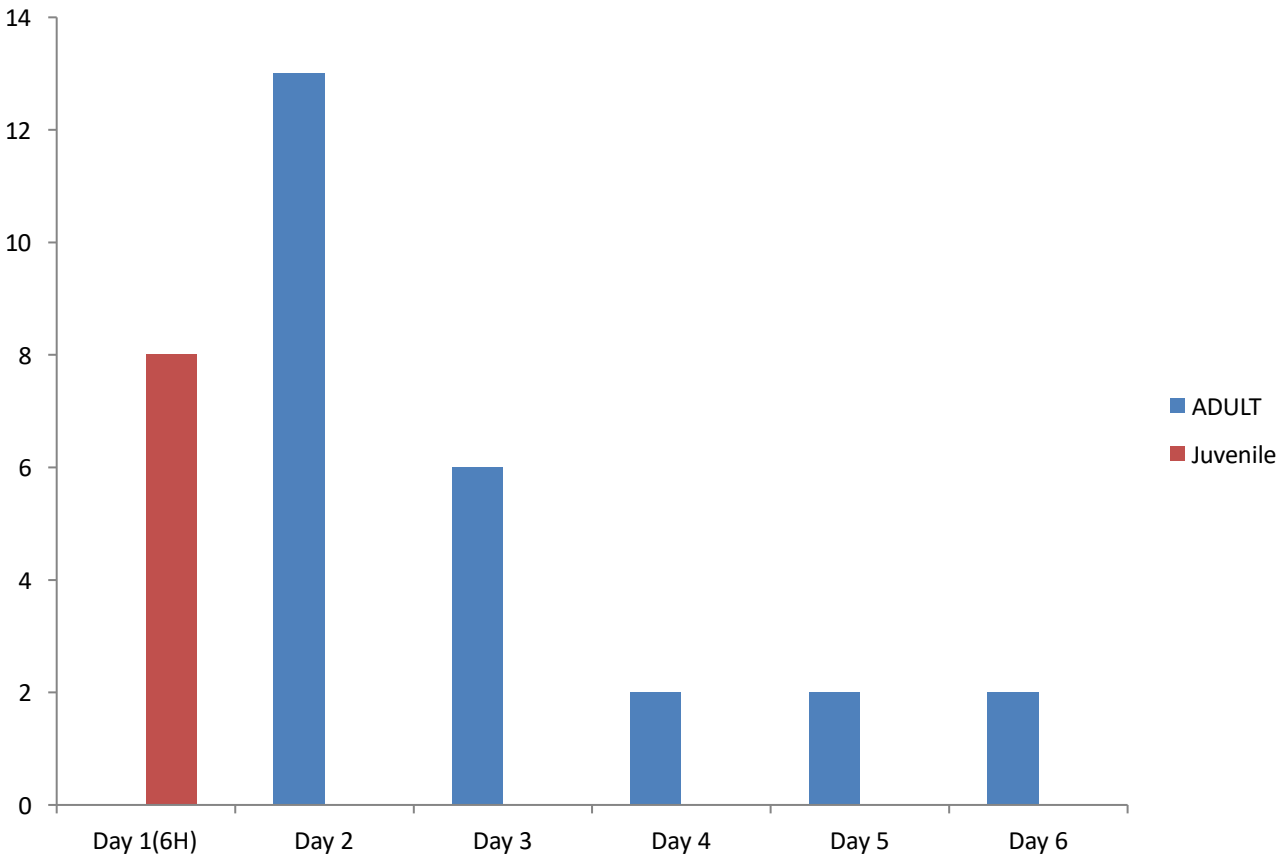

Fig 1. Mortality pattern of *Clarias gariepinus* infected with *Pseudomonas aeruginosa*

Gross pathology of juvenile and adult Clarias gariepinus experimentally infected with Pseudomonas aeruginosa

Gross pathology was most frequent in experimentally infected adult *Clarias gariepinus*. Conversely, gross pathology was not evident in experimentally infected juveniles. The organ that was most frequently impacted was the liver. Hepatomegaly (enlargement of the liver) and pale yellow to brown discolouration (Fig. 2H) was documented in 28 fish (56%). Widespread haemorrhages were seen in the skin, around the urogenital openings and gonads (26%) (Figs. 2C, 2D, 2F and 2G) and within abdominal musculature (24%). 14 fish (28%) showed ascites/peritonitis. 28% (14 fish) were anaemic and exhibited severe pallor of the gills and depigmentation of the skin (Figs. 2A and 2H). Other gross lesions of less frequency include liver necrosis (4%), hyperaemic and haemorrhagic gills and arborescent organs (4%) (Fig. 2E), skin ulcer (Fig. 2B), muscle and fin necrosis (8%), fin and skin haemorrhages (16%) (Fig 2C). The results of the gross pathology of adult and juvenile *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* are summarized in table 2.

Table 2. Gross pathology of juvenile and adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa*

S/NO	GROSS LESIONS	ADULT (*P.aeruginosa*) (N=50)	JUVENILE (*P.Aeruginosa*) (N=50)
1	Pale carcass, gills/Skin depigmentation	14 (28%)	0
2	Fin/skin haemorrhages	8 (16%)	0
3	Skin ulcer, muscle and fin necrosis	4 (8%)	0
4	Haemorrhagic/hyperaemic gills and arborescent organs	2 (4%)	0
5	Pale yellow to brown enlarged liver	28 (56%)	0
6	Liver necrosis	2(4%)	0
7	Ascites/ Peritonitis	14(28%)	0
9	Haemorrhagic abdominal muscle	12 (24%)	0
10	Haemorrhagic gonads and urogenital openings	13 (26%)	0
11	Gastritis	1 (2%)	0

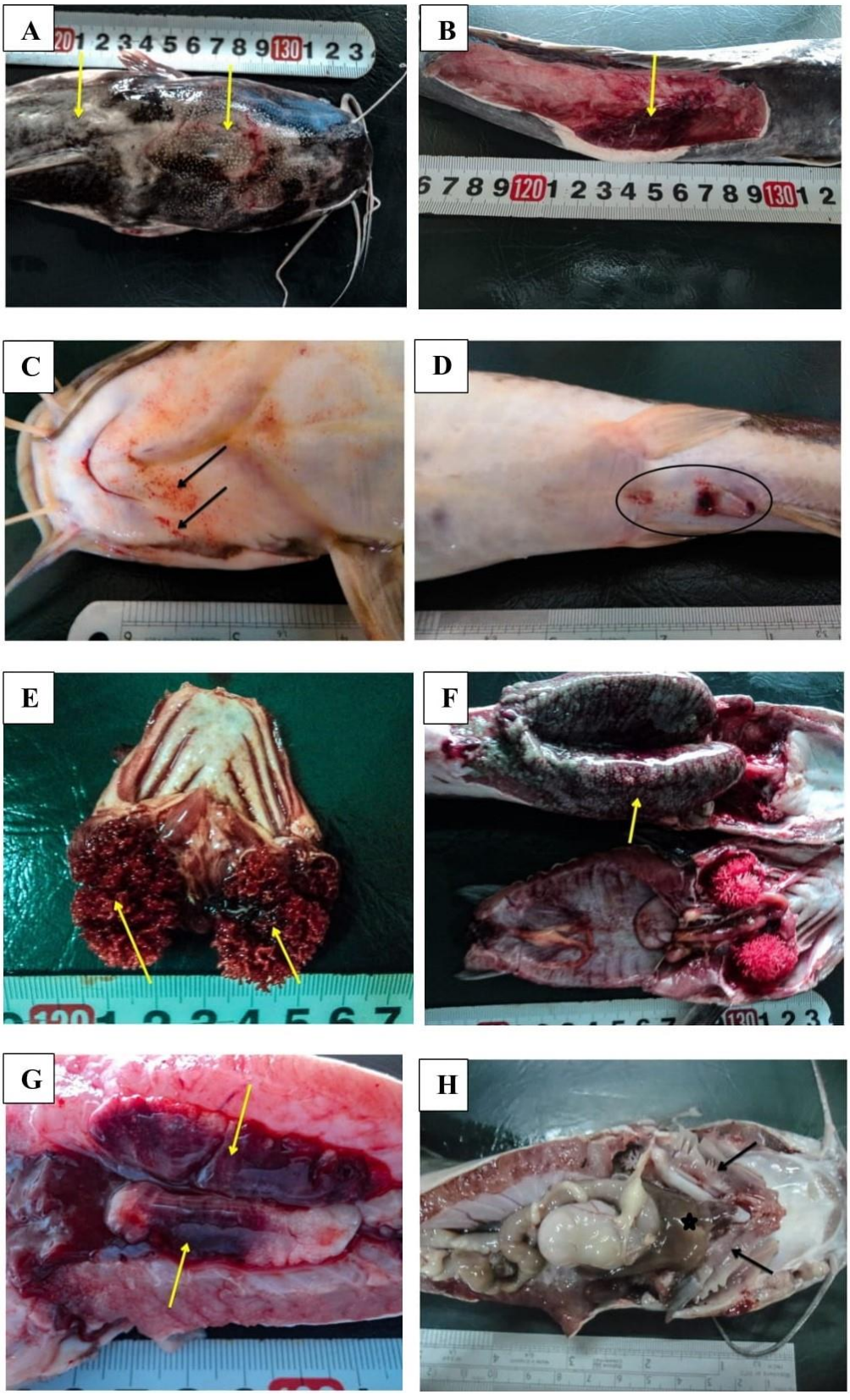
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Fig 2. Gross lesions observed in Adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa*

A. Adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* showing depigmentation of the skin (arrows)

B. Adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* showing severe ulceration of the skin and severe haemorrhage in the exposed skeletal muscle (arrow)

C. Adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* showing multifocal petechial haemorrhages on the ventral aspect of the head and operculum (arrows)

D. Adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* showing severe haemorrhage around the urogenital opening (encircled)

E. Adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* showing severely haemorrhagic arborescent organs (arrows)

F. Adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* showing moderately haemorrhagic ovaries (arrow)

G. Adult *Clarias gariepinus* experimentally infected with Pseudomonas aeruginosa showing severely haemorrhagic testes (arrows)

H. Adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* showing severely pale gills (arrows) and pale brown discoloured liver (star).

Histopathology of juvenile and adult Clarias gariepinus experimentally infected with Pseudomonas aeruginosa

In the experimentally infected adults, the liver most frequently showed histological lesions with a total of 17 fish (34%) exhibiting severe diffuse hepatocellular vacuolar change (Fig. 3A) with occasional focal necrosis. 54% (27 fish) of adult fish showed histological lesions in the gills. The histological lesions ranged from moderate (18%, 9 fish) to severe hyperplasia and fusion of secondary lamellae (28%, 14 fish) (Fig 3B) and severe lamellar haemorrhage (8%, 4 fish). Strikingly, a total of 27 adult fish (54%) showed arborescent organ histopathology. The lesions observed included moderate epithelial vacuolar degeneration and hyperplasia (18%, 9 fish) ` to severe epithelial vacuolar degeneration and hyperplasia and inflammation (36%,18 fish) (Fig. 3C). A total of 16 adult fish (32%) showed lesions in the skin and skeletal muscles. The histopathology in these tissues was mixed. The observed lesions include mild mucous cell hyperplasia and erosion, myofibre necrosis (Fig 3D), epidermal and skeletal muscle haemorrhage (Fig. 3H). Organs that presented severe but less frequent histological lesions include the kidney which exhibited severe acute nephritis and haemorrhage (10%, 5 fish) and the intestine which showed severe fibrinous serositis and haemorrhage (4%, 2 fish). Lesions were also observed in the heart of a few infected adults. The cardiac lesions included a variety of

inflammatory conditions ranging from lymphocytic pericarditis/ myocarditis to heterophilic epicarditis (8%, 4 fish) and also haemorrhages in the pericardium, epicardium (Fig. 3F) and myocardium (10%, 5 fish). Other notable lesions that occurred less frequently include haemorrhage in the eye chambers and retina (6%, 3 fish) (Fig 3G) and testicular degeneration, haemorrhage and congestion (6%, 3 fish) (Fig 3 E). The histopathology of adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* is summarized in tables 3A, 3B and 3C

In the experimentally infected juveniles, the gills were most frequently impacted histologically with 50% (25 fish) showing gill lesions. The most consistent gill lesion was moderate hyperplasia and fusion of the secondary lamellae (44%, 22 fish). Additionally, 4% (2 fish) and 2% (1 fish) showed severe hyperplasia and fusion of the secondary lamellae and lamellar haemorrhage respectively. Only 4 % (2 fish) of experimentally infected juveniles exhibited liver histopathology. The liver pathology consisted of severe diffuse hepatocellular vacuolar change and parenchymal haemorrhage. The histopathology of juvenile *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* is summarized in table 4.

Table 3A: Histopathology and lesion score for *Clarias gariepinus* adults experimentally infected with *Pseudomonas aeruginosa*

ORGAN/TISSUE	HISTOPATHOLOGY	SCORE	FREQ (%)
ARBORESCENT ORGAN	No visible lesion	0	23 (46%)
	Epithelial vacuolar degeneration and hyperplasia	++	9 (18%)
	Epithelial vacuolar degeneration and hyperplasia and inflammation	+++	18 (36%)
EYE	Haemorrhage in the chambers and retina	++	3 (6%)
	No visible lesion	0	47 (94%)
GILLS	Hyperplasia and fusion of secondary lamellae	++	9 (18%)
	Hyperplasia and fusion of secondary lamellae	+++	14 (28%)
	Lamellar haemorrhage	+++	4 (8%)
	No visible lesion	0	23 (46%)

GONAD	Ovarian haemorrhage	+	1 (2%)
	Testicular degeneration, haemorrhage and congestion	+++	3 (6%)
	No visible lesion	0	46 (92%)

Key: + mild; ++ moderate; +++ severe

Table 3B: Histopathology and lesion score for *Clarias gariepinus* adults experimentally infected with *Pseudomonas aeruginosa*

ORGAN/TISSUE	HISTOPATHOLOGY	SCORE	FREQ (%)
HEART	Epicardial haemorrhage	+	1 (2%)
	Pericardial and epicardial haemorrhage	++	2 (4%)
	Endocardial/epicardial/myocardial haemorrhage	+++	2(4%)
	Lymphocytic pericarditis	+	2 (4%)
	Lymphocytic myocarditis	+++	1 (2%)
	Heterophilic epicarditis	++	1 (2%)
	No visible lesion	0	41 (82%)
INTESTINE	Fibrinous serositis and haemorrhage	+++	2 (4%)
	Acute enteritis	+++	1 (2%)
	No visible lesion	0	47 (94%)
KIDNEY	Renal tubular vacuolar change and haemorrhage	+++	2 (4%)
	Necrosis of renal tubular epithelium and depletion of haematopoietic cells	+++	5 (10%)
	No visible lesion	0	43 (86%)
LIVER	Random hepatocellular vacuolar change, capsular haemorrhage and necrosis"	+	2 (4%)
	Multifocal hepatocellular vacuolar change and necrosis	++	3 (6%)
	Diffuse hepatocellular vacuolar change with necrosis	+++	17 (34%)
	Acute hepatitis with bacteria colonies	+++	1 (2%)
	No visible lesion	0	27 (54%)

Key: + mild; ++ moderate; +++ severe

Table 3C: Histopathology and lesion score for *Clarias gariepinus* adults experimentally infected with *Pseudomonas aeruginosa*

ORGAN/TISSUE	HISTOPATHOLOGY	SCORE	FREQ (%)
SKIN/SKELETAL MUSCLE	Focal epidermal haemorrhage, mucuous cell hyperplasia and erosion	+	5 (10%)
	Focal epidermal haemorrhage	++	2 (4%)
	Epidermal and muscle haemorrhage	+++	3 (6%)
	Myofibre necrosis	++	3 (6%)
	Myofibre necrosis	+++	3 (6%)
	Skin depigmentation	+++	15 (30%)
	No visible lesion	0	34 (68%)
SPLEEN	Acute splenitis with erythrophagocytosis	+++	1 (2%)
	Lymphoid depletion	++	2 (4%)
	Capsular haemorrhage with acute splenitis and mild haemosiderosis	+++	3 (6%)
	No visible lesion	0	44 (88%)
STOMACH	Acute gastritis	+++	1 (2%)
	Congestion and serosal haemorrhage	+++	3 (6%)
	No visible lesion	0	46 (92%)

Key: + mild; ++ moderate; +++ severe

Table 4: Histopathology and lesion score for Juvenile *Clarias gariepinus* infected with *Pseudomonas aeruginosa*

ORGAN/TISSUE	HISTOPATHOLOGY	LESION SCORE	FREQUENCY (%)
Liver	Diffuse hepatocellular vacuolar change	+++	1 (2%)
	Parenchymal haemorrhage	+++	1 (2%)
	No visible lesion	0	48 (96%)
Gills	Hyperplasia and fusion of secondary lamellae	++	22 (44%)
	Hyperplasia and fusion of secondary lamellae	+++	2 (4%)
	Lamellar haemorrhage	+++	1 (2%)
	No visible lesion	0	25 (50%)

Key: + mild; ++ moderate; +++ severe

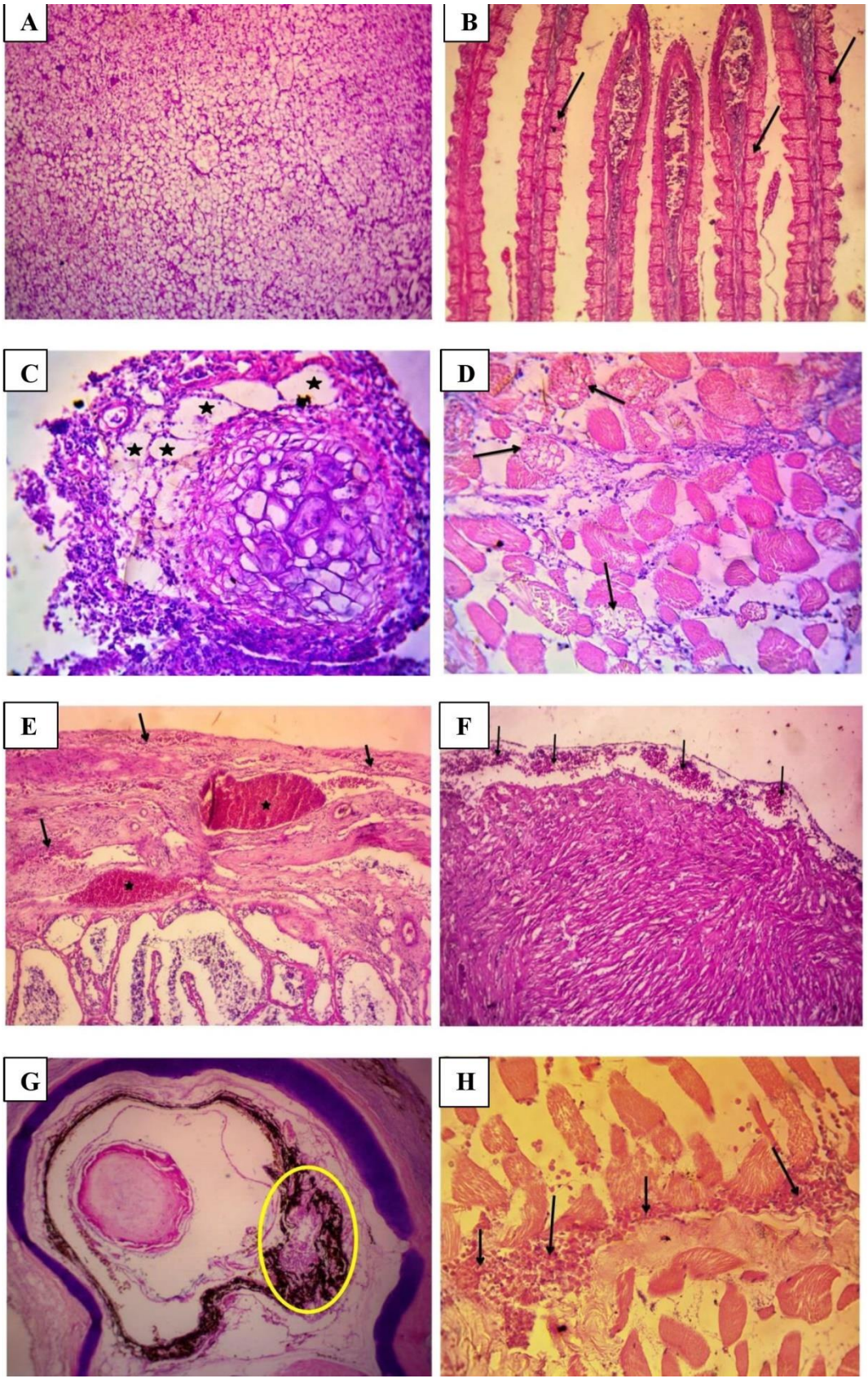
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Fig 3. Histological lesions observed in Adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa*

A. Liver of adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* showing severe diffuse hepatocellular vacuolar change. H&E, x150

B. Gill of of adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* showing severe hyperplasia and fusion of secondary lamellae (arrows) H&E, x60

C. Arborescent organ of adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* showing multiple large vacuoles in the epithelium (stars). There is also moderate infiltration of the hyperplastic epithelium by mononuclear cells. H&E, x400

D. Skeletal muscle of adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* showing vacuolation and necrosis of myofibres (arrows) and moderate numbers of macrophages and a few lymphocytes in the endomysium. H&E, x150

E. Testis of adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* showing severe haemorrhage within the tunica albuginea connective tissue (arrows). There is also congestion of the blood vessels (stars) H&E, x400

F. Heart of adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* showing severe subepicardial haemorrhage (arrows) H&E, x150

G. Eye of adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* showing disruption of the retinal pigment epithelium by severe haemorrhage (encircled) H&E, x60

H. Skeletal muscle of adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* showing severe haemorrhage in the endomysium (arrows).H&E, x600

Haematology of adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa*

48 hours following the experimental infection of adult *Clarias gariepinus* with *Pseudomonas aeruginosa,* there was a severe anaemia characterized by a significant decline in the packed cell volume (PCV 31.42±7.62% vs. control 39.60±5.62%) and Haemoglobin concentration (10.57 ± 2.54 g/dl vs. Control: 13.10 ± 1.79 g/dl). A downward trend, though not statistically significant, was also observed in the RBC count. There was also a leukocytosis (WBC 18085.42±1810.75 /µL vs. control 17060.00±936.84/µL) and thrombocytosis (PLT 157083.33±32521.45/µL vs. control 103100.00±19941.30/µL). Similarly, the haematology of surviving fish at day 35 post-infection revealed anaemia (PCV 33.40±5.78% vs. control 39.60±5.62 %,), (HB10.89± 1.95 g/dl vs.control13.10±1.79g/dl), leukopaenia (WBC 15,795.33±1,050.09/µl vs. Control 17,060.00±936.84 /µl) and thrombocytosis (PLT 133,133.33±33,915.58 vs. control 103,100.00±19,941.30).

The haematology of adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* are summarized in table 5.

Table 5. Haematological changes in adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* (Independent samples t-test)

		T+48h		Day 35	
Parameter	**48h Control (10)**	***P.aeruginosa*(n=24)**	**p-value**	***P. aeruginosa* (n=15)**	**p- value**
Packed Cell Volume (%)	39.60±5.62	**31.42±7.62**	**0.005**	**33.40±5.78**	**0.014**
RBC ($\times 10^6$/µl)	3.62±0.46	3.18±0.73	0.090	3.37±0.60	0.27
Haemoglobin (g/dl)	13.10±1.79	**10.57±2.54**	**0.007**	**10.89±1.95**	**0.008**
WBC (/µl)	17,060.00±936.84	**18,085.42±1,810.75**	**0.038**	**15,795.33±1,050.09**	**0.005**
Platelet (/µl)	103,100.00±19,941.30	**157,083.33±32,521.45**	**0**	**133,133.33±33,915.58**	**0.019**
Lymphocyte (/µl)	10,343.25±1,016.76	10,746.77±2,430.51	0.618	9,783.59±1,076.06	0.206
Heterophil (/µl)	5,467.55±1,066.78	6,004.21±1,816.97	0.391	4,836.55±923.95	0.129
Monocyte (/µl)	500.00±195.55	623.19±228.88	0.147	415.49±185.06	0.285
Eosinophil (/µl)	713.40±168.07	665.00±282.85	0.619	696.50±376.26	0.896
Basophil (/µl)	35.80±75.57	46.25±81.90	0.731	63.20±97.02	0.46

Serum protein chemistry of adult Clarias gariepinus experimentally infected with Pseudomonas aeruginosa

48 hours following the experimental infection of adult *Clarias gariepinus* with *Pseudomonas aeruginosa*, there was a notable hypoproteinaemia (TP 6.08±0.97g/dl vs. control 7.08±0.74g/dl) due to hypoalbuminaemia (ALB 1.66±0.45 vs. control 2.18±0.57g/dl) and hypoglobulinaemia (GLB 4.42±0.71 vs. control 4.90±0.22g/dl). However at day 35 post-infection, surviving fish showed hypoproteinaemia (TP 6.46±0.59g/dl vs. control 7.08±0.74g/dl) attributable to hypoglobulinaemia (GLB 4.58±0.31g/dl vs. control 4.90±0.22) and a low Albumin/Globulin ratio (A/G 0.34±0.08 vs.

control 0.42±0.10). The serum protein chemistry of adult *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa* are summarized in table 6

Table 6: ***Serum protein chemistry in adult Clarias gariepinus infected with Pseudomonas aeruginosa (Independent samples t-test)***

	48h			**Day 35**	
Parameter	**48h Control(n=10)**	***P.aeruginosa*(n=24)**	**p-value**	***P. aeruginosa* (n=15)**	**p-value**
Total Protein (g/dl)	7.08±0.74	6.08±0.97	**0.007**	6.46±0.59	**0.029**
Albumin (g/dl)	2.18±0.57	1.66±0.45	**0.008**	1.88±0.35	0.115
Globulin (g/dl)	4.90±0.22	4.42±0.71	**0.047**	4.58±0.31	**0.011**
Alb/Glob Ratio	0.42±0.10	0.45±0.55	0.848	0.34±0.08	**0.043**

Bacteria re-isolation and identification from fish carcasses

Bacteria isolate that released a bluish green pigment into the TSA medium and emitted a sweet , grape-like odor were recovered from the dorsal kidneys of the freshly dead fish. The isolates were identified to be *Pseudomonas aeruginosa* by biochemical tests. Table 7 shows the biochemical characteristics of the bacteria.

Table 7. Biochemical characteristics of *Pseudomonas aeruginosa* isolated from carcasses of experimentally infected *Clarias gariepinus* adults and juveniles

FEATURES	***Pseudomonas aeruginosa***
Gram reaction	-
Motility	+
Oxidase	+
Nitrate reduction	+
Lysine	-
Ornithine	-
H_2S production	-
Glucose	-
Mannitol	-
Xylose	-
ONPG	-
Indole	-
Urease	-
Citrate	+
Gelatin	+
Sorbitol	-
Rhamnose	-
Sucrose	-
Lactose	-
Arabinose	-
Arginine	+

DISCUSSION

In this study, the 48-hour period between infection and onset of clinical signs and mortality in experimentally infected *C. gariepinus* adults is contrary to the findings of Amrevuawho *et al.,* (2014) in a similar experimental infection of *Clarias gariepinus* sub-adults in which they started recording clinical signs at 21 days post infection. This observation could be due to the administration of a smaller inoculation dose of *P.aeruginosa* (10^6 cells/ml) by those researchers via a different route (oral) in contrast to a much larger inoculation dose (1.7x 10^9 CFU/ml) administered via the intraperitoneal route in the present study. The markedly lower mortality and pathology observed in juvenile *C. gariepinus,* in this study, compared to adults contrasts with some reports for other bacterial pathogens in the same age group of *Clarias gariepinus* (Mohamad *et al.,* 2022). However, it is well established that the innate immune system which is the first line of defence in fish is robust and can be exceptionally effective in early life stages. (Magnadóttir, 2006; Zapata *et al.*, 2006). The rapid containment of the infection in juveniles shown by the cessation of mortality after 6 hours and the absence of gross lesions, suggests a potent and effective innate immune response may have been mounted. In contrast, the severe systemic pathology observed in infected adults could indicate a dysregulated and overwhelming immune response, leading to fatal septicaemia.

Anaemia, leukocytosis and thrombocytosis observed in adult *Clarias gariepinus* 48 hours post-infection align with the haematological changes reported by Amrevuawho *et al.,* (2014) in *Pseudomonas aeruginosa* infected *Clarias gariepinus* sub-adults at three weeks post-infection. The anaemia observed in this study, could be due to the release of inflammatory cytokines such as Interleukin-6 (IL-6) that disrupt iron metabolism, suppress the production of erythropoietin and ultimately inhibit erythropoiesis (Weiss and Goodnough,2005). The thrombocytosis observed in the current study is not in agreement with findings by El-Wafai *et al.* (2024) who reported thrombocytopaenia in Nile tilapia, 12 days post-infection with *Pseudomonas aeruginosa*. The thrombocytosis observed in infected adults could be attributed to the action of the acute phase cytokine Interleukin-6 (IL-6) in stimulating the liver to increase the production of thrombopoietin (TPO), which in turn accelerates platelet production (Kaser *et al*., 2001). Anaemia and thrombocytosis, first seen at 48 hours post-infection, persisted in surviving fish at day 35. This anaemia suggests ongoing haemolysis, suppressed erythropoiesis, or a combination of both, indicating the fish hadn't fully recovered. The leukopaenia observed at day 35 could indicate suppression of haemopoiesis in the head kidney (pronephros) and the spleen due to chronic and overwhelming *P.aeruginosa* infection. The changes observed in the serum protein values (hypoproteinaemia with hypoalbuminaemia and hypoglobulinaemia) 48 hours post-infection are in agreement with findings by Magdy *et al*. (2014) in a similar study. These serum protein findings suggest that inflammatory cytokines (IL-1, IL-6 and TNF α) inhibited the liver's ability to

synthesize albumin and overwhelmed the immune system's capacity to synthesize antibodies (Gruys *et al*., 2005). The hypoproteinaemia and hypoglobulinaemia observed at day 35 post-infection suggests a failure of the surviving fish to mount a sustained antibody response against *P.aeruginosa*, probably due to immunosuppression or the overwhelming nature of the infection. Magdy *et al*., (2014) also reported immunosuppression in *Clarias gariepinus* experimentally infected with *Pseudomonas aeruginosa*.

The gross and histological lesions in the current study are in tandem with observations made by Amrevuawho *et al*., (2014) and Magdy *et al*., (2014), in similar experimental infection studies. These authors documented widespread haemorrhages, degenerative and necrotic lesions in the skin, liver, kidneys, spleen and testis. The liver, gills and arborescent organs, were the most severely affected by the *Pseudomonas aeruginosa* infection in adult *Clarias gariepinus.* Degeneration, necrosis and inflammation in these target organs could be linked to the direct cytotoxic effects of virulence factors produced by *Pseudomonas aeruginosa*. *P. aeruginosa* produces virulence factors such as proteases, pyocyanin, and exotoxin A, which are known to be cytotoxic and disrupt cellular metabolism, leading to cellular degeneration and necrosis (Pavlovskis *et al*., 1978; Cheluvappa, 2007; Saleh, 2013; Liao *et al*., 2022). The widespread haemorrhages observed in infected adults indicate that liver damage from *Pseudomonas aeruginosa* virulence factors compromised the production of essential clotting factors. It is important to note that testicular degeneration was observed in 3 (6%) experimentally infected adult African catfish. This indicates a potential detrimental effect of *P. aeruginosa* on the fertility of adult male African catfish.

CONCLUSION

From this study, we can conclude that the isolated *Pseudomonas aeruginosa* are highly pathogenic to Adult and Juvenile African catfish (*Clarias gariepinus*) causing very high and rapid mortalities within the acute phase of infection. In the current study, juvenile *Clarias gariepinus* had far less mortality and pathology compared with infected adults, suggesting some degree of resistance to the bacterium. This sharp contrast in the course of the disease and pathological features between infected adults and juveniles will inform targeted disease management strategies. Furthermore, haematological and serum chemistry changes indicate that adult *Clarias gariepinus* surviving the acute infection may become immunosuppressed. The detailed pathological and haematological data presented in this study will serve as an important reference for diagnosis and future research endeavors. The profound pathology induced by this bacterium on the respiratory organs (gills and arborescent organ) and liver in both adult and juvenile *C. gariepinus* led to rapid and massive mortality in affected fish stocks and consequently massive financial losses. This bacterium can be considered as a pathogen of economic importance in commercial African catfish culture in Nigeria.

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