Isolation of *Aeromonas hydrophila* and Evaluation of Its Pathological Effects on Koi Fish (*Cyprinus carpio*)

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ABSTRACT

In the summer of 2018, this study was performed on 50 Koi fish transferred to Razef Research Complex. In this study, after observing macroscopic symptoms in fish, including lethargy and immobility, imbalance, darkening of the skin, skin wounds, petechiae in the abdomen, opacity of the eye and anophthalmia, anorexia, and sitting on the bottom of the aquarium, sampling was performed to diagnose the pathogens and examine the pathological changes. According to microbial tests on skin samples and internal organs, *Aeromonas hydrophila* was reported, and septicemia in 48% of the studied fish. In necropsy specimens, the organs were pale, the liver was enlarged, and petechiae were observed on the surface of the liver. The microbial contamination also led to considerable tissue damages, including the destruction of gill filaments, nuclear pycnosis in the liver, and the infiltration of inflammatory cells into the heads of all fish. Considering the significant losses due to the pathogenicity of *Aeromonas hydrophila* in different fish species and the importance of Koi fish, observing quarantine and hygienic principles to prevent sepsis was very important. Early diagnosis and use of appropriate treatment protocols for this disease are recommended.

Keywords: *Aeromonas hydrophila*, Koi fish, Pathology

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Introduction

*Aeromonas hydrophila* is a Gram-negative bacillus found in aquatic environments, drinking water, sewage, and food (1). This bacterium can be isolated from estuaries, seawater, and freshwater (1-3). Freshwater environments, especially those rich in mineral materials, are the natural habitat of *A. hydrophila*; however, recent evidence suggests that this organism can also be found as a part of intestinal flora in fish (4).

The bacterium is known to be a pathogenic microorganism in fish, causing a wide range of diseases, including motile *Aeromonas* septicaemia in carp, tilapia, catfish, and salmon, the red spot disease in bass and carp, and wound-associated infections (e.g., Epizootic ulcerative syndrome) in catfish, cod, and carp (5).
**Aeromonas septicemia** usually occurs when fish are under stressful conditions such as high-temperature and poor-quality water, hypoxia, parasitic infections, high density, manipulation, and transport (6, 7); it may cause chronic inflammation for several weeks, gradually increasing the mortality rate. Factors like mortality associated with this bacterial infection, its management, and its treatment costs, highly infect the fish farming industry (8, 9). Also, *A. hydrophila* can cause localized tissue damage in the host, including tissue degeneration and inflammation (10, 11).

Another important point about this bacterium is that in addition to fish, it can lead to zoonotic disease in amphibians and reptiles, which can also be pathogenic for mammals, including humans (2, 8, 11, 12). In fact, it is known as an emerging pathogen for humans (1). Consequently, the presence of drug-resistant *A. hydrophilic* in aquariums becomes important regarding multiple public health aspects (13).

Koi (fancy carp *Cyprinus carpio* L. var. Koi) is an expensive ornamental freshwater fish that is often infected by infectious diseases, such as *A. hydrophila*, eventually leading to their death (14), raising environmental concerns (15) and zoonotic hazards (13). Based on the above and the importance of *A. hydrophila* infection in Koi fish, which leads to high mortality in fish, in the present study, we reported the isolation of *A. hydrophila* bacteria from the Koi fish transferred from farm ponds to the aquariums of a research center. The bacteria were identified using microbial culture methods, and pathological changes in the gills, liver, and kidneys were assessed to determine the pathological effects of this infection on this valuable ornamental fish.

**Case Report**

In the summer of 2017, 50 pieces of Koi fish (with an average weight of 25 ± 2 grams and a mean length of 24 ± 3 cm) were transferred from one of the concrete ponds of the Koi Fish Farming and Breeding Center in Shahriar city to the Razef Research Center, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran. Then, the fish were randomly divided into five tanks (170 L) (10 fish per tank). After a few days after the transfer, all the fish developed similar macroscopic symptoms, including darkening of the skin, abdominal petechiae, skin lesions, corneal opacity and anophthalmia, lethargy, immobility, imbalance, anorexia, and seating on the floor of the aquarium. After observing these clinical signs, we took the necessary measures (including autopsy for obtaining samples from the skin and internal organs for pathological and microbial examination). As this study was a case report, seven fish presenting these clinical signs were randomly caught from each tank (a total of 35 fish). After anesthesia by clove powder, sampling was performed to obtain specimens from the skin and internal organs and identify the pathogenic agent. All the histological and microbial examinations were conducted under completely sterile conditions.

Liver, gill, and kidney samples were initially washed with the physiological serum and then completely homogenized using a tissue homogenizer. Afterward, the specimens were cultured on Brain Heart Infusion Agar (BHI Agar) medium and incubated at 28°C for two days. After this step, the grown colonies were re-cultured on BHI Agar medium; the motile, oxidase-positive, and glucose fermenter bacteria suspected as *Aeromonas* spp., underwent further biochemical and physiological analyses. The confirmatory tests at this step included: *H₂S* production, urease test, citrate reduction, indole production, nitrate reduction, growth on MacConkey medium, and the fermentation of carbohydrates (glucose, inositol, sucrose, sorbitol, arabinose, and mannitol) (16).

For histological examinations, gill, liver and kidney samples were initially fixed in formalin buffer 10% for 48 hours. Then the fixed samples were placed in paraffin after dewatering and clearing with xylene. Tissue sections with a diameter of 5 μm were prepared using a microtome device and stained by hematoxylin-Eosin (H&E). The prepared histological slides were examined by optical microscope (Olympus BX51; Olympus, Tokyo, Japan) to evaluate pathological features (17).

The results of the microbial tests showed that 48% of septicemia cases (24 samples) were caused by *A. hydrophila* (Figure 1). In autopsy specimens, in addition to petechiae and skin darkening and ulcerative lesions, the internal organs were pale, and the liver was enlarged with observable petechiae on its surface (Figure 2). In the microscopic examination of the gills, we observed collapsed and swollen secondary lamellae, the swelling, and shortening of lamellae and their complete destruction, as well as the edema of gill filaments. In addition, the epithelial detachment was observed along with the detachment of the lamellar epithelium outer layer. Congestion was evident in the primary and secondary lamellae (Figure 3A and B). In the kidney, the infiltration of inflammatory cells (neutrophils) was evident in vessels and renal parenchyma (Figure 3C). The most prominent changes in the liver were irregular hepatic fibers, nuclear pyknosis, and cytoplasmic vacuolation. In addition, varying degrees of pancreatic cell degeneration were evident in some parts (Figure 3D).
Figure 1. The identification of *Aeromonas hydrophila* bacterium in the studied samples

Figure 2. The darkening of the skin (A), ulcerative skin lesions (B), petechiae on the skin surface (the white arrow) (C), the enlargement of the liver, pale internal organs, and petechiae on the liver surface (the black arrow) (D).
### Discussion

The present study identified *A. hydrophila* bacteria in Koi fish, which is known as one of the most critical lethal pathogens in various carp fish (9). Aeromonas bacteria are a part of the water’s natural flora, as well as fish. Still, they can become opportunistic pathogens causing super-acute epidemics (e.g., *Aeromonas hydrophila* pathotype) under certain conditions, including environmental stresses. This event can cause considerable casualties in the aquaculture industry (18, 19), often occurring within a few hours of the disease onset (20). In the present study, it can be said that transport stress and reduced immune function of fish could have been contributors to the outbreak. This is in line with the notion that Aeromonas bacteria are opportunistic pathogens that can only cause disease in immunocompromised fish (21). In Iran, several studies have investigated *A. hydrophila* infection in various fish, including goldfish (22), angelfish (23), and common carp (24).

In the autopsy examination of the obtained samples, wounds and bleeding spots were evident on the body’s surface, as well as the abdomen and internal organs. These findings were similar to the symptoms observed in the study of Stratev et al. in 2015 (11). Many studies have shown that chronic infection with *A. hydrophila* is associated with ulcerative skin lesions and local bleeding and inflammation (10).

In our study, histological examinations showed multiple tissue damages in the gills, liver, and kidneys. These included necrosis, inflammation, and hemorrhage in the gills, inflammation in the kidney, necrosis, and nuclear pyknosis in hepatocytes. In several studies examining the histological features of *A. hydrophila* infection, similar findings have been reported (11, 25-29). In this regard, degenerative histological changes due to *A. hydrophila* infection have been reported in organs such as the gills, kidneys, and liver (10). Also, in line with the present study in which tissue damages in the gills were prominent, Al Yahya et al. (2018) (10) and Cookyaei et al. (2012) (30) also reported necrosis in gill blades and hemorrhage in gills due to *A. hydrophila* infection. One of the significant issues in the histopathological features of *A. hydrophila* infection is that most tissue damage due to this bacterium has been observed in the liver and kidneys (11). In fact, the liver can be considered the target organ of *A. hydrophila* (10). In the histological evaluation of the
kidney, similar to the study of Sellegounder et al. (2018), infiltration of inflammatory cells was observed (31). In a study considering Nile tilapia, bleeding foci were observed in the kidney due to a bacterial infection (32). In the examination of the liver and pancreas tissues, we noticed vacuolation and nuclear pyknosis, as well as the necrosis of hepatocytes and pancreatic cells, which were similar to the results of other studies (10, 31, 33). In a study, Afifi et al. (2000) attributed these changes to the toxins and extracellular compounds released by A. hydrophila, including hemolysin, proteases, and elastase, in the liver, leading to severe tissue damage and necrosis (34).

**Conclusion**

The results of the present study showed that freshwater fish are always prone to Aeromonas spp. infections. According to our research, pathological changes in the gills, liver, and kidneys can be suitable biomarkers for assessing the pathogenicity of A. hydrophila in Koi fish. In addition, Aeromonas species can be a threat to public health, especially for those who are in contact with sick fish. Therefore, the proper management of water quality and food health and the quarantine of new fish can play a significant role in preventing and controlling A. hydrophila infection in fish and avoiding its transmission to humans.

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**Conflict of Interest** The authors declared no conflicts of interest.
جداوی آگریموناس هیدروفیلا و بررسی اثرات آسیب‌شناسی ناشی از این پاتوژن (Cyprinus carpio) در ماهی کوی (Majallah-i mikonbod-shaasai-e iran Majallah-i mikonbod-shaasai-e iran)

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چکیده

این مطالعه در کلیت سال 1399 بر روی ۵۰ تیکت ماهی کوی سطحی شده به مجموعه تحفیظی راز، صورت یافت. در این سری پس از مشاهده علائم میکروپکست و اعمال مبارزه در این میکروپکس، روت‌های پوستی، پنیشه در ناحیه کنگری، کروپ ایجایی، یا ایجاد ایجاد ته افزایش فعالیت میکروپکس در ماهی‌ها، اعمال میکروپکس نسبت به واحدهای زیست‌شناسی، وارده و اندازه‌های طبیعی فرموله‌های میکروپکس در سه گروه ماهی کوی. با توجه به کاهش تعداد لاش‌ها و تغییرات الیاف لیمفا، ایده‌آل‌ترین بیماری یک‌پوش‌گیری در بازی‌های ماهی کوی، مطالعه اصلی قناعتی بود. با میکروپکس‌های موجود در ته‌آمیختی، به‌سیاوش سری‌طلب ایجاد شد. در کنار این آزمایشات، میکروپکس در هم‌سری به شکستن چربی از ماهی‌ها که به‌صورت استحکام‌دار و به‌طور کامل گردید، در این ماهی‌ها، باعث از شکستن لایه‌های چربی و مصرفینی‌های شیونی شد. آزمایشات مثبت‌اند. با توجه به این نتایج، این مقاله به‌عنوان اصوله‌برداری، به‌دست آمده در ماهی‌ها بوده و تشخیص به‌ویژه دریافت و پیش‌بینی نمایه‌شناختی، با کندی معنادار است.

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مقوده

کیور، سالنژ، مایاه ماهی و سالمان، بیماری‌های کوی در قرمز و کبیر و غنی‌گونه‌های هماهنگ، مانند سردم‌پوشی یا پروتویلگی در گربه ماهی، یکی از مشاوره‌های اصلی است. در این پژوهش، با استفاده از چهار نوع جهاده شیوا علی‌زاده، آرمان قربان‌زاده، جهاد پیروان، دانشکده دانشگاه آزاد اسلامی، رودهن، ایران

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نتهای قابل توجه دیگر در رابطه با این بیانکی است که این یافته نشان می‌دهد که تاکنون این بیماری در هر یک از استان‌های کشور ثبت نشده است. از این رو، برای اطمینان از ممکن بوده این بیماری در دیگر استان‌ها نیز بوجود آید، لازم به اطلاع از هیئت‌های زبان‌نویسی و کمیته‌های بهداشتی است. به این ترتیب، نتایج این کار، به عنوان یک پژوهش اسپرسی در بخش‌های مختلف بیماری‌ها و بستری‌های سلامتی مطرح می‌گردد.
سیده شیوا علیزاده و همکاران | آنتی‌موناس هیدرفیلا در ماهی گویی

بر این، در جات مختلف در نیزهای پانکراس جهت در چنین بخش مشهود بود (شکل D.2)。

شکل 1: شناسایی باکتری آنتی‌موناس هیدرفیلا در نمونه‌های مورد مطالعه
شکل ۲. تیره شدن سطح بدن (A)، ضایعات اولسانتوی پوست (B)، فلش سفید: پشتی روی سطح پوست (C)، بزرگ شدن کبد، رنگ برندگی اندازه‌ها، فلش سیاه: پشتی سطح کبد (D).

شکل ۳. آسیب ناشی نمونه‌های ایشی، کلیه و کبد: NO: نیتروژنی دی‌اکسید، Ne: نیتروژنی اکسید، Ca: نیکلک. 

A: صورت غیرکناری، B: صورت ناراحتی، C: پرندگی، D: پرندگی. 

A: آب، B: آب، C: آب، D: آب. 

A: آب، B: آب، C: آب، D: آب. 

A: آب، B: آب، C: آب، D: آب. 

A: آب، B: آب، C: آب، D: آب.
نتیجه تحقیق حاضر به شناسایی باکتری آتروموناس هیدروفیلا در انسان‌های کوی کناری جمعیتی که این باکتری به عنوان یکی از عوامل بیماری‌زا یا درمانی مبتنی بر دندان از کوی‌های ماهیان شناخته شده است (9). آتروموناس یا قلو طبیعی آرزآ و دندان‌های هستند اما در اثر همبستگی داخلی در جمله اکسترس ماهی‌ای می‌توانید به بیانی‌های فرتیلیتی نشان دهنده که این می‌تواند با باین‌های فرتیلیتی نشان دهنده که این آگاهانه تأثیر سیاسی در صنعت آب‌پروری گردیده (18, 19) که اغلب طی جنگ‌سایت از روز بیماری مجری به موجب و می‌شود (20)، به این ترتیب در نسبت به خطر می‌باشد. در حال حاضر که در این رابطه در ایران مطالعات مندرج در بررسی آتروموناس هیدروفیلا در ماهیان مختلف از جمله ماهی قرمز (22، فشرده ماهی (23) و کیور معمول (24) به‌پرداخته.

در بررسی کلیدگذاری نمونه‌های اخیر شده شاهد زخم و نقش خونریزی روزی سطح بدن، به شکستگی ماهی و اندام‌های داخلی و بودیم که شناسایی باLEM مشاهده شده در بررسی Strateev و همکاران در سال 2015 بوده (11) و مطالعات بسیاری نشان داده که غوطه زنده ماهی‌های آتروموناس هیدروفیلا منجر به ضایعات اولارزیوی و بیماری‌های مختلف از جمله ماهی قرمز (22، فشرده ماهی (23) و کیور معمول (24) به‌پرداخته.

نتایج این مطالعه نشان می‌دهد ماهیان آب شیرین همیشه در معرض ابتلا به غوطه زنده ماهی‌های دانش‌های بین‌آب و کیلوی می‌تواند بیمار‌پردازی رئیس و برنامه‌ریزی در ماهی‌های آتروموناس هیدروفیلا در ماهیان کوی بناشد. علاوه بر این گونه‌های آتروموناس می‌تواند تغییرات در سیستم ایمنی ماهی‌های بیماره‌های به حساب باید. فرد سازمانی که قبلاً توجهی به پیشگیری و گسترش غوطه زنده از آتروموناس هیدروفیلا در ماهیان داشته و در نتیجه آن به انسان جلوگیری کند.

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تعارض در منابع

حقوق تعارض در منابع از سوی نویسندگان اعلام شده است.

منابع مالی

وجود ندارد.

(2018) Siad SamCookiyaee (2018) و SamCookiyaee (2018) و همکاران (2018) و
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