ISSN 2689-8268 | Volume 6

American Journal of Surgery and Clinical Case Reports

Research Article Open Access

Study of Aeromonas Hydrophila Infections in Pelteobagrus Fulvidraco: In the Morphological and Hematological Symptoms

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Received: 20 July 2023
Accepted: 19 Aug 2023
Published: 23 Sep 2023
J Short Name: AJSCCR

Citation:

Chen S. Study of Aeromonas Hydrophila Infections in Pelteobagrus Fulvidraco: In the Morphological and Hematological Symptoms. Ame J Surg Clin Case Rep. 2023; 6(15): 1-6

Keywords:

Aeromonas hydrophila; *Pelteobagrus fulvidraco*; Blood cell concentration; Infection; Tissue damage

1. Abstract

Aeromonas hydrophila is a gram-negative conditional pathogen of the genus Aeromonas in the family Vibrionaceae that generates exotoxins and other virulence factors that can cause pathological alterations in the tissues and organs of numerous aquatic animals. *A. hydrophila* infection can cause large-scale illness in *Pelteobagrus fulvidraco* aquaculture, which can cause fish mortality and financial losses for the aquaculture sector. In this study, different concentrations of *A. hydrophila* (9.00×10^2, 9.00×10^5, and 9.00×10^8 CFU/mL) were used to infect healthy *P. fulvidraco* at room temperature, and the pathological changes and blood cell concentrations of the diseased fish were investigated using morphological observation and blood cell count. The findings revealed that *A. hydrophila* infection caused lesions in the gills, kidney, liver, and stomach of *P. fulvidraco*, manifesting as hemorrhage, ascites, and septicemia. As the concentration of *A. hydrophila* infection increased, the tissue damage of *P. fulvidraco* became more serious and the hemocyte concentration tended to decrease. However, there was a recovery in the decrease of hemocyte concentration at a lower bacterial infection concentration (9.00×10^2 CFU/mL). The results of this study demonstrated that *P. fulvidus* hemocyte concentrations were resistant to low concentrations of *A. hydrophila* (9.00×102 CFU/mL), and the morphological and blood cell concentration changes can provide a scientific basis for determining and identifying *A. hydrophila* infection in aquaculture production.

2. Introduction

Pelteobagrus fulvidraco belongs to the genus *Pelteobagrus* in the Siluriformes family (Bagridae), also known as Bagrid catfishes. It is an important economic fish in rivers and lakes [1, 2, 3]. *P. fulvidraco* is preferred by aquaculturists because of its short growth cycle, wide feeding habit, high population reproduction, and high economic benefits. It is also a high-quality farmed fish, rich in protein, high-quality fat, various trace elements, and other nutrients. Its tender meat, few spines, and delicious taste led to an increasing demand for *P. fulvidraco*, thus further driving the expansion of the artificial culture market. As a major freshwater fish species, the farming industry of *P. fulvidraco* in Huzhou, Zhejiang Province, has been rapidly developing, with farmed production averaging already about 20% per year from 56,000 tonnes in 2015 to reach 120,200 tonnes in 2019 [4]. Contrarily, infections prevalent in yellow catfish during farming operations have had a significant negative impact on the sector [5, 6]. Bacterial diseases are the most prevalent, with *Aeromonas hydrophila*, *Aeromonas sobria*, *Sluggish* Edwardsiella tarda are the common bacterial illnesses [7, 8, 9]. *A. hydrophila* belongs to the genus *Aeromonas* in the family Vibrionaceae and is a short gram-negative bacterium that is found in freshwater ecosystems and is widespread on the surface of water bodies, soil, aquatic plants, and the digestive tracts of animals [10, 11]. *A. hydrophila* can produce highly toxic exotoxins such as hemolysins, histotoxins, proteases, necrotoxins, cytotoxic enterotoxins, and cytoexcitatory enterotoxins, which are the most important pathogenic factors of the bacterium and are closely related to the pathogenicity of *A. hydrophila* [12, 13]. According to the analysis of actual cases, these toxins reach various tissues and organs of aquatic animals through blood circulation, reducing the immune function of animals and thus causing pathological changes leading to mass mortality, clinically characterized by acute hemorrhagic septicemia, which is the main cause of bacterial septicemia in freshwater animals such as fish, shrimp, and frogs

and is also the main source of infection in humans. It is a typical human-animal-fish pathogenic bacterium [14, 15, 16], which can lead to acute gastroenteritis, traumatic infections, and sepsis in immunocompromised individuals under direct or indirect infection. Due to the diversity of genetic structural variation in this bacterium, the genes for its exotoxins vary greatly, and although the virulence factors of *A. hydrophila* are complex and numerous, they act in synergy on each organism [17].

In this study, different concentrations of *A. hydrophila* suspensions were injected intraperitoneally into healthy artificial *P. fulvidraco* for a period of two weeks to complete a preliminary description of the pathogenicity of *A. hydrophila* and the pathological changes in the diseased fish by observing the morphological characteristics of the diseased fish, analyzing the changes in blood cell concentrations, and observing the re-identification of the strain. The results of this study will provide a scientific basis for the identification and diagnosis of *A. hydrophila* infections in aquaculture.

3. Materials and Methods

3.1. *P. fulvidraco* and bacteria species used in this study

The experimental *P. fulvidraco* were purchased from the Neijiang Aquatic Products Market on March 21, 2022, randomly divided into five groups, and then temporarily reared in an indoor room temperature tank for one week with an average body length of 14.44±1.31 cm and an average body weight of 27.56±5.38 g (Figure 1). During the temporary and experimental period, oxygen was supplied by an aerated pump, and the fish were fed twice a day with a Tongwei special diet for *P. fulvidraco* at 3% of their body weight. The experimental *A. hydrophila* were purchased from Shanghai Luwei Technology Co., Shanghai, China.

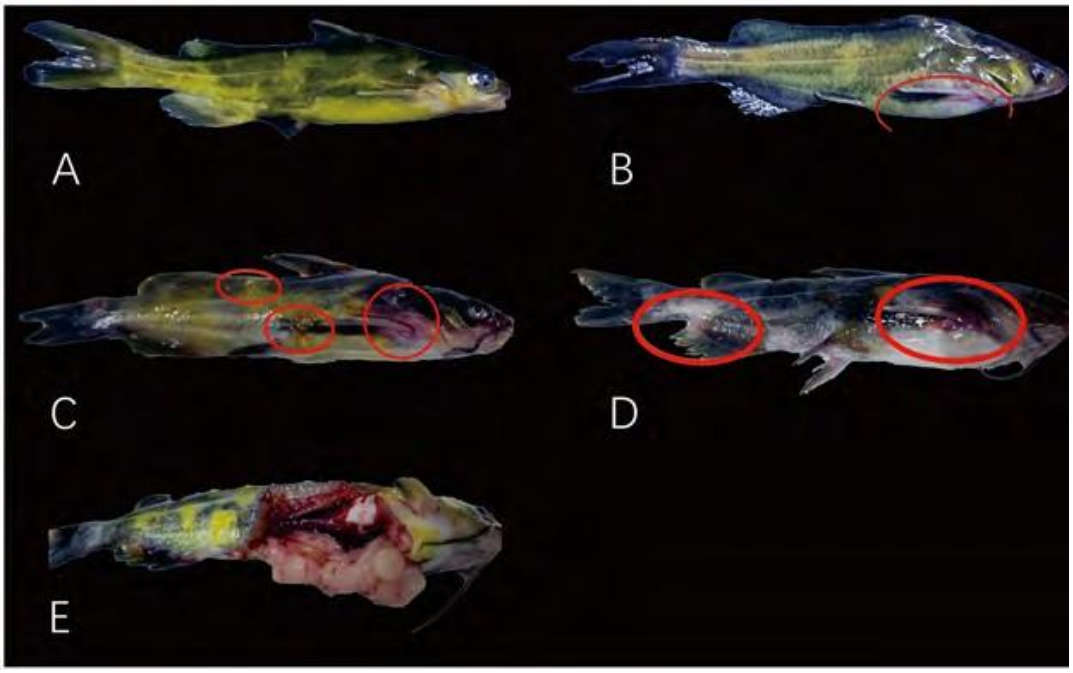

Figure 1: Significant symptoms of sick *P. fulvidraco* infected with *A. hydrophila*. A. control, B. abdominal oedema (Group I day 2), C. yellow spots on the body surface and ulceration (Group III Day 6), D. congestion at the base of the fins (Group I Day 4), E. abdominal hemorrhage with congested and enlarged liver (Group II Day 4).

3.2. Experimental methods and procedures

In order to obtain the experimental solution of *A. hydrophila*, it was first cultivated by using the plate scribing method and incubated in a constant temperature chamber at 28 ℃. The culture medium was incubated in a basal conical flask, with the date of inoculation and the name of the strain, and incubated in a shaker at 28 °C for 16-24 h until the logarithmic phase. The preparation of an *A. hydrophila* suspension was done by the gradient dilution method. Seven sterile test tubes were taken, and 9 ml of sterile saline was pipetted into each tube and plugged with cotton plugs, labeled as 1, 2, 3, 4, 5, 6, and 7, corresponding to different dilutions of bacterial suspension. Pipette 1 ml of the bacterial suspension into test tube 1, and shake the tube slightly to mix the suspension with the saline. The concentration of the bacterial suspension in tube 1 was initially determined to be 9.00×10^8 CFU/mL by Mackenzie's turbidimetric method. 1 mL of the bacterial suspension was pipetted from tube 1 to tube 2 as described above, and the above operation was repeated. The concentration of the suspension was used as the experimental solution.

Experimental *P. fulvidraco* were injected intraperitoneally at, and the prepared bacterial suspensions of groups I, II, III with concentrations of 1 9.00×10^8 CFU/mL, 4 9.00×10^5 CFU/mL, and 9.00×10^2 CFU/mL. Four groups of *P. fulvidraco* were taken for experiments after indoor temporary rearing and divided into experimental groups I, II, III, and the control group; the other group was the reserve group. The experimental groups I, II, and III were

injected with 0.20 mL of bacterial suspension at concentrations of 9.00×108 CFU/mL, 9.00×105 CFU/mL, and 9.00×102 CFU/mL from the base of the ventral fin of the fish body, respectively, while the control experiment injected 0.20 mL of 0.85% sterile saline for each fish (Table 1). Infections were observed for two consecutive weeks, and hematocrits were counted.

3.3. Blood cell count determination

The blood counts of *P. fulvidraco* were determined as soon as the experimental group showed up dead. Centrifuge tubes containing 1499.00 μL of 0.85% sterile saline were prepared in advance for the dilution of whole blood from the three experimental and control groups; empty centrifuge tubes were prepared to hold the blood samples to be tested. To avoid hemolysis, the fish are cut off from the tail, and 1.00 μL of whole blood is drawn into the prepared centrifuge tubes for dilution. The blood was counted on a hemocytometer plate. The diluted blood was gently shaken again, and a small amount of diluted blood was added to the edge of the coverslip with a dropper. The surface tension of the diluted blood was used to quietly wait for the liquid to fill the entire counting area.

Table 1: Injection concentrations in different experimental groups

Groups	Control group	Bacterial injection groups		
		I	II	III
Bacterial concentration (CFU/mL)	0.85% Stroke-Physiological Saline Solution	9.00×108	9.00×105	9.00×102
Injection volume (mL/individual)	0.2	0.2	0.2	0.2
Number of injections	10	10	10	10

3.4. Isolation, purification, and re-assay of *A. hydrophila*

In order to ensure the accuracy of the experiment, the diseased *P. fulvidraco* were dissected in each group under aseptic conditions, and samples were taken from the liver of the diseased fish with an inoculation loop, inoculated by scribing on agar medium, and incubated in a constant temperature chamber at 28 ℃ for 16~24 h to observe whether there was colony growth and to determine whether the source of the disease was *A. hydrophila*.

4. Results

4.1. Symptoms of diseased *P. fulvidraco*

The symptoms of *P. fulvidraco* infected with *A. hydrophila* were similar and varied among individuals. The overall symptoms included bleeding on the surface of the fish, patches of body color, increased mucus, loss of balance in swimming, decreased feeding or no feeding, solitary swimming and floating on the surface of dying fish, and abdominal oedema in most fish (Figure 1B), with the most obvious symptoms in Experiment I. The fish in each experimental group showed yellow patches or ulcers on the body surface (Figure 1C); the fin bases were congested with blood, accompanied by rotten tails and fins (Figure 2D). Dissection of the diseased fish revealed the presence of reddish ascites and yellow jelly-like material in the abdominal cavity, thinning of the intestinal wall, and varying degrees of congestion and enlargement of the liver (Figure 1E).

This experiment identified the predominant symptoms of *A. hydrophila* infections in *P. fulvidraco*, which included bleeding on the surface, patches of body color, increased mucus, loss of balance in swimming, decreased feeding or no feeding, solitary swimming and floating on the surface of dying fish, and abdominal oedema in most fish.

4.2. Effects of *A. hydrophila* on blood cells in *P. fulvidraco*

The hemocyte concentrations of *P. fulvidraco* infected with *A. hydrophila* at room temperature were counted and compared, and the hemocyte concentrations of individuals in experimental groups I, II, and III decreased on day 4 compared to the control group, and the hemocyte concentrations of individuals infected with high concentrations of the bacterial solution decreased to lower levels compared to those of the low concentration group and were lower than those of the control group. The trend was the same as on day 4. The mean hemocyte concentrations in fish from group I were lower than those in the control group on day 4 and day 8 compared to day 14, while those in group II were not significantly different from those in the control group on all three occasions. Overall, the blood cell concentration of *P. fulvidraco* infected with *A. hydrophila* decreased compared with the control group and was always smaller than the control group, and the blood cell concentration decreased as the concentration of infected bacteria increased but recovered at group III, indicating that *P. fulvidraco* had some resistance to the lower concentration of *A. hydrophila* infection.

Figure 2 Blood cell concentration statistics of *P. fulvidraco* after infection with *A. hydrophila* at room temperature. I. Experimental group I (bacterial concentration 9.00×10^8); II. Experimental group II (bacterial concentration 9.00×10^5); III. Experimental group III (bacterial concentration 9.00×10^2); Control group. Control group (0.85% saline); n = 5, significant difference between groups with different letters on the column ($p < 0.05$).

4.3. Results of isolation and purification of *A. hydrophila* and validation

After the inoculation of liver samples from experimental groups I, II, and III, the colonies that formed in the medium were of the same shape, forming slightly convex and smooth, milky white colonies with a certain degree of transparency and a special odor, and there was no significant difference with the previously activated colonies, while no colonies were formed in the control group after inoculation. This again confirmed that the causative agent of the diseased *P. fulvidraco* was *A. hydrophila* (Figure 3).

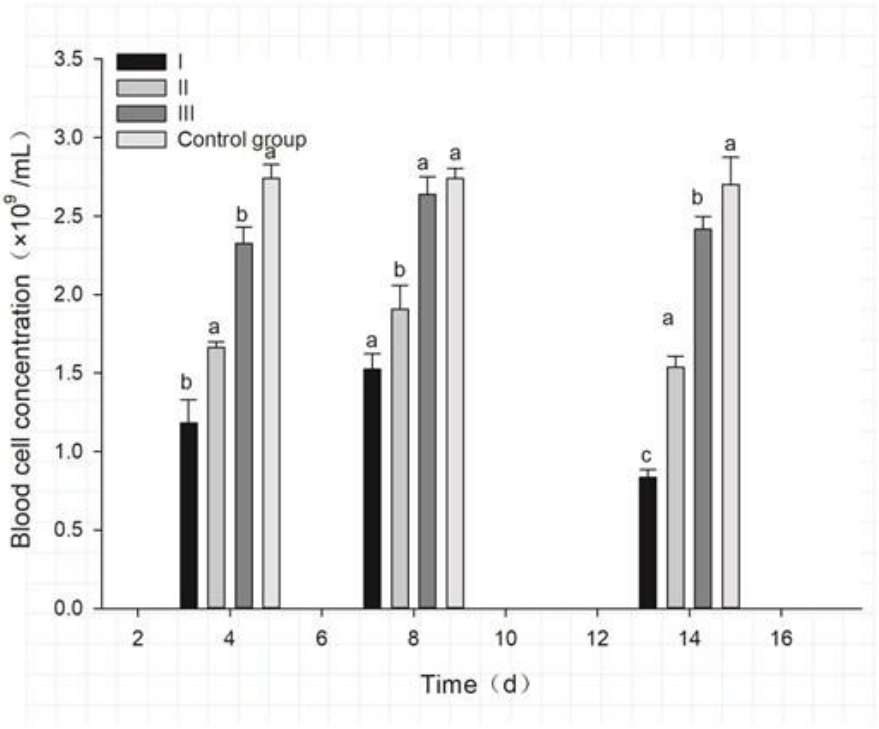

Figure 2: Blood cell concentration statistics of *P. fulvidraco* after infection with *A. hydrophila* at room temperature.

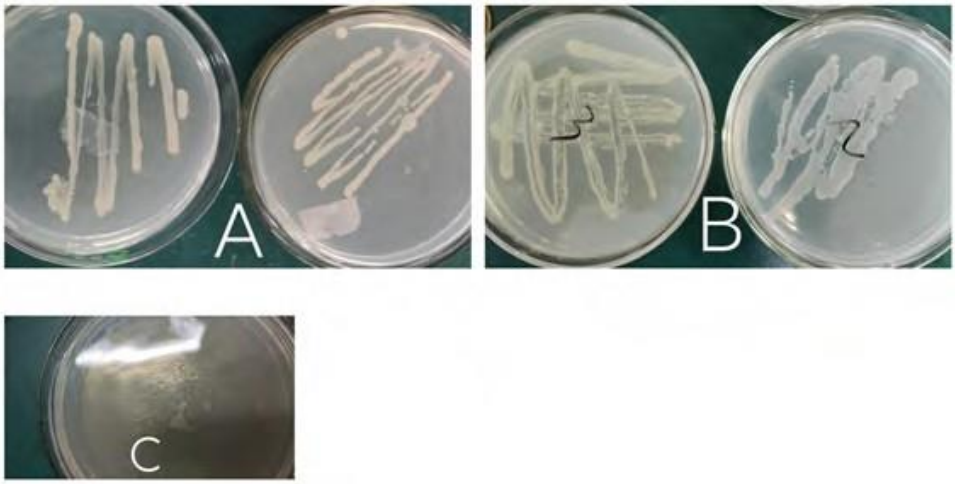

Figure 3: Colony morphology of *A. hydrophila*. A. Activated *A. hydrophila*; B. Morphology of liver inoculum colonies of diseased fish; C. Control group.

5. Discussion

A. hydrophila is widely distributed in the aquatic environment and is a major pathogen causing disease and mortality in aquatic organisms [3, 18]. Studies have shown that aquaculture species such as groupers (*Megalobrama amblycephala*), carp (*Cyprinus carpio*), crucian carp (*Carassius auratus*), yellow eels (*Monopterus albus*), grass carp (*Ctenopharyngodon idella*), *Mylopharyngodon piceus*, and *Pelodiscus sinensis* are commonly affected by diseases caused by *A. hydrophila* [19-22]. The increased demand for *P. fulvidraco* as a high-quality freshwater fish has resulted in the growth of *P. fulvidraco* culture, however numerous domestic *P. fulvidraco* farms face recurrent disease outbreaks. According to relevant investigations on freshwater fish pathogen research, the pathogenic bacteria that trigger ascites, hemorrhagic oedema disease, and septicemia in *P. fulvidraco* are *A. hydrophila* [4]. From the experiments conducted by Zhang T et al, 2009 [23], it was revealed that the infected *P. fulvidraco* had obvious pathological changes in various organs and tissues. When the parenchymal cells are severely degenerated and necrotic, local blood circulation undergoes stressful changes, causing damage to the blood vessel walls, leading to closure and rupture and forming blood spots; the epithelial cells of the gill lamellae are hyperplastic and hypertrophic, leading to bleeding and local necrosis, which affects the osmoregulatory effect of the gills, thus accelerating the death of diseased *P. fulvidra-*

co. In the present investigation, the main clinical symptoms of the diseased *P. fulvidraco* were oedema in the abdomen, congestion and enlargement of the liver, hemorrhage in the abdominal cavity after dissection, yellowish fluid, and a yellowish gelatinous body after dissection; increased mucus on the surface of the fish, yellow spots and ulceration, hemorrhage at the base of the fins, bulging eyes, and difficulty in feeding and movement. It was difficult to maintain locomotor balance. The results of this research were consistent with those of other authors and are mainly caused by the numerous virulence factors of *A. hydrophila*.

In a previous study on the changes in blood in freshwater fish caused by *C. aeruginosa* infection, it was found that the number of blood cells in fish decreased to different degrees after infection with *C. aeruginosa*. For example, [24] studied the effect of artificial infection with *C. aeruginosa* on the blood cell concentration of eels and found that the blood cell concentration in eels infected with *C. aeruginosa* showed a trend of increasing and then decreasing with the increase of the bacterial solution [24]. In a study of the Japanese eels artificially infected with *A. hydrophila*, the hematocrit of Japanese eels also showed a trend of first increasing and then decreasing, while the hematocrit of carp infected with *A. hydrophila* was significantly reduced [25]. Lu Hongda's study on turtle septicemia with *A. hydrophila* showed that *A. hydrophila* secretes hemolytic and enterotoxic exotoxins, which cause severe hemolytic anemia, rupture, and lysis of blood cells in the blood vessels and organs of the turtle [26]. In this study, the blood cell count of *P. fulvidraco* decreased in comparison with the control group after infection with *A. hydrophila*, recovered with time in the low concentration infection group, but was still lower than the control group; comparing different concentrations of *A. hydrophila*-infected *P. fulvidraco*, the higher the concentration of the infected bacteria, the lower the blood cell count. The results of this study were similar to those of other species of *P. fulvidraco*.

The study showed that the clinical symptoms of *A. hydrophila* infection in *P. fulvidraco* at room temperature were ascites and septicemia; the blood cell concentration of the affected fish showed a tendency to decrease after infection, and the higher the concentration of the bacterial suspension, the lower the blood cell concentration and the more serious the damage to the fish, providing a scientific basis for the determination and identification of *A. hydrophila* infection in aquaculture production.

6. Acknowledgements

We would like to give special thanks to the Neijiang Normal University, Sichuan, China, for their help in carrying out this study. We also express our thanks to the staff of the American Journal Experts language service for their academic editing services. Research Grant supported this study was from Natural Science Foundation of Sichuan Province (2022NSFSC1721, 2021YFN0033), Science and Technology Program Projects of Sichuan Provincial Science and Technology Department (2021YFN0028), Neijiang Normal University Science and Technology Foundation (2019FM05) and the Neijiang Normal University School-level Research project (2023YB12).

7. Statement of Conflict of Interest

The authors have declared no conflict of interest.

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