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**Research Article** 

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# Clinical and Pathological Characteristics of Aeromonosis (*Aeromonas hydrophila*) in Sturgeon Fish under Conditions of Recirculating Aquaculture Systems and Study of the Therapeutic Effect of Bitter Wormwood (*Artemisia absinthium L.*)

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### ABSTRACT

The aim of our study was to determine clinical and pathological characteristics of aeromonosis in sturgeon fish in the conditions of recirculating aquaculture systems and to study the therapeutic effect of bitter wormwood. Fish with clinical signs of the disease were studied, external lesions, behaviour, and reactions were examined, pathological and anatomical analysis was performed, and the degree of lesions was assessed. To investigate the therapeutic properties, 5 experimental groups (n=20) were created. For group 1, an aqueous infusion of wormwood herb was used in the form of therapeutic baths. For group 2, an aqueous wormwood herb infusion was administered orally in therapeutic baths. Group 3 was a complex preparation. For group 4, antibiotics were administered. For group 5, table salt was used. It has been established that at recirculating aquaculture systems, aeromonosis runs in acute and subacute form with clinical signs of bacterial-hemorrhagic septicemia. More than 70% of morbidity in acute form was found mainly in fry and yearlings. In the subacute form, fish over a year old and older, less frequently fried and yearlings were predominantly affected. The study of therapeutic properties showed that a low mortality rate was observed in the 1st and 3rd groups. No fish mortality was observed in group 2. The survival rate of fish in the 1st, 2nd and 3rd groups was higher (from 90%) than in the 4th (75%) and 5th (60%). No side effects, irritation, or recurrence were observed with wormwood infusion. The results of studies may be helpful for researchers and specialists in fish farming and aquaculture.

Key words: Aeromonas hydrophila, sturgeons, pathology, clinical sign, Artemisia absinthium L.

### INTRODUCTION

The family of sturgeons (*Acipenseridae*) is one of the most endangered fish of commercial value. The current catastrophic decline in the number and commercial stocks of sturgeons is a worldwide trend, leading to the almost universal cessation of sturgeon fishing. The intensive reduction of wild sturgeon populations has led to the development of sturgeon aquaculture as a means of artificial breeding for reintroduction and obtaining sturgeon products (Grozea et al. 2023).

In the West Kazakhstan region, various species of sturgeons are grown in the sturgeon fish farm «Educational and Scientific Complex of Experimental Aquaculture Production» LLP in recirculating aquaculture systems (RAS) (Absatirov et al. 2019). Even under controlled housing conditions, violation of any of the essential indicators of RAS becomes the cause of mass diseases and death of fish (Zhang et al. 2022).

Bacterial diseases are the leading cause of fish mortality. The most common bacterial agents associated with diseases of fish reared in RAS are Aeromonads and Gram-negative bacilli. Currently, the genus *Aeromonas* is considered not only as an essential pathogen of fish and other cold-blooded species but also as an etiologic agent responsible for various infectious complications in humans (Dharmaratnam et al. 2018; Radosavljević et al. 2019).

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Aeromonas hydrophila is an important zoonotic pathogen that affects a wide range of organisms. In humans, infection by A. hydrophila is also a serious problem, especially for immuno-compromised individuals. Transmission of the bacterium is possible through contact with contaminated water, consumption of infected food, or ingestion of the microorganism in wounds. Understanding the zoonotic potential of Aeromonas hydrophila is important for medicine, veterinary medicine, and ecology. Only an integrated approach, including monitoring, prevention, and timely treatment of infections, will minimize the risks associated with this microorganism (Ahmed et al. 2018; Pal 2018; Ziarati et al. 2022).

Treatment methods using environmentally safe substances with therapeutic and prophylactic activity available for widespread use are preferred for preventing and treating fish diseases in confined conditions. Preparations from raw plant materials have several advantages, particularly due to their absence of side effects and low toxicity to the organism, though they can have a complex impact (Adel et al. 2021; Nurzhanova et al. 2021; Li et al. 2023).

Wormwood (Artemisia absinthium L.) is an herbaceous perennial of the inflorescence family. It contains bitter glycosides, essential oil, flavonoids, phytoncides, tannins, lignans, organic acids (succinic, malic), carotene and ascorbic acid (Bordean et al. 2021; Kabdulkarimova et al. 2022; Bordean et al. 2023). The variety of biologically active substances in wormwood causes a wide range of therapeutic effects (Trifan et al. 2022; Ismael et al. 2024). Bitter lactones absintine and anabsintine cause anti-inflammatory and antimicrobial properties of the herb. Unsaturated hydrocarbon capillin has fungicidal properties and is effective in diseases caused by pathogenic fungi. Artemisia bitterica exhibits a wide range of pharmacotherapeutic effects: anti-inflammatory, antibacterial, wound healing and antitoxic (Minda et al. 2022; Mohammed 2022; Radulović et al. 2022; Benmimoune et al. 2023; Al-Assaf et al. 2024; Aydın et al. 2024; Trinh et al. 2024).

The aim of our study was to determine the clinical and pathological characteristics of aeromonosis (*Aeromonas hydrophila*) in sturgeon fish under conditions of RAS and to study the therapeutic effect of bitter wormwood (*Artemisia absinthium* L.).

### MATERIALS AND METHODS

#### **Ethical approval**

The content of experimental animals and all manipulations carried out comply with the Commission Recommendations 2007/526/EC of June 18, 2007, on the maintenance and care of animals used for experimental and other scientific purposes. Conclusion of the local commission on biological ethics of the «Kazakh Research Veterinary Institute» LLP branch of the West Kazakhstan Research Veterinary Station" (Minutes No. 1 of January 16, 2020).

#### **Research period and location**

The research was carried out under conditions of RAS based on «Training and Research Complex of Experimental Industrial Aquaculture Production» LLP, engaged in cultivating various species of sturgeon fish. The study period was from February to May 2020. City of Uralsk, Republic of Kazakhstan.

### Clinical, pathological anatomical and bacterial examination

For bacterial examination and diagnosis of bacterial diseases, diseased fish with visible clinical signs were selected. Clinical, pathological and bacterial examinations of fish were carried out. During clinical examination, we performed an external assessment of sick fish, paid attention to the presence of hemorrhages, ulcers, depth, shape, and size, and evaluated their behavior and reactions to external stimuli.

When performing pathological anatomical examination, attention was paid to pathological changes in internal organs (appearance, structure of organs, blood filling, presence of haemorrhages, oedema). A bacteriological examination was carried out using the generally accepted methods. The percentage of affected fish and the form of the disease course were determined (Ture et al. 2018; Sergaliyev et al. 2019; Basankin et al. 2024).

#### Therapeutic study

The analogue approach was used to establish five experimental groups (n=20) of fish with aeromonosis to investigate the preparations' therapeutic qualities. For the 1st experimental group, an aqueous infusion of crushed wormwood herb was used as a therapeutic bath. The 2nd experimental group used aqueous infusion of crushed wormwood herb in the form of therapeutic baths and orally. A complicated concoction was employed for three experimental groups to treat bacterially induced infectious illnesses in fish. The fourth experimental group was given antibiotics. Table salt was utilized for the fifth experimental group.

#### Fish housing and feeding

All groups were kept in quarantine pools under the same conditions, corresponding to the fish breeding technology. All studied fish were fed in the standard mode.

### Preparation of an aqueous infusion of crushed wormwood herb

Aqueous infusion of crushed wormwood herb taken in the ratio of 1:20 (1 part of wormwood and 20 parts of distilled water) was used as an antibacterial herbal preparation. The preparation was prepared in the given ratio on a boiling water bath for 30 minutes, strained, and cooled. The preparation was stored in the refrigerator for 7 days. The obtained initial solution was applied in therapeutic baths, externally and orally (for per os application, the initial solution was diluted with distilled water in a ratio of 1:4).

#### Study groups

# Aqueous infusion of crushed wormwood herb in the form of therapeutic baths

(Experimental group 1). For this group, we used an aqueous infusion of crushed wormwood herb as therapeutic baths. The initial infusion was added to the bath in the amount of 500mL/40L of water once daily. Sick fish were kept in treatment baths for 30 minutes daily.

# Aqueous infusion of crushed wormwood herb in the form of therapeutic baths and orally

(Experimental group 2). For this group, aqueous infusion of crushed wormwood herb was used in the form of therapeutic baths and orally. Sick fish kept in quarantine pools were subjected to daily external treatment with the initial solution and given orally diluted 1:4 initial solution in the amount of 2mL.

# Complex preparation for treatment of infectious diseases of fish of bacterial etiology

(Experimental group 3). This group prepared a complex preparation for treating infectious diseases of fish bacterial etiology. As an active substance, we took metallic iodine, potassium iodide and vitamins: A (retinol acetate), E (alpha-tocopherol acetate), B<sub>1</sub> (thiamine hydrochloride), B<sub>2</sub> (riboflavin), B<sub>6</sub> (pyridoxine hydrochloride), B<sub>12</sub> (cyanocobalamin), iron carbonate, magnesium phosphate, manganese sulfate, copper sulfate, zinc sulfate, cobalt chloride, sodium chloride, succinic acid, glucose, rectified ethyl alcohol (96°) in specified quantities and given with feed at a dose of 1.00-1.50mg/1kg of fish weight, once a day, for 5-7 days (National Center for Biotechnology Information 2024).

### Antibiotics

(Experimental group 4). Antibiotics were used for this group. Application of levomycetin and biomycin was used orally (by mouth) with 3% starch suspension at 50mg/kg of fish weight for prophylactic purposes 1-2 times and for therapeutic purposes 3-4 times with an interval of 16-18 hours.

#### Table salt

(Experimental Group 5). For this group, table salt was used for treatment. A strong salt solution of 2-2.5% (20-25kg/1,000) was used, exposure - 10-15min, in the form of therapeutic baths.

#### RESULTS

#### **Evaluation of clinical signs**

The presence of single or multiple ulcers of various shapes with deepening into the underlying musculature down to the bone in the abdomen, back, at the base of the pectoral and pelvic fins; hyperemia around the eyes and exophthalmia; erosion of scales (lepidortosis); lesions of the tail and fins; protrusion of the anus; enlargement of the abdomen was noted in sick fish. The gills were slimy and hyperemic. Some fish had head lesions. Several fish showed tail stems fall off due to deep penetrating ulcers, erosions and perforation of gill covers, and extensive and deep necrosis of fins. Sick fish gathered near the pool's edge, swam upside down, fed poorly, and showed no reaction to light or food (Fig. 1).

The illness was both acute and chronic. Thirty percent of fish died. The acute form was characterized by inflammation of internal organs, fins and skin, especially in the head and abdomen, erosion of scales, and exophthalmia. The manifestation of hydrocele and formation of ulcers of different sizes and depths, rot and necrosis of fins with the destruction of interradial membranes characterized the subacute form.



**Fig. 1:** A) erosion of the base of the gill cover and hyperemia around the eyes; B) deep multiple ulcers with exposure to the wound bed; C) loosening of the edge and disintegration of the interradial septa of the pectoral fins; D) deep ulcerative lesion of the caudal peduncle when the disease progresses to a generalized form; E) fish swimming upside down at the edge of the pool; F) sick fish in the pool.

#### Pathological anatomical studies

At pathological anatomical necroscopy in the abdominal cavity, there was an accumulation of bloody exudate; the liver, kidneys, and spleen were swollen, flabby, hyperemic, catarrhal and hemorrhagic inflammation of the stomach and intestines, blood stasis in parenchymatous organs and pinpoint hemorrhages were noted (Fig. 2).



**Fig. 2:** A) presence of ascitic fluid in the abdominal cavity; B) catarrhal-hemorrhagic inflammation of the intestinal mucosa.

More than 70% of acute form incidence was found mainly in fry and yearlings. The majority of fish afflicted by the subacute type were yearlings and adult fish, less frequently fry. In fry, the bacterial disease was complicated by a secondary fungal infection saprolegniosis. Hyphae of the fungus affected the traumatized parts of the fry body and then moved to uninjured parts (Fig. 3).

Microstructural changes in diseased fish were expressed in the skin epidermis's destruction and necrosis

and muscle fibres' disintegration. The liver and kidneys observed Foci of inflammatory infiltration with eosinophils and epithelium necrosis.



**Fig. 3:** A) acute form of aeromonosis in fry B) complication of saprolegniosis with caudal fin destruction.

#### **Bacteriological studies**

During bacteriological studies, bacteria of *Aeromonas hydrophila* species were isolated in samples from diseased fish. Isolates of microorganisms were isolated from the liver, intestine, spleen, and washes from ulcerative lesions. When cultured in meat-peptone broth, a uniform opacity was formed on the surface of the medium, with a slimy precipitate and film, and moire waves appear when shaken. They formed round, small colonies on meat-peptone agar with bluish or whitish-matte cast in transmitted light (Fig. 4).



**Fig. 4:** Growth of cultures on: A) Meat Peptone Broth; B) Meat Peptone Agar.

A. hydrophyla bacteria are Gram-negative, motile, short, thickened in the middle with rounded ends of bacilli, ranging in size from 0.5 to  $2.0\mu$ m. They have a capsule, microcapsule, and flagella. The A. hydrophila pathogen is oxidase- and catalase-positive, produces hydrogen sulfide, breaks down to form acid and gas, and ferments maltose, sucrose, rabinose, lactose and glucose.

Studying the patterns of sturgeon fish infection, it was found that disease outbreaks were caused by abiotic factors, namely poor water quality (increased ammonia concentration) and temperature.

### Therapeutic study

## Aqueous infusion of crushed wormwood herb in the form of therapeutic baths

On days three and four after applying therapeutic baths to fish, experimental group 1 showed a reduction in inflammation surrounding wounds and ulcers, the formation of epithelization around the edges of wounds and ulcers, a tightness of the skin, and active feeding of the fish. Complete healing of wounds was noted on 7-10 days of daily application of therapeutic baths (Fig. 5).



**Fig. 5:** Dynamics of wound healing on the back after therapeutic baths: A) at the beginning of the experiment; B) on the 3rd day of the drug application; C) on the 5th-6th day of the drug application; D) at the end of the experiment.

# Aqueous infusion of crushed wormwood herb in therapeutic baths and orally

In experimental group 2, on the 2nd and 3rd day of combined external and internal preparation application, inflammatory phenomena in ulcers and wounds decreased, the edges of ulcers tightened, and epithelisation of the wound surface was activated. Further, ulcers and wounds healed in 5-7 days. No fish deaths were observed during the treatment period (Fig. 6). No side effects or irritation were observed during the application of the drug. No cases of repeated disease were observed during the observation of fish in experimental groups.

# Complex preparation for treatment of infectious diseases of fish of bacterial etiology

In experimental group 3, after 3-4 days of using the complex preparation on fish, inflammation around

wounds and ulcers decreases, epithelisation of the edges of lesions begins, the skin gradually tightens, and fish actively eat food. Total wound healing occurs after 6-8 days of daily application.



**Fig. 6:** Dynamics of tail part healing after oral and external application of the drug: A) at the beginning of the experiment; B, C) on 3-4 days of application; D, E) at the end of the experiment.

#### Antibiotics

In group 4, due to the constant use of antibiotics, we observed the emergence of resistance of microorganisms to them. As a result, the use of these drugs became ineffective, and we observed a case of recurrent morbidity. Inflammation and swelling of skin tissues on the border with the wound surface did not pass for a long time, excessive mucous discharge was observed and in most cases, signs of necrosis and rejection of dead tissues developed later and ulcers became larger.

#### **Table salt**

In the 5th experimental group, pronounced clinical signs of the disease were still observed in the first days

after salt treatment. Fish were lethargic and weakened. In this group, repeated disease and fish death were observed. In dead fish, ulcerative lesions were bleeding and loose. The condition of the fish worsened, food refusal increased, and external symptoms of the disease increased. At transition to the generalized form, most of the diseased fish died. The solution of table salt could have been more effective.

#### DISCUSSION

### Clinical, pathological anatomical and bacterial examination

As a result of clinical, pathological anatomical and bacterial examination, we found that the fish were sick with earomonosis. Fish of all age groups were affected. The mortality of fish amounted to 30%. It was found that aeromonosis runs in acute and subacute forms with clinical signs of bacterial-hemorrhagic septicemia.

More than 70% of acute form incidence was found mainly in fry and yearlings. In the subacute form, fish older than a year and older fish, less often fry, and yearlings were predominantly affected. In fry, the bacterial disease was complicated by secondary fungal infection-saprolegniosis, which resulted in high mortality among fry.

It has been revealed that fish disease is caused by abiotic factors, such as violation of conditions of fish kept in RAS, increased ammonia concentration, and temperature factors. Ammonia is one of the leading indicators of water quality affecting fish health (Di et al. 2018; Luo et al. 2018; Zhu et al. 2023; Chen et al. 2023; Duman and Şahan 2023).

#### Therapeutic study

During the study period in the 1st, 2nd, and 3rd experimental groups, no deviations in fish behaviour or nutritional disorders were observed. Improvement in the fish condition, the gradual disappearance of clinical signs of the disease, and fish willingly eating food were noted. Complete recovery in these experimental groups was observed in the bulk of fish within 5-10 days from the beginning of the experiment. Side effects and irritation were not observed. No complications or cases of repeated disease occurred during the observation period for the fish in the 1st, 2nd, and 3rd experimental groups. In the 1st and 3rd experimental groups, low mortality rate was observed. No fish mortality was observed in the 2nd group. Fish survival rate in the 1st, 2nd and 3rd experimental groups was higher (from 90%) than in the 4th (75%) and 5th (60%) (Table 1).

 Table 1: Therapeutic efficacy of drugs during the period of experiments

| № Groups | Period of the experiment (days) |         |         |          |          |          | Cases of recurrent disease, % | Survival rate, % |  |
|----------|---------------------------------|---------|---------|----------|----------|----------|-------------------------------|------------------|--|
|          | 3rd day                         | 5th day | 7th day | 10th day | 15th day | 20th day |                               |                  |  |
|          | Number of dead fish             |         |         |          |          |          |                               |                  |  |
| 1        | 1                               | 1       | -       | -        | -        | -        | Not noted                     | 90               |  |
| 2        | -                               | -       | -       | -        | -        | -        | Not noted                     | 100              |  |
| 3        | 1                               | -       | -       | -        | -        | -        | Not noted                     | 95               |  |
| 4        | 2                               | 1       | 1       | -        | 1        | -        | Noted                         | 75               |  |
| 5        | 4                               | 2       | 1       | -        | 1        | -        | Noted                         | 60               |  |

Each group comprised of 20 fish

It should be noted that in the 1st and 2nd experimental groups using water solutions and in the 3rd experimental group, the fish showed intensive healing and scarring of ulcers. Within 3 to 5 days of application of therapeutic baths, the fish display a decrease in inflammation and swelling around the ulcers, epithelisation of the edges of the ulcers with the formation of a scar and a tightening from the borders to the centre of the ulcers, with the first signs of healthy granulation tissue appearing. The affected area of the wound remains slightly darker than the surrounding skin. A pronounced effect on the change of the wound surface area was observed in the 1st, 2nd, and 3rd groups treated with wormwood and a complex preparation, respectively.

The results obtained when using an aqueous solution of wormwood showed high therapeutic efficacy (90-100%), which we attribute to the presence of biologically active components in the plants with antimicrobial, antiinflammatory and immunostimulating effects (Batiha et al. 2020; Szopa et al. 2020; Yousefi et al. 2021; Nurlybekova et al. 2022; Berganayeva et al. 2023).

Thus, the aqueous solution of wormwood slide has a pronounced antibacterial, anti-inflammatory, and wound healing effect, stimulating regeneration processes and accelerating wound healing, and is prepared from available, harmless and effective local plant raw materials (Moslemi et al. 2012; Boudjelal et al. 2020; Benkhaled et al. 2020; Sultan et al. 2020). Complex preparation is also adequate. However, the disadvantage of this preparation is its multicomponent composition, labour-intensive preparation and high cost (Hunter and Isaza 2017). Antibiotics are used as preparations for treating and preventing bacterial diseases of fish by injection, treatment in treatment baths, and orally (Sheng and Wang 2021; Aisa and Parlier 2022; Yasin et al. 2023). With the widespread use of antibiotics, the emergence of resistant forms of microorganisms are often observed, making the use of these drugs ineffective (Anagnostopoulos et al. 2022; Sun et al. 2022; Shi et al. 2023; Bondad-Reantaso et al. 2023; Dinev et al. 2023; El-Hossary et al. 2023). Many antibiotics have a direct toxic effect. Excessive use of antibiotics also increases their accumulation in fish products, which generates risks of negative impact on consumers and the environment. Also, the disadvantage of these drugs is their poor efficacy in case of severe fish damage (Sobierajski et al. 2022; Vidovic and Vidovic 2020; Ruzante et al. 2022; Caneschi et al. 2023; Damborg et al. 2024). The use of table salt solution does not have a practical therapeutic effect in the treatment of aeromonosis, and it can be toxic in frequent use and overdose (Tavares-Dias 2022).

#### Conclusion

Thus, unfavourable environmental conditions in recirculating aquaculture systems, such as reduced water quality and temperature fluctuations, favor the reproduction of the Aeromonas pathogen and increase fish vulnerability to disease outbreaks. In industrial fish farming conditions, the risk of fish infection can be reduced by maintaining optimal hydrochemical conditions in the aquatic environment, stable temperature, and compliance with the norms of fish farming technology. The results of our clinical, pathological anatomical and bacterial studies showed that the etiologic factor of the fish disease outbreak in the sturgeon fish farm of «Educational and Scientific Complex of Experimental Aquaculture Production» LLP is the causative agent of aeromonosis - *Aeromonas hydrophilusa*. We used the aqueous infusion of wormwood herb with a pronounced antibacterial, anti-inflammatory, and wound healing effect, stimulating regeneration processes and accelerating wound healing. It is prepared from available, harmless and effective local plant raw materials. It can be used to prevent and treat bacterial diseases of fish in RAS. The results of this research may be helpful for researchers and specialists in fish farming and aquaculture.

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Author's contribution: NF, AG, GN and KAK conceptualized the research study, designed and implemented the experimental protocols. KA, SB, SN, SY contributed to the data collection and analysis. NF, SBT, KA, SY and MA interpreted the results and drafted the manuscript. NF, AG and GN supervised the project. All authors have reviewed and approved the final manuscript for publication.

**Data availability statement:** The data that supports the findings of this study are available in the material of this article. There is no other supporting data.

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