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Potential of *Pasteurella multocida* (serotype A1) Isolated from Diseased Chicken to Establish Infection in African Catfish (*Clarias gariepinus*)

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Abstract

In integrated poultry cum fish systems, the droppings and offals from the chicken are used to fertilize the pond and feed the fish. These droppings and offals may sometimes harbour pathogenic microorganisms such as *Pasteurella multocida*. This study aimed to ascertain if *P. multocida* of *avian* origin can establish and cause infection in *Clarias gariepinus.* One hundred and twenty, apparently healthy 6 weeks old *C. gariepinus* juveniles were divided into three treatment groups and used for the study. Serotype A1 *P. multocida* isolated from infected chicken was homogenized in a sterile phosphate buffered saline and the turbidity adjusted to correspond to 0.5 McFarland's turbidity standard. Treatment group A consists of fish infected by immersion in homogenized bacterial inoculums administered at 1 ml/ liter of water while group B were injected intraperitoneally with 0.2ml of the bacterial inoculums. Group C were injected with sterile buffered saline to serve as control. Monitoring of the experimental fish for any clinical sign of disease was carried out daily. Blood samples were collected from 12 fish per treatment group for determination of haematological parameters and blood chemistry analysis. No clinical signs of disease were observed in all the groups for 28 days post infection; the growth rates and morphometric parameters did not differ significantly among treatment groups. There was also no significant change ($p>0.05$) in haematological and serum biochemical parameters. This study has demonstrated that serotype A1 *Pasteurella multocida* did not establish an infection in *C. gariepinus;* hence, it did not result in any haemato-biochemical alteration in the fish.

Keywords: Avian; Catfish; Clarias gariepinus**;** Pasteurella multocida; poultry

1. Introduction

African catfish is a very popular fish species among consumers in Nigeria and other Sub-Saharan African countries (Adah *et al*., 2021). As a result of increase in demand, most farmers operate integrated farms where they keep birds and fishes. Integrated fish cum livestock husbandry is practiced with a view to bringing together normally exclusive farming systems. In such farming system, waste from one enterprise (livestock) becomes input for the other subsystem (fish), thereby maximizing input utilization (Delmendo, 1980; Shoko *et al*., 2019). In Asia and other countries where integrated farming is widely practiced, integration of fish with chicken and duck are the most popular (Sahoo and Singh, 2015). The fish cultured in such system benefits from abundant nutrients arising from natural food which develops from the fertilization of the pond with the livestock manure (Bashir *et al*., 2020). In some integrated poultry cum fish farms, the poultry house overlays the ponds, and the droppings from the birds are used to fertilize the pond. Some farmers also pour poultry faeces and offals, especially intestine, into the fish pond as feed for fish. These droppings and offals from chicken may sometimes harbour pathogenic microorganisms that cause various diseases in chickens such as *Pasteurella multocida, Escherichia coli, Salmonella enteritidis* and parasites. These parasites and microorganisms may sometimes be a source of infection to susceptible fishes cultured in such integrated farms.

Pasteurella multocida is one of the bacterial infections commonly encountered in poultry industry in Sub-Saharan Africa. The *P. multocida* was first discovered in 1878 in birds exhibiting signs of cholera while Louis Pasteur first isolated it in 1880. *Pasteurella multocida* is small, penicillin-sensitive, Gram-negative, non-motile coccobacilli (Zhao *et al*., 2021). The bacterium causes infections in humans through the bites and scratches of cats or dogs (Kannangara *et al*., 2020). In humans, *P. multocida* has been associated with numerous primary or secondary respiratory syndromes (Pak *et al*., 2018) indicating that the organism is of zoonotic importance. The organism is a normal microbiota of the upper respiratory tract of mammals and birds such as cats, dogs, rabbits, cows, swine and chicken. The bacteria are present in a wide range of environments including aquatic habitat.

Pasteurella multocida causes fowl cholera, an acute septicemic infection in birds that results in significant economic losses to the poultry industry, especially in turkey production (Mostaan *et al*., 2020). Pasteurellosis of

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fishes, especially those caused by *Pasteurella piscida*, have existed for a long time. In 1963 at Chesapeake Bay, USA, the disease was reported to have caused heavy losses in white perch (*Roccus americanus*) and striped bass (*M. saxatilis*) production (Snieszko *et al.,* 1964). Also in Japan in 1964 and 1972, it was reported to be responsible for massive mortality of cage-cultured yellowtails (*Seriola quinqueradiata*) in excess of 340 metric tons (Matsusato, 1975). Recently, the incidence of pasteurellosis that caused significant economic losses has been reported in lumpsuckers (*Cyclopterus lumpus* L) in Norway affecting fishes of all life stages (Ellul *et al*., 2019). *Pasteurella* infections have been variously described as bacterial tuberculosis and pseudotuberculosis (Egusa, 1983).

There is a need to study the transmission and pathogenicity of *P. multocida* in fish since poultry manure is used as organic fertilizers in fish ponds. From the literature reviewed, there is no record of *P. multocida* infection among *Clarias gariepinus*. Therefore, this study aimed to ascertain if *P. multocida* of *avian* origin can cause infection in *C. gariepinus* and its effects on the haematological parameters, serum biochemistry, growth and survival of the fish species.

2. Materials and Methods

2.1. Experimental animals and culture system maintenance

The study was undertaken at the Aquaculture Research Facilities, Department of Veterinary Medicine, University of Nigeria, Nsukka situated at latitude 6°45' and 7°N and longitude 7°12' E. The climate is considered as being tropical, with marked periods of rainfall and dry seasons with minimum temperature (16^0C) in December/ January and maximum temperature (34^0C) in March/ April (Source: World Climate Online). One hundred and twenty apparently healthy 6weeks old *Clarias gariepinus* juveniles were procured from a reliable farm in Nsukka, Enugu state. Prior to the commencement of the experiment, the experimental fish were acclimatized for two weeks, during which 2mm fish basal diet was provided at the rate of 3% of their body weight twice daily (Sarka and Rahid, 2012). Borehole water was used for the experiment, and the culture water was changed once in 2 days by gradual removal and addition to ensure adequate oxygenation. The experiment was carried out in 6 tarpaulin fish ponds, constructed with metallic framework. Each of the ponds is 52x48x58.3cm (LxWxH) and has a capacity of 145.5L. Uneaten food and solid wastes were removed from the culture water by siphoning. Water quality parameters such as dissolved oxygen, temperature and pH were monitored weekly. Portable dissolved oxygen meter JPB – 607 A was used to monitor the dissolved oxygen and the water temperature while pocket-sized pH meter manufactured by Hanna instruments was used to monitor the pH of the culture water. The water quality parameters obtained include mean dissolved oxygen 5.6 ± 0.7, mean temperature 25.3 ± 1.38^0 and mean pH of 6.5 ± 0.12. All the parameters measured were within the tolerable limits for fish culture (Timmons and Losordo, 1994).

2.2. Bacterial strains

Serotype 1 characterized *Pasteurella multocida* isolate was obtained from Department of Pathology and Microbiology, National Veterinary Research Institute, Vom Plateau state, Nigeria. Upon arrival, it was sub-cultured on a blood agar at the laboratory of the Department of Veterinary Medicine, University of Nigeria, Nsukka. Biochemical test such as citrate test, H_2S test and methyl red test were performed to re-confirm the organism.

2.3. Infection of Catfish

120 African catfish (*Clarias gariepinus*) used for the study were randomly assigned into three treatment groups (A, B and C) of 40 catfish per group. Each group was further subdivided into two replicates and the stocking density were 20 fish per replicate for ease of handling and reared in tarpaulin pond of 145.5L capacity with iron framing at the 4 sides as stated above. *Pasteurella multocida* was homogenized in a sterile phosphate buffered saline and the turbidity was adjusted to correspond to 0.5 McFarland's turbidity standard (equivalent to 1 x 10^8 colony forming units/ml) in accordance with Zapata and Ramirez-Arcos (2015). Fish in group A were infected by immersing them in appropriate tank containing 1ml/liter of bacterial inoculums for 5 minutes while group B were injected intraperitoneally with 0.2ml of the bacterial inoculums. Group C were injected with sterile buffered saline only to serve as control.

2.4. Observation of critical outcomes and sampling frequency

The infected fish were monitored daily for signs of ill health: sluggishness, off feed, skin lesions, morbidity and mortality. At the end of the experimental period of 4 weeks, the weight and lengths (total and standard) of 6 fish per replicate (12 fish per treatment group) were determined. Tissue samples were taken from 6 fish per replicate for bacteriological assay. Isolation and identification of *P. multocida* using ovine blood agar selective media and *P. multocida* specific PCR methods (Dziva *et al*., 2008) were also employed for detection of the bacterium in fish samples from each treatment group. Also, blood samples were collected from 6 fish per replicate for determination of haematology and blood chemistry analysis.

Sampling and taking of the morphometric (total length, standard length and weight) of a representative sample in each replicate was undertaken every week, and the sample size was 6 fish per replicate. The final sampling and taking of the morphometric of a representative sample in each replicate was undertaken at the 28^{th} day, and the mortality in each treatment was recorded so as to calculate the survival rate.

Condition factor, growth and mortality rates were determined.

The condition factor (K) of each fish was calculated using the formula:

$$K = \frac{100W}{L^3}$$ (Ighwela *et al*., 2011)

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

Specific growth rate was calculated using the formula:

$$SGR = \frac{LnW_f - LnW_i}{T \text{ (days)}} X100$$

(Gharaei *et al*., 2020)

Where W_f = final weight and W_i= initial weight

$$\text{Survival rate} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} X100$$

2.5. Gross pathology

Fish in all the groups were examined for presence of skin lesions. Three fishes from each group were selected and sacrificed 4 weeks post infection. Following death, the fishes were dissected, and the internal organs were examined for the presence of gross lesions.

2.6. Determination of Haematological Parameters

Six fishes were randomly selected from each replicate for haematology and serum biochemistry. Universal bottles treated with ethylene diamine tetra acetic acid (EDTA) were used for blood collection for haematological determination. Blood samples were collected from the caudal circulation (Lawrence *et al*., 2020) with the aid of heparinized 3ml disposable plastic syringes and a 21 gauge disposable hypodermic needle. Collected blood was mixed well with the anticoagulant, and the following haematological studies were carried out on the blood sample. The packed cell volume (PCV) was determined by the micro-heamatocrit method (Schalm *et al.,* 1975). The haemoglobin concentration (Hb) was determined by the cyanometmoglobin method (Kachmar, 1970). The erythrocyte count was determined by haemocytometer method (Dacie and Lewis, 1991). The total white blood count was determined by the haemacytometer method (Dacie and Lewis, 1991). Smears for the differential white blood cell count were prepared on clean slides and studied by the Leishman technique (Schalm *et al.,* 1975).

2.7. Serum biochemistry determination

Non-heparinized blood samples were collected from the caudal circulation from six fish per replicate for the serum chemistry analysis. The collected bloods were allowed to clot, and the clotted blood meant for serum biochemistry was separated from clear serum by centrifugation. All serum biochemistry determination was carried out following standard procedures. Serum Aspartate aminotransferase (AST) and Serum alanine aminotransferase (ALT) were determined using the standard colorimetric method of Reitman and Frankel (1957). This is an in-vitro method of determining AST and ALT using Randox Glutamic-oxaloacetic transaminase test kit and Randox Glutamic-pyruvic transaminase test kit respectively. Total proteins were determined by the direct Biuret method (Lubran, 1978) for the in-vitro determination of total protein in serum or plasma using the total proteins test kit while Albumin was determined using the Bromocresol Green method (Reinhold, 1953)**.** Serum urea nitrogen was determined by the modified Berthlot-Searcy method for the in-vitro determination of urea in serum or plasma using urea test kit.

2.8. Analysis of data

Clinical outcome, morbidity and mortality rates were determined and presented in tables. Data on absolute body weight, condition factor, specific growth rate, survival rate, haematology and serum biochemistry among the four experimental groups were compared using one way analysis of variance (ANOVA). Significant difference was accepted at $p<0.05$.

3. Results

3.1. Clinical signs

No clinical sign of disease was observed in all the groups for 28 days post infection of the *Clarias gariepinus* with *Pasteurella multocida* as shown in Table 1. There were also no gross pathological lesions observed in all the organs examined at the end of the experimental period. Tissue samples taken from each treatment group at the end of the experimental period for bacteriological assay yielded no *P. multocida* isolates.

Table 1. Clinical signs of infection in *Clarias gariepinus* infected with *Pasteurella multocida*.

	Experimental group		
Clinical parameter	A (*P. multocida* injected I.P	B (*P. multocida* infected in water)	C (Unifected Control)
Sluggish Movement	Absent	Absent	Absent
Anorexia	Absent	Absent	Absent
Morbidity rate	Absent	Absent	Absent
Curdling together	Absent	Absent	Absent
Emaciation	Absent	Absent	Absent
Growth rate	Good	Good	Good
Mortality rate	0%	0%	0%

3.2. Changes in Morphometric, Haematologic and Serum biochemistry paremeters

The mean weight of the fish infected with *Pasteurella multocida* are presented in Table 2. Fish in group C gave the least mean weight of 245.64±24.55 twenty eight days post infection. Fish in group A gave a lower mean weight of 254.94±48 compared to those in group B which gave 261.04±9.9 twenty eight days post infection. The results gotten from calculation of the condition factor and specific growth rate also showed that both morphometric parameters did not vary significantly ($p>0.05$) 28 days post infection.

The result of the haematology as outlined in Table 3 showed that there was no significant change ($p>0.05$) in haematological parameters of *Clarias gariepinus* infected with type A1 *Pasteurella multocida*.

Also, the result of the serum biochemistry as outlined in Table 4 showed that there was no significant change ($p>0.05$) in the serum biochemistry parameters of *Clarias gariepinus*.

Table 2: Effect of *Pasteurella multocida* infection on the mean (± SD) morphometric parameters and specific growth rate of *Clarias gariepinus*

Parameter	Day 0 post infection			Day 14 post infection			Day 28 post infection		
	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B	Group C
Weight (g)	152.90±11.7	179.60±11.8	181.50±22.5	235.81±19.5	235.47±15.2	235.34±29.2	254.94±5	261.04±9.9	245.64±24.5
Standard length (cm)	27.05±0.74	28.85±0.43	28.52±0.35	30.71±0.92	30.60±0.70	30.31±1.14	30.79±1.22	32.00±0.67	31.25±0.71
Condition factor	0.67±0.09	0.77±0.03	0.78±0.11	0.90±0.08	0.83±0.04	0.82±0.04	0.81±0.10	0.80±0.03	0.71±0.05
Specific growth Rate							0.68±0.26	0.48±0.09	0.68±0.21

Table 3: Effect of *Pasteurella multocida* infection on the mean (± SE) Haematological parameters of *Clarias gariepinus*

Parameters	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B	Group C
RBC ($\times10^6$/µL)	2.25±0.10	2.27±0.15	2.32±0.22	1.73±0.6	1.55±0.10	1.76±0.16	1.78±0.15	1.42±0.16	1.50±0.88
WBC ($\times10^9$/µL)	11.46±0.15	11.87±0.20	11.90±0.11	12.14±0.32	12.02±0.29	12.32±0.24	12.50±0.35	12.49±0.40	12.77±0.35
HG (g/dl)	8.56±0.35	8.56±0.40	8.40±0.47	8.03±0.49	8.83±0.22	8.64±0.35	8.13±0.24	8.71±0.38	7.83±0.60
PCV (%)	36.63±3.81	38.00±4.06	39.13±4.58	25.75±5.73	34.25±3.74	40.00±6.34	36.13±5.20	24.25±4.71	36.88±3.96
Lymphocyte (%)	91.00±1.85	91.50±2.60	93.13±2.03	91.00±2.10	89.88±2.56	90.79±1.14	90.00±1.85	90.50±2.60	92.13±2.03
Neutrophils (%)	6.38±1.22	6.13±1.50	4.88±1.36	6.13±1.36	6.63±1.69	5.50±1.10	7.38±1.22	7.00±1.22	5.88±1.36
Eosinophils (%)	1.63±0.53	1.00±0.50	1.00±0.46	2.13±0.64	2.88±0.88	1.75±0.53	1.88±0.61	1.25±0.73	1.38±0.65
Monocytes (%)	0.99±0.27	1.37±0.82	0.99±0.59	0.74±0.64	0.61±0.22	1.96±0.5	0.74±0.25	1.25±0.65	0.61±0.30
MCH(pg)	38.04±3.86	37.71±4.23	36.21±4.42	46.24±3.54	56.97±4.93	49.09±2.45	45.67±3.68	61.34±6.34	52.20±4.23
MCV(fl)	162.8±10.22	167.4±8.23	168.66±12.54	148.83±13.11	220.97±13.00	227.27±13.12	202.98±9.31	170.77±14.12	245.87±12.21
MCHC(g/dl)	23.37±2.74	22.53±3.74	21.47±4.12	31.18±3.11	25.78±3.11	21.60±2.30	28.00±4.23	22.13±2.20	35.92±4.23S

The Mean parameters determined did not vary significantly (p>0.05) among the group

Table 4: Effect of *Pasteurella multocida* infection on the mean (± SE) blood serum chemistry of *Clarias gariepinus*

Parameters	Group A	Group B	Group C	Group A	Group B	Group C	Group A	Group B	Group C
Total protein(g/dl)	4.45±0.19	3.70±0.39	5.27±0.33	4.56±0.48	4.54±0.46	4.13±0.20	3.81±0.36	4.92±0.16	4.78±0.29
Albumin(g/dl)	0.83±0.22	1.21±0.34	0.48±0.19	1.69±0.37	0.44±0.14	0.37±0.12	0.68±0.32	0.61±0.16	0.91±0.17
Globulin(g/dl)	3.62±0.08	2.49±0.21	4.79±0.52	2.870±0.21	4.10±0.25	3.76±0.08	3.13±0.12	4.31±0.13	3.87±0.21
Alb/ Glo ratio	0.23±0.08	0.49±0.10	0.10±0.03	0.59±0.06	0.11±0.08	0.10±0.02	0.18±0.03	0.14±0.08	0.24±0.07
ALT (IU/L)	13.00±2.22	13.38±7.41	15.13±2.39	20.75±3.06	13.50±2.76	18.00±2.97	15.13±4.11	15.13±3.27	16.25±3.56
AST (IU/L)	54.75±8.81	53.37±8.88	60.50±6.70	65.37±11.90	53.37±8.88	60.00±9.43	55.50±7.79	53.00±8.96	56.25±8.48
Urea (mg/	4.58±0.68	6.12±0.71	4.94±0.63	5.17±0.81	4.66±0.51	4.98±0.86	5.49±0.67	3.95±0.84	5.70±0.65

The Mean parameters determined *did not vary significantly (p>0.05) among the group*

4. Discussion

Establishment of bacterial infection in fish results in specific signs and symptoms severity of which is dependent on the virulence and pathogenicity of the bacterium involved (Omeje *et al*., 2019). In this study, *Clarias gariepinus* experimentally infected with serotype A1 *P. multocida* of poultry origin were investigated in order to evaluate its infectivity and haemato-biochemical effect on the fish. There was total absence of common clinical signs of bacterial disease, which may include sluggish movement, anorexia, morbidity, curdling together, and emaciation in the group infected by immersion which indicates that they were not diseased. The same observance was made on Group A in which the fish was infected by injecting 0.02ml/g of the organism intraperitoneally. Supposedly, the fish in this group should have a greater influence of the bacterium because intraperitoneal infection -which is a parenteral route- ensures prompt absorption of injected material into the liver through the mesenteric vessels (Al Shoyaib *et al*., 2020). Although intravenous injection is quicker in eliciting response, intraperitoneal injection was used because intravenous access will be challenging for the juvenile fish being used for the experiment. For both groups, the morbidity and the mortality rates were zero; there was absence of observable clinical signs of infection throughout the 28 days post infection which was the same as uninfected control group C. Bacteriological assay employing normal bacteria isolation in blood agar and specific PCR methods yielded no *P. multocida* isolates, which indicates that the bacterium did not establish an infection in *Clarias gariepinus*. The results of the study also indicated that the specific growth rate (SGR) and the Condition Factor (CF) of the fish in the groups were normal and did not differ significantly ($p>0.05$) in the three groups 28 days post infection. This result is in agreement with Nizan and Hammerschlag (1993) who reported that *Pasteurella multocida* has been isolated from Tilapia hybrids (*Oreochromis spp)* with no clinical sign which showed that the infection was subclinical and the source of infection was suggested to be the poultry manure used as organic fertilizer in the ponds. However, *Pasteurella piscicida* was reported to cause Pasteurellosis in fish species such as sea bass, white bass, yellowtail, striped bass and gilthead sea-bream (Thune *et al.,* 1993). In poultry industry pasteurellosis due to *P. multocida* has been recognized as one of the diseases of veterinary importance. A variety of domestic livestock including mammals and birds are susceptible to *P. multocida* (Abdulrahman and Davies, 2021), but there has not been any reported case of *P. multocida* infection in African catfish (*Clarias gariepinus).* Although they isolated *P. multocida* from natural pond water, Prince and Brand (1984) suggested that it may be due to the presence of waterfowl carcasses which shed the bacteria. A research done by Backstrand and Botzler (1986) showed that even though the capability of water in transmitting *P. multocida* organism from infected to susceptible avian host (waterfowl) is not in doubt, they postulated that the concentration of the organism declines rapidly in water. The low survival of *P. multocida* in water hypothesized by these scientists was reflected in the Group B of this study which was infected by immersing the fish into a tank containing 1 ml/ litre of the bacterial inoculum.

Changes in blood parameters are known to occur in fishes under disease, agitation or nutritive stress (Blaxhall, 1972; Dossou *et al*., 2018). The result of the haematology showed that the bacterium, *Pasteurella multocida* did not induce changes in haematological parameters. The packed cell volume, haemoglobin concentration, red blood cell count, white blood cell count, eosinophils, neutrophils and lymphocytes did not differ significantly on days 0, 14 and 28 post infections for the three groups. This may also suggest the absence of any other infection (parasitism, allergy, dermatitis). This is in agreement with the work of Adedeji *et al* (2000) who reported that haematological parameters are crucial and serve as a possible indicator of physiological or pathological changes in disease investigation and fishery management.

Plasma enzymes are useful as appropriate markers of tissue (organ) damage (Ramesh *et al*., 2018). An elevated level of plasma enzyme may suggest damage to tissue or organ. Damage to the liver may cause poor detoxification and deamination, which can lead to poor feed conversion, loss of weight and mortality. Based on the result of serum biochemistry, there were no significant differences in the plasma enzymes (alanine aminotransferase and aspartate aminotransferase) analyzed, after 28 days post infection for the group A, B and C. This is indicative that there was no gross pathological change or damage in the liver of the fish since the *P. multocida* organism did not establish to cause infection in the *C. gariepinus*. The serum total protein, total albumin and blood urea nitrogen also did not differ significantly ($p>0.05$).

5. Conclusion

This study has demonstrated that type 1 *Pasteurella multocida* did not establish an infection in *Clarias gariepinus.* Hence, it did not result in any haemato-biochemical alteration in *Clarias gariepinus*.

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Ethical Statements

All experiments in this study associated with fish complied with animal welfare ethical approval (FVM-UNN-IACUC-2019-0925) obtained from Faculty of Veterinary Medicine Institutional Animal Care and Use Committee, University of Nigeria, Nsukka.

Conflict of Interests

The authors declare that there is no conflict of interests.

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