

Original Research Articles

Identification and pathogenicity of *Aeromonas veronii* isolated from sexually mature female *Hypophthalmichthys molitrix*

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Keywords: *Aeromonas veronii*, bacterial septicemia, histopathology, *Hypophthalmichthys molitrix*, immune-related enzymes

<https://doi.org/10.46989/001c.74696>

Israeli Journal of Aquaculture - Bamidgeh

Vol. 75, Issue 1, 2023

An outbreak of bacterial septicemia in female silver carp *Hypophthalmichthys molitrix*, which caused significant death of the fish in the Yantian Reservoir, was investigated. The pathogen was isolated from diseased fish and identified as *Aeromonas veronii* utilizing biochemical characteristics and molecular methods analyses. An artificial infection experiment indicated that the strain caused 100% mortality of juvenile silver carp and mature female fish with eggs but no death of the mature male fish. Silver carp challenged with *A. veronii* showed similar clinical signs with naturally infected fish. The histopathological study revealed that *A. veronii* infection caused the increment of hemosiderin granules and the vacuole formation in tissues of the spleen and livers, as well as the collapse, deformation, and disintegration of egg cells. The ACP, AKP, and CAT enzyme activities in the serum of both naturally and artificially infected silver carp were decreased significantly with the severe infection. In this study, *A. veronii* was isolated and identified as the primary bacterial pathogen causing the mass death of sexually mature female silver carp with eggs in Yantian Reservoir.

INTRODUCTION

Hypophthalmichthys molitrix, also named silver carp, is a popular planktivorous fish species in freshwater aquaculture for its faster growth rate and lower feeding cost than other fish species.¹ It is also introduced into ponds, reservoirs, and lakes worldwide to control the phytoplankton biomass, prevent blooms, and promote nutrient regeneration. In China, silver carp is one of the Four Major Chinese carp, with a total production of 3.81 million tons in 2020, which was the second-highest production fish among all fish species. However, intensive aquaculture practices and the deterioration of water quality often give rise to bacterial diseases in fish, causing economic loss to the aquaculture industry.² The commonly reported bacterial pathogens in silver carp are *Plesiomonas shigelloide*,³ *Aeromonas sobria*,⁴ and *Acinetobacter* spp., which have become the limiting factors for the silver carp aquaculture industry.

The genus *Aeromonas* is composed of various species, mainly pathogens of various fish species, and is widespread in the aquatic environment worldwide.⁵ *Aeromonas veronii*, an important opportunistic pathogen, could cause diseases in various kinds of fish, including largemouth bass *Mi-*

cropterus salmoides,² crucian carp *Carassius auratus*,⁶ sheatfish *Silurus glanis*,⁷ Nile tilapia *Oreochromis niloticus*,⁸ and channel catfish *Ictalurus punctatus*.¹⁰ The clinical signs caused by *A. veronii* include hemorrhagic ulcers, abdominal ascites, abdominal distension, redness, and swelling of the anus (He et al., 2019). In 2011, *A. veronii* was reported in silver carp for the first time.¹¹ At the same time, there were limited reports on the infection of *A. veronii* in silver carp from then on, especially regarding its pathogenicity.

In May 2018, a disease was an outbreak in wild silver carp in Yantian Reservoir, Guangdong Province, China, which resulted in mass mortality of female silver carp with eggs. We preliminarily considered that the death of the fish was caused by bacterial disease and isolated an *A. veronii* strain from infected female silver carp. The present work aimed to identify the pathogenic bacteria, study its pathogenicity and conduct histopathological and immune-related enzyme activity analyses.

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MATERIALS AND METHODS

ISOLATION AND IDENTIFICATION OF THE BACTERIA STRAIN

Moribund broodfish of silver carp weighing 0.5-1.5 kg ($n=22$), with a body length of 31-35 cm, were sampled from Yantian Reservoir, Guangdong province, China, where broken out fish disease. The temperature, dissolved oxygen, and pH in water were 26.5°C, 9.5 mg/L, and 8.4, respectively. The fish were transported immediately to the laboratory to isolate pathogenic bacteria. The infected fish were anesthetized with 100 mg/L of tricaine methanesulfonate (MS-222), then their skins were disinfected with 75% ethyl alcohol. Next, the blood samples were collected from the caudal vessel, and the ascites in the ovary were collected post-dissection. The obtained blood and ascites samples were spread on the plates containing beef extract-peptone solid medium (10 g/L peptone, 3 g/L beef extract, 5 g/L NaCl, 16 g/L agar, pH 7) to isolate pathogenic bacteria.¹² The plates were cultured at 28°C for 24 h. The dominant bacteria were purified using streak cultivation on the beef extract-peptone solid medium three times. The single colony named WSQ-1 was selected and inoculated in beef extract-peptone broth at 28°C for 18 h. The bacterium solution was preserved at -80°C after adding sterile glycerol at a final concentration of 40% (v/v).

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION

Morphological observation of the WSQ-1 isolate strain was carried out using Gram staining. Biochemical identification and characterization of the isolated strain WSQ-1 were carried out using 21 biochemical and physiological test reactions, the standard strain *Aeromonas veronii* GDMCC 1.893 was purchased from Guangdong Microbial Culture Collection Center. The identification of WSQ-1 was according to the method of the Identification Manual of Common Bacterial Systems.¹³ The details of these 21 biochemical tests are shown in [Table 1](#).

MOLECULAR CHARACTERIZATION

Genomic DNA of WSQ-1 was extracted using an UNIQ-10 column DNA extraction kit (Sangon, China) following the manufacturer's instructions. A pair of universal primers 27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5'-GGT-TACCTTGTACGACTT-3'¹⁵ were used for amplification of the 16S *rRNA* gene, *gyrB*3F: 5'-TCCGGCGGTCTGCACG-GCGT-3' and *gyrB*14R: 5'-TTGTCCGGTTGTACTCGTC-3' were used for amplification of the *gyrB* gene.¹⁶ The PCR amplification conditions were initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 98°C for 30 s, annealing at 54°C for 45 s, and extension at 68°C for 45 s, as well as a final extension at 68°C for 7 min. The amplified products were analyzed with 1% agarose gel electrophoresis, and the sequence was cloned into the pMD-18T vector (TakaRa, Japan) and sequenced by BGI Genomics (Shenzhen City, China). A blast search for the se-

quence was carried out via the NCBI website. The phylogenetic tree was established using the Neighbor-Joining method in the MEGA 6.0 software package.

EXPERIMENTAL INFECTION

Healthy juvenile silver carp weighing 30-50 g were purchased from a commercial fish farm at Huadu, Guangzhou City, Guangdong province. 180 healthy adult silver carp weighing 1.5-4 kg were obtained from Zhenkou Reservoir, Jiangxi province, China. Before the experiment, the fish were treated with copper sulfate at a concentration of 2 mg/L for about 20 min to eliminate all pathogens on fish gills and surfaces. Five individuals of the fish were randomly selected for bacterial and parasitic examination to ensure that the fish had no pathogen, then were temporarily reared for a week in 9 of 2 cubic meters tanks with aerated and dechlorinated freshwater in the Aquatic Animal Technology Laboratory of Jinan University, each tank contained 20 fish and 1.8 cubic meter water. The healthy adult and juvenile silver carp were fed daily with powder feed at 1% of fish weight. The water temperature was kept at 23.0 ± 0.3°C with an air conditioner, the dissolved oxygen was higher than 6.0 mg/L, and the pH of the water was 8.1.

A total of 240 healthy juvenile silver carps (30-50 g) were randomly divided into four groups: (a) Injected infection group: each silver carp was injected with 100 µL WSQ-1 suspension by intraperitoneal injection at a concentration of 3×10⁶ CFU/mL¹⁷; (b) Bathed infection group: the fish were bathed with WSQ-1 at a concentration of 3×10⁷ CFU/mL; (c) Injected control group: each fish was injected with 100 µL 0.65% saline water; (d) Bathed control group: the fish were treated with 0.65% saline water. Each group was carried out in triplicate.

Female fish with eggs ($n=90$) were divided into an infected group ($n=45$), and a control group ($n=45$), and male fish ($n=90$) were also divided into an infected group ($n=45$) and a control group ($n=45$). Each group contained three tanks with 15 fish in each tank. The fish have injected with 100 µL WSQ-1 suspension at a concentration of 3×10⁷ CFU/mL per kilogram in infected groups and injected with 100 µL 0.65% saline water per kilogram in the control group.

The bacteria were sub-cultured in 10 ml of beef extract-peptone medium, and the suspension was incubated at 28°C for 24 h. After incubation, the culture was centrifuged for 5 min at 5000 rpm. The supernatant was removed, and the bacteria were washed twice with normal saline (NS) solution and then resuspended in 10 ml of NS. Next, 1 ml of cell suspension dilution was made up to 10⁻⁷ in NS, and the diluted suspension was spread on the medium and cultured at 28°C for 24 h to determine the number of cells/. All fish were raised at 23°C for 14 days to observe and record pathological symptoms. The fish were inspected daily for clinical signs and mortality. The blood and ascites from infected fish were collected to re-isolate the pathogenic bacterium.

HISTOPATHOLOGICAL EXAMINATIONS

The spleen, liver, and gonads of moribund fish from the artificially and naturally infected silver carp were sampled.

Table 1. Physiological and biochemical characteristics of strain WSQ-1

Characteristics	WSQ-1	<i>A. veronii</i> *	<i>A. veronii</i> ^a	
			<i>A. veronii</i> biovar <i>sobria</i>	<i>A. veronii</i> biovar <i>veronii</i>
Motility	+	+	+	+
Hemolysis	+	+	+	+
Oxidase	+	+	+	+
Voges-Proskauer	+	+	+	+
Citrate	+	+	+	+
Malonate	-	-	-	-
Indole	+	+	+	+
Cellobiose	+	+	d	d
Phenylpyruvic acid production	+	+	+	+
D-Sorbitol	-	-	-	-
L-Arabitol	-	-	d	-
Esculin hydrolysis	+	+	-	+
Methyl red	+	+	+	+
D-Raffinose	-	-	-	-
Lactose	-	-	-	-
Sucrose	+	+	+	+
H ₂ S from gelatin cysteine throsulfate	-	-	d	d
Gluconate oxidation	+	+	+	+
Arginine dihydrolase	-	-	+	-
Lysine decarboxylase	+	+	+	+
Ornithine decarboxylase	+	+	-	+

Note: “*”: standard strain; a: strains described in Bergey’s Bacteria Identification Manual¹⁴; “+”: positive; “-”: negative; “d”: different.

The collected samples were then fixed in Bouin’s fixative, dehydrated in ethanol, embedded in paraffin wax blocks, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E) for histopathological observation under a light microscope.¹⁸

SERUM ENZYMES ACTIVITY

Blood samples from the healthy, artificially infected, and naturally infected fish were obtained from the caudal vein after the fish were anesthetized with 100 mg/L MS-222. Then the collected blood was centrifugated at 1469 g for 10 min at 4°C. The serums were obtained and stored at -20°C before use. The serum activities of acid phosphates (ACP), alkaline phosphatase (ALP), and catalase (CAT) were measured using Kits (Nanjing Jiancheng Bioengineering Institute, China) following the instructions.

STATISTICAL ANALYSIS

The data were analyzed with Excel 2016 and SPSS Statistics 24 (International Business Machines Corporation). The comparison of the difference between experimental groups and control groups was analyzed by chi-square test and one way-ANOVA. A significant difference was considered at $p < 0.05$. Sequence analysis was performed using MEGA 6.0 software.

RESULTS

CLINICAL SYMPTOMS OF NATURALLY INFECTED FISH

Twenty-eight silver carp were collected in May 2018, of which 22 were sexually mature females with eggs, and all had diseased symptoms, while the male individuals showed no clinical symptoms. Clinical symptoms of the naturally diseased fish showed abdominal distension and anal congestion. After dissection, a substantial amount of ascitic fluid filled the abdominal cavity, and the fish’s liver and spleen were swollen and darkened. The ovary membrane was broken, then the eggs scattered and changed from slight to full transparency ([Figure 1](#)).

BIOCHEMICAL CHARACTERIZATION

WSQ-1 was isolated from naturally diseased fish with colony characteristics of grey-white, smooth surface, the translucent, slightly convex, and regular margin on the agar plates ([Figure 2A](#)). The strain belonged to a Gram-negative bacterium with red color and blunt ends without spores ([Figure 2B](#)). The physiological and biochemical characteristics of WSQ-1 were the same as those of the standard strain. The esculin hydrolysis and ornithine decarboxylase results were negative, and the arginine dihydrolase and L-Arabitol results were positive, which indicated that WSQ-1 was consistent with *Aeromonas veronii* biovar *veronii*. Thus,

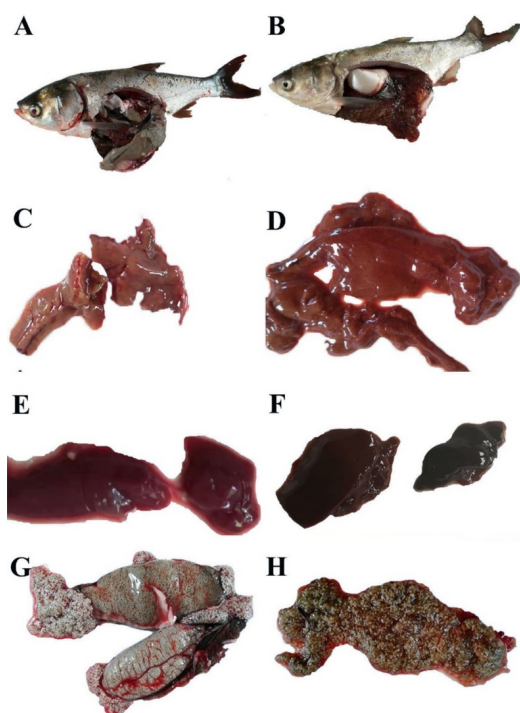


Figure 1. Healthy and diseased mature female silver carp with eggs infected by WSQ-1. A: Healthy fish; B: Diseased mature female with eggs; C, E, and G: the livers, spleen, and ovary from healthy fish, respectively; D, F, and H: the livers, spleen, and ovary from naturally infected fish, respectively. The livers and the spleen were darkened, the eggs changed from slight transparency to full transparency.

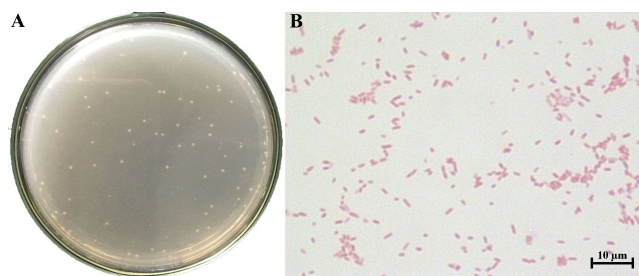


Figure 2. Colony morphology and gram staining of the pathogenic bacterium WSQ-1. A: The colony morphology showed grey-white, smooth surface, translucent, slight convex, and regular margin on the agar plate; B: The bacteria were red, rod-shaped, and non-endospore under microscopy (100×) post gram staining.

the pathogenic bacterium WSQ-1 was identified as *Aeromonas veronii* biovar *veronii* (Table 1).

MOLECULAR IDENTIFICATION OF THE ISOLATE

The taxonomic position of the isolated bacterium was based on sequence analysis of the 16S rDNA and gyrB. The phylogenetic tree indicated that WSQ-1 belonged to the *Aeromonas* and clustered into an independent branch

with *A. veronii* (MK584927.1) (Figure 3A) and *A. veronii* (LC311646.1) (Figure 3B). The NCBI blast results indicated that WSQ-1 shared 99.5% and 98.41% identity with the reference strain *A. veronii*.

EXPERIMENTAL INFECTION

The present results indicated that *A. veronii* caused 100% death of juvenile silver carp challenged with intraperitoneal injection and the pathogenic bacterium suspension bath. In addition, *A. veronii* resulted in 100% mortality of sexually matured female silver carp with eggs post intraperitoneal injection, but it caused no mortality of sexually matured male individuals (Table 2). The clinical signs of infected female fish or juvenile fish showed an enlarged abdomen with ascites accumulation in the abdominal cavity and hyperemia of the pectoral fins and abdomen. The male fish showed no clinical signs. The pathogen was re-isolated from the ascites and blood of the infected fish and identified as *A. veronii*. The bacteria were re-isolated from the female rather than the male fish.

IMMUNE-RELATED ENZYME ACTIVITY

The ACP, ALP, and CAT serum enzyme activities showed no significant difference between healthy mature females with eggs and healthy mature females without eggs ($p > 0.05$). Still, they decreased significantly in the diseased mature females with eggs ($p < 0.05$) (Table 3). In the artificial infection of silver carp, the serum ACP, ALP, and CAT levels dropped significantly on day 6 (Table 4).

HISTOPATHOLOGICAL FINDINGS

Severely damaged tissues could be detected in both of naturally and artificially infected female silver carp. The livers and spleen showed hemosiderin deposition and vacuolar degeneration (Figures 4 and 5). The ovary histopathological section revealed egg cells' disordered arrangement, wavy membrane deformation, and even broken egg cells (Figure 6).

DISCUSSION

Aeromonas species, opportunistic pathogens, are widespread in aquatic environments and can cause acute or chronic infection in aquatic animals.¹⁰ Among them, *Aeromonas hydrophila* is the pathogenic bacterium mainly studied, resulting in significant economic loss in aquaculture. While other isolates, such as *A. veronii*, *A. sobria*, *A. caviae*, and *A. punctate*, are also potential pathogens of freshwater fish in aquaculture.⁸ *Aeromonas* spp. infection usually causes similar clinical symptoms of diseased fish, such as hemorrhage, ascites, redness, and swelling of the anus. For example, *A. veronii* results in ulcerative syndrome, fin rot, and hemorrhagic septicemia in freshwater fish¹⁹; *A. hydrophila* causes hemorrhagic ascites, enlargement of the swim bladder, as well as intestinal and renal congestion of the moribund fish²⁰; *A. sobria* infection leads to the clinical symptoms of redness and swelling of the

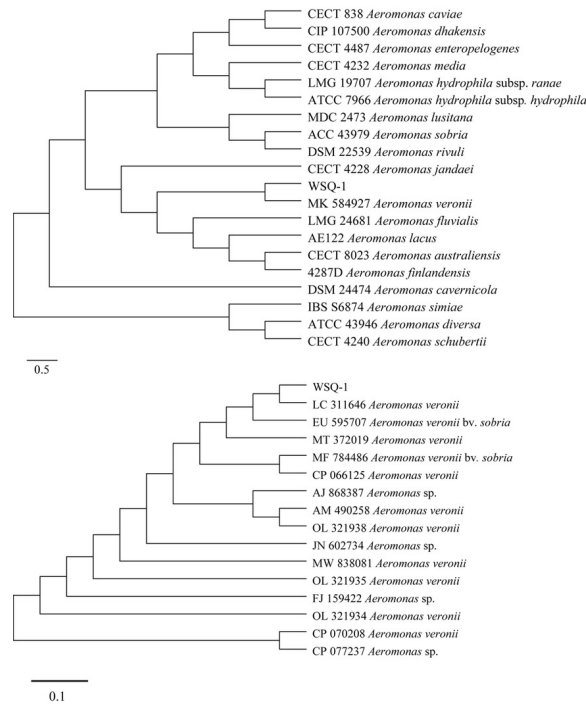


Figure 3. The phylogenetic tree based on the gene sequences of the 16S rRNA gene and *gyrB* gene of strain WSQ-1. A: 16S rRNA gene; B: *gyrB* gene; Phylogenetic tree was generated using the MEGA6.0 software with the neighbor-joining method. The number in the parentheses indicates the GenBank accession number of each species.

Table 2. Cumulative mortality of silver carp, including juveniles and matured females with eggs challenged with *Aeromonas veronii* during the period of 14 days.

Challenge route	Experiment groups	Number of fish (group × number)	Challenge dose	Mortality (mean ± SD)
Bath	Experimental juvenile group	3×20	3×10 ⁷ CFU/mL	100%±0.0
	Control group	3×20	0.65% saline water	0%±0.0
Injection	Experimental juvenile group	3×20	3×10 ⁶ CFU/mL	100%±0.0
	Control group	3×20	0.65% saline water	0%±0.0
	Experimental matured female group	3×15	3×10 ⁶ CFU/mL	100%±0.0
	Experimental matured male group	3×15	3×10 ⁶ CFU/mL	0%±0.0
	Matured female control group	3×15	0.65% saline water	0%±0.0
	Matured male control group	3×15	0.65% saline water	0%±0.0

anus, severe hemorrhage of the intestine in fish.⁴ Thus, it is difficult to distinguish the practically pathogenic *Aeromonas* species according to the clinical symptoms of diseased fish. The pathogenic bacterium isolated from diseased silver carp was identified as *Aeromonas veronii* biovar *veronii* with morphological and biochemical characteristics and 16S rDNA and *gyrB* gene DNA sequence analyses. The isolated strain was identified as **A. veronii**, which had been reported in silver carp. However, its pathogenicity in

the fish was still lacking.^{6,11} Therefore, the challenge experiments were performed to study the potential pathogenic ability of *A. veronii* in silver carp.

The healthy juvenile fish were challenged with *A. veronii* by intraperitoneal injection and bath with the strain suspension. The results indicated that silver carp showed 100% mortality in the infection group. The infected juvenile fish's clinical symptoms were similar to those of the naturally infected fish, such as abdominal distension and anal con-

Table 3. Serum enzyme activities of silver carps with or without natural infection by *Aeromonas veronii*.

Enzymes	Healthy females without eggs	Healthy mature females with eggs	Diseased mature females with eggs
ACP activity (U L ⁻¹)	115.20±10.36 ^a	120.17±3.11 ^a	61.70±4.42 ^b
ALP activity (U L ⁻¹)	45.05±0.10 ^a	42.89±0.47 ^a	17.66±0.12 ^b
CAT activity (U L ⁻¹)	18.93±2.23 ^a	13.53±0.15 ^a	4.23±0.03 ^b

Values are means ± SD of three replications. Values in the same row with different superscript letters differ significantly ($p < 0.05$).

Table 4. Serum enzyme activities of unmatured silver carps artificially infected with *Aeromonas veronii* at different times.

Enzymes	0 d	3 d	4 d	6 d
ACP activity (U L ⁻¹)	123.93±1.25 ^a	115.20±10.36 ^a	120.17±3.11 ^a	61.70±4.42 ^b
ALP activity (U L ⁻¹)	42.44±0.59 ^a	45.05±0.10 ^a	42.89±0.47 ^a	17.66±0.12 ^b
CAT activity (U L ⁻¹)	17.03±0.22 ^a	18.93±2.23 ^a	13.53±0.15 ^a	4.23±0.03 ^b

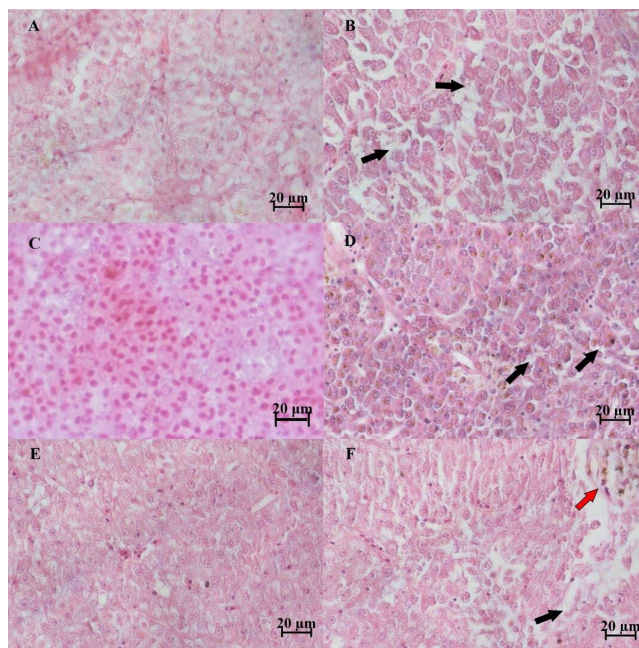


Figure 4. Histopathological analysis of the livers of *Hypophthalmichthys molitrix* infected by *Aeromonas veronii*. (A): healthy mature female silver carp with eggs; (B): naturally diseased mature female silver carp with eggs; (C): healthy mature female silver carp with eggs; (D): artificially infected mature female silver carp with eggs; (E): healthy juvenile silver carp; (F): artificially infected juvenile silver carp; Black arrow: vacuolar degeneration; Red arrow: hemosiderin deposition; Scale bar: 20 μm.

gestion, and a substantial amount of ascitic fluid filled the abdominal cavity. *A. veronii* could be re-isolated from the infected host, establishing Koch's postulates. These results demonstrated that the isolated strain was a pathogen.

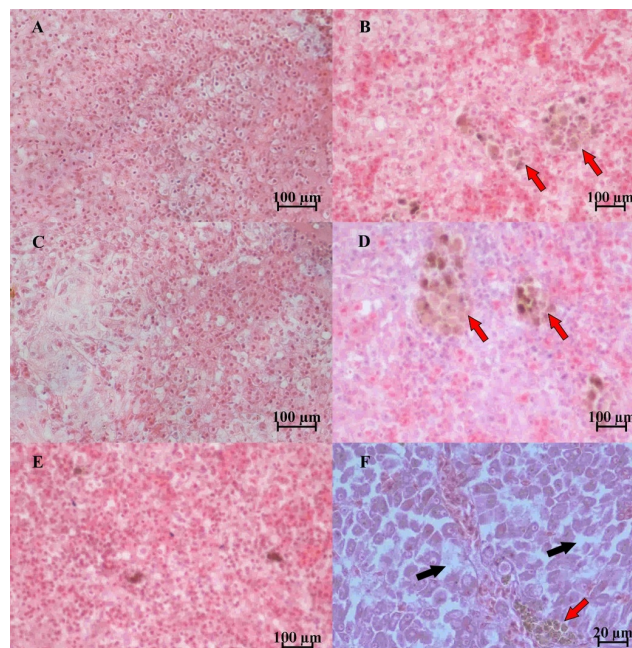


Figure 5. Histopathological analysis of the spleen of *Hypophthalmichthys molitrix* infected by *Aeromonas veronii*. (A): healthy mature female silver carp with eggs; (B): naturally diseased mature female silver carp with eggs; (C): healthy mature female silver carp with eggs; (D): artificially infected mature female silver carp with eggs; (E): healthy juvenile silver carp; (F): artificially infected juvenile silver carp; Black arrow: vacuolar degeneration; Red arrow: hemosiderin deposition; Scale bar: A-E is 100 μm, while F is 20 μm.

During the investigation of *A. veronii* in Yantian Reservoir, all diseased and dead silver carp were sexually mature female fish with eggs. All sexually mature male fish, however, showed no *A. veronii* infection. This phenomenon was

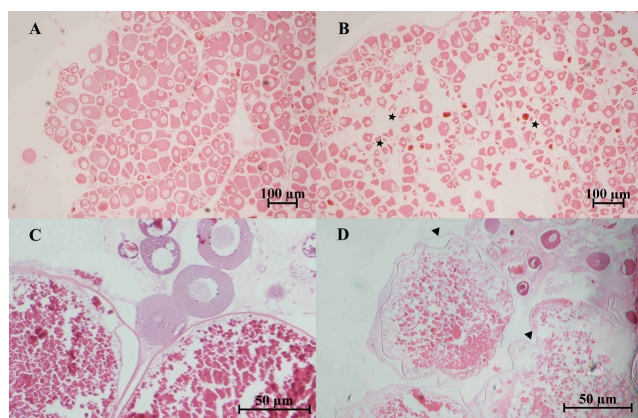


Figure 6. Histopathological analysis of the ovary of *Hypophthalmichthys molitrix* infected by *Aeromonas veronii*. (A): healthy mature female silver carp with eggs; (B): naturally diseased mature female silver carp with eggs; (C): healthy mature female silver carp with eggs; (D): artificially infected mature female silver carp; Penta star: broken egg fragments and disordered arrangement of egg cells; Triangle: wavy deformation of eggs membranes. Scale bar: A-B is 100 µm, while C-D is 50 µm.

demonstrated using the challenge experiments. The results displayed that *A. veronii* resulted in 100% mortality of matured female silver carp and no death of the male fish. These results indicated that sexually mature female silver carp with eggs were much more susceptible to *A. veronii* than sexually mature male silver carp.

In mammals, the sex hormones of females during pregnancy cause the female to be vulnerable to pathogens, and the clinical and experimental evidence showed that estrogens affect the immunity of female individuals.²¹ A similar phenomenon was also reported in rainbow trout.²² Previous studies demonstrated an increment in plasma estrogen levels during the pre-spawning period of females.²³ Elevating sex steroid levels made fish more vulnerable to pathogens during the spawning season, subsequently increasing fish mortality.²⁴ In the present study, there was no sex difference in the mortality of juvenile fish. Only sexually mature female silver carp with eggs were infected by *A. veronii*, indicating that the sex steroid might cause the female fish to be more vulnerable to *A. veronii*. Further study should investigate the exact mechanism of the present phenomenon.

Furthermore, this study is the first report on the histological changes in silver carp infected by *A. veronii*. *Aeromonas* infections could result in various histological changes, such as cell necrosis, cell punctate vacuolation, cell swelling, necrosis, intravascular congestion, hepatocellular steatosis, and hemorrhage.^{25,26} The pathological changes of infected silver carp were characterized by septicemia and irreversible lesions to the spleen, livers, and ovary. Bacterial cells with high density caused tissue vacuolation (Gaafar et al., 2021). Hemosiderin accumulation might result from blood cell destruction, iron release from hemoglobin, and increasing hemosiderin.²⁷ Cell membrane

deformation and cells (eggs) broken in the ovary were also observed in other organs of fish infected by *Aeromonas* spp.^{8,26,28,29}

The levels of various active enzymes in blood closely relate to fish's health status and anti-infection immunity.³⁰ ACP and AKP are the marker enzymes of macrophages, which are involved in the transfer and metabolism of phosphate groups. The level of ACP and AKP reflects the activation degree of macrophages. The reactive oxygen species (ROS) play an essential role in the anti-infection of invading pathogens, but the excessive production of ROS leads to oxidative stress damage to the body. CAT can protect the cells from the grievous effects of oxidative stress to avoid the adverse effects of ROS.³¹ A previous study indicated that AKP and CAT could be used as biochemical indexes to evaluate the resistance of tilapia to *Streptococcus agalactiae* disease. The lower expression levels of ALP and CAT indicated lower resistance after infection with *Streptococcus agalactiae*.³² Moreover, the increased CAT activity in the livers and ACP and AKP activities in the plasma enhanced the antioxidant capability, innate immunity, and disease resistance of *Megalobrama amblycephala*.⁶ The present study showed that the ALP, ACP, and CAT activities of *H. molitrix* kept a normal fluctuation range in healthy female fish but were decreased significantly in diseased matured female individuals with eggs. This suggested that the resistance was reduced significantly in the fish infected by the pathogenic bacterium. This was an essential factor to result in the death of diseased fish.

In conclusion, the present study demonstrated that the isolated pathogen WSQ-1 was identified as *A. veronii* biovar *veronii*, which was highly pathogenic to silver carp and caused the mass death of sexually mature female fish with eggs. The findings in this study will provide new insights into the diagnosis and the sexual susceptibility caused by *A. veronii* and help understand the pathogenesis of *Aeromonas* spp. in fish.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China [32173013]; the Shellfish and Large Algae Industry Innovation Team Project of Guangdong Province [2022KJ146]; the Key-Area Research and Development Program of Guangdong Province [2019B020217001].

CONFLICT OF INTEREST

The authors have no competing interests to declare.

ETHICAL STATEMENT

The usage of fish was approved by the Jinan University Laboratory Animal Ethics Committee [20200511-11]. The authors followed the Guide for the Care and Use of Laboratory Animals and the National Standards of Laboratory Animals of the People's Republic of China.

Submitted: December 26, 2022 CST. Accepted: March 21, 2023

CST. Published: May 03, 2023 CST.



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REFERENCES

1. Tang Y, Zhao L, Cheng Y, Yang Y, Sun Y, Liu Q. Control of cyanobacterial blooms in different polyculture patterns of filter feeders and effects of these patterns on water quality and microbial community in aquacultural ponds. *Aquaculture*. 2021;542:736913. doi:[10.1016/j.aquaculture.2021.736913](https://doi.org/10.1016/j.aquaculture.2021.736913)
2. Pei C, Song H, Zhu L, et al. Identification of *Aeromonas veronii* isolated from largemouth bass *Micropterus salmoides* and histopathological analysis. *Aquaculture*. 2021;540:736707. doi:[10.1016/j.aquaculture.2021.736707](https://doi.org/10.1016/j.aquaculture.2021.736707)
3. Behera BK, Bera AK, Paria P, et al. Identification and pathogenicity of *Plesiomonas shigelloides* in silver carp. *Aquaculture*. 2018;493:314-318. doi:[10.1016/j.aquaculture.2018.04.063](https://doi.org/10.1016/j.aquaculture.2018.04.063)
4. Dar GH, Dar SA, Kamili AN, Chishti MZ, Ahmad F. Detection and characterization of potentially pathogenic *Aeromonas sobria* isolated from fish *Hypophthalmichthys molitrix* (Cypriniformes: Cyprinidae). *Microbial Pathogenesis*. 2016;91:136-140. doi:[10.1016/j.micpath.2015.10.017](https://doi.org/10.1016/j.micpath.2015.10.017)
5. Beaz-Hidalgo R, Figueras MJ. *Aeromonas* spp. whole genomes and virulence factors implicated in fish disease. *J Fish Dis*. 2013;36(4):371-388. doi:[10.1111/jfd.12025](https://doi.org/10.1111/jfd.12025)
6. Zhang DX, Kang YH, Zhan S, et al. Effect of *Bacillus velezensis* on *Aeromonas veronii*-induced intestinal mucosal barrier function damage and inflammation in crucian carp (*Carassius auratus*). *Front Microbiol*. 2019;10:02663. doi:[10.3389/fmicb.2019.02663](https://doi.org/10.3389/fmicb.2019.02663). PMID:31798571
7. Hou XC, Li XX, Li AJ, Sun JF, Sun YJ. Characterization and pathology of *Aeromonas veronii* Biovar Sobria from Diseased Sheatfish *Silurus glanis* in China. *Israeli Journal of Aquaculture - Bamidgeh*. 2019;71. doi:[10.46989/001c.20989](https://doi.org/10.46989/001c.20989)
8. Dong HT, Techatanakitarnan C, Jindakittikul P, et al. *Aeromonas jandaei* and *Aeromonas veronii* caused disease and mortality in Nile tilapia, *Oreochromis niloticus* (L.). *J Fish Dis*. 2017;40(10):1395-1403. doi:[10.1111/jfd.12617](https://doi.org/10.1111/jfd.12617)
9. Raj NS, Swaminathan TR, Dharmaratnam A, Raja SA, Ramraj D, Lal KK. *Aeromonas veronii* caused bilateral exophthalmia and mass mortality in cultured Nile tilapia, *Oreochromis niloticus* (L.) in India. *Aquaculture*. 2019;512:734278. doi:[10.1016/j.aquaculture.2019.734278](https://doi.org/10.1016/j.aquaculture.2019.734278)
10. Hoai TD, Trinh ThiT, Nguyen VT, Nguyen Thi Huong G, Kim Van V. *Aeromonas veronii* caused disease and mortality in channel catfish in Vietnam. *Aquaculture*. 2019;513:734425. doi:[10.1016/j.aquaculture.2019.734425](https://doi.org/10.1016/j.aquaculture.2019.734425)
11. Li GQ, Wang Y, Li JN, Shao S. Identification of pathogens in fulminant hemorrhagic disease of cultured fish in Anhui. *Zhongguo Weishengtaxixue Zazhi/Chinese Journal of Microecology*. 2011;23(6):496-499. doi:[10.13381/j.cnki.cjm.2011.06.009](https://doi.org/10.13381/j.cnki.cjm.2011.06.009)
12. Fisher TW, Garczynski SF. Isolation, culture, preservation, and identification of entomopathogenic bacteria of the Bacilli. In: Lacey LA, ed. *Manual of Techniques in Invertebrate Pathology*. Academic Press; 2012:75-98. doi:[10.1016/b978-0-12-386899-2.00003-8](https://doi.org/10.1016/b978-0-12-386899-2.00003-8)
13. Dong XZ, Cai MY. *Manual of Identification of Common Bacterial Systems*. Science Press. ISBN; 2001.
14. Buchanan RE, Gibbons NE. *Bergey's Bacteria Identification Manual*. 8th edn. Science Press; 1984.
15. Jensen S, Bergh Ø, Enger Ø, Hjeltne B. Use of PCR-RFLP for genotyping 16S rRNA and characterizing bacteria cultured from halibut fry. *Can J Microbiol*. 2002;48(5):379-386. doi:[10.1139/w02-033](https://doi.org/10.1139/w02-033)
16. Yáñez MA, Catalán V, Apráiz D, Figueras MJ, Martínez-Murcia AJ. Phylogenetic analysis of members of the genus *Aeromonas* based on gyrB gene sequences. *International Journal of Systematic and Evolutionary Microbiology*. 2003;53(3):875-883. doi:[10.1099/ijs.0.02443-0](https://doi.org/10.1099/ijs.0.02443-0)
17. Y. S, X. H, A. L, et al. Isolation and characterization of *Aeromonas veronii* from ornamental fish species in China. *Israeli Journal of Aquaculture-Bamidgeh*. 2017;69:1446. doi:[10.46989/001c.21023Tang](https://doi.org/10.46989/001c.21023Tang)
18. Han Z, Sun J, Jiang B, et al. Concurrent infections of *Aeromonas veronii* and *Vibrio cholerae* in koi carp (*Cyprinus carpio* var. koi). *Aquaculture*. 2021;535:736395. doi:[10.1016/j.aquaculture.2021.736395](https://doi.org/10.1016/j.aquaculture.2021.736395)

19. Mallik SK, Joshi N, Shahi N, et al. Characterization and pathogenicity of *Aeromonas veronii* associated with mortality in cage farmed grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844) from the Central Himalayan region of India. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*. 2020;113(12):2063-2076. doi:[10.1007/s10482-020-01478-3](https://doi.org/10.1007/s10482-020-01478-3)
20. Di J, Zhang S, Huang J, et al. Isolation and identification of pathogens causing haemorrhagic septicemia in cultured Chinese sturgeon (*Acipenser sinensis*). *Aquac Res*. 2018;49(11):3624-3633. doi:[10.1111/are.13830](https://doi.org/10.1111/are.13830)
21. Yang C, Yu W, Bi Y, et al. Increased oestradiol in hepatitis E virus-infected pregnant women promotes viral replication. *J Viral Hepat*. 2018;25(6):742-751. doi:[10.1111/jvh.12865](https://doi.org/10.1111/jvh.12865)
22. Currie JLM, Woo PTK. Susceptibility of sexually mature rainbow trout, *Oncorhynchus mykiss* to experimental cryptobiosis caused by *Cryptobia salmositica*. *Parasitol Res*. 2007;101(4):1057-1067. doi:[10.1007/s00436-007-0586-8](https://doi.org/10.1007/s00436-007-0586-8)
23. Jerez S, Rodríguez C, Cejas JR, Bolaños A, Lorenzo A. Lipid dynamics and plasma level changes of 17 β -estradiol and testosterone during the spawning season of gilthead seabream (*Sparus aurata*) females of different ages. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*. 2006;143(2):180-189. doi:[10.1016/j.cbpb.2005.11.002](https://doi.org/10.1016/j.cbpb.2005.11.002)
24. Campbell JH, Dixon B, Whitehouse LM. The intersection of stress, sex and immunity in fishes. *Immunogenetics*. 2021;73(1):111-129. doi:[10.1007/s00251-020-01194-2](https://doi.org/10.1007/s00251-020-01194-2)
25. Chen F, Sun J, Han Z, et al. Isolation, Identification and Characteristics of *Aeromonas veronii* From Diseased Crucian Carp (*Carassius auratus gibelio*). *Front Microbiol*. 2019;10(2742). doi:[10.3389/fmicb.2019.02742](https://doi.org/10.3389/fmicb.2019.02742). PMID:32038507
26. Wang B, Mao C, Feng J, et al. A first report of *Aeromonas veronii* infection of the sea bass, *Lateolabrax maculatus* in China. *Front Vet Sci*. 2021;7:600587. doi:[10.3389/fvets.2020.600587](https://doi.org/10.3389/fvets.2020.600587). PMID:33553279
27. Qamar A, Waheed J, Zhang Q, Namula Z, Chen Z, Chen JJ. Immunotoxicological effects of dioxin-like polychlorinated biphenyls extracted from Zhanjiang Bay sediments in zebrafish. *Environ Monit Assess*. 2020;192(7):1-10. doi:[10.1007/s10661-020-08427-7](https://doi.org/10.1007/s10661-020-08427-7)
28. Abd El Latif AM, Elabd H, Amin A, Eldeen AIN, Shaheen AA. High mortalities caused by *Aeromonas veronii*: identification, pathogenicity, and histopathological studies in *Oreochromis niloticus*. *Aquacult Int*. 2019;27(6):1725-1737. doi:[10.1007/s10499-019-00429-8](https://doi.org/10.1007/s10499-019-00429-8)
29. Kaur A, Holeyappa SA, Bansal N, Kaur VI, Tyagi A. Ameliorative effect of turmeric supplementation in feed of *Labeo rohita* (Linn.) challenged with pathogenic *Aeromonas veronii*. *Aquacult Int*. 2020;28(3):1169-1182. doi:[10.1007/s10499-020-00518-z](https://doi.org/10.1007/s10499-020-00518-z)
30. Cai XH, Liu XJ, Peng YH, Wang Z, Huang G. Serum enzyme activity of *Mugil cephalus* Linnaeus artificially infected by *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio harveyi*. *Journal of Southern Agriculture*. 2014;45(1):137-142. doi:[10.3969/j](https://doi.org/10.3969/j)
31. Elvitigala DAS, Premachandra HKA, Whang I, et al. Marine teleost ortholog of catalase from rock bream (*Oplegnathus fasciatus*): Molecular perspectives from genomic organization to enzymatic behavior with respect to its potent antioxidant properties. *Fish and Shellfish Immunology*. 2013;35(4):1086-1096. doi:[10.1016/j.fsi.2013.07.13](https://doi.org/10.1016/j.fsi.2013.07.13)
32. Ao Q, Luo Y, Lu M, Zhu J. Effects of *Streptococcus agalactiae* infection on blood and hepatopancreatic tissue biochemical indices in different species of tilapia. *Progress in Fishery Sciences*. 2020;41(4):167-173. doi:[10.19663/j.issn2095-9869.20190610001](https://doi.org/10.19663/j.issn2095-9869.20190610001)