CONTROL OF *AEROMONAS SALMONICIDA* INFECTION IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) REARED IN RECIRCULATING AQUACULTURE SYSTEM

Angelica Docan[1*], V. Cristea[1], Lorena Dediu[1], Aurelia Nica[1]

[1] *Dunarea de Jos University, Galati, Romania*

Abstract

Recirculating systems create unique environments for fish culture which may provide favorable conditions for disease occurrence or the reproduction of opportunistic microorganisms. Stressful conditions in recirculating systems, such as poor water quality or high stocking densities in the culture tanks, may contribute to disease outbreaks. The present study was conducted in the context of pathogen bacteria proliferation reported in the case of Onchorhynchus mykiss reared in a recirculating system. First clinical signs of disease were evident in terms of lesion: bleeding ulcers on the skin surface, bruising at the base of fins, bleeding oral region. A. salmonicida has been isolated from the kidneys, blood and furuncle of sick fish. Furunculosis is a bacterial infection of salmonids and other fishes caused by Aeromonas salmonicida. Aeromonas salmonicida strains isolated from diseased specimens of trout, showed an antibiotic resistance-type "multi-Steep" being sensitive to chloramphenicol, kanamycin, streptomycin and resistant to penicillin, ampicillin, tetracycline, oxacillin and erythromycin. Following application of different treatment schemes, the best results were obtained with chloramphenicol for 7 days.

Key words: *Oncorhynchus mykiss, Aeromonas salmonicida,* recirculating aquaculture system

INTRODUCTION

The global research carried out on the species *Oncorhynchus mykiss,* underlined the fact that they are not too particular to weather conditions, they have a good degree of fodder assimilation, a rapid growth within captivity. These technological aspects are an important argument for the growing of the consumption trout in a super intensive recirculating system.

The technological performance of an aquaculture recirculating system highly depends on the efficient control of diseases and stress conditions [2]. There can be in the water culture of a recirculating system a series of pathogenous organisms, in different stages of life. High stocking densities, specific to aquaculture recirculating systems, can damage the health condition and the resistance of culture biomass. [9], [7].

Although the growth is permanently controlled within the aquaculture recirculating systems by monitoring and maintaining median parameters within optimum limits, the practice has demonstrated that the continual operation is not always possible because of various chemical and biological factors which affect water recirculating process. The most frequent diseases in the recirculating system are bacterial infections which, in a proportion of 90%, are caused by fish pathogenic germs, by poor water quality or nutritional factors which have an important role in the immune system depression hence declining fish resistance to pathogenous germs. Thus, the bacterial hemorrhagic septicemia was signaled on the species *Acipenser stellatus* which was grown within a recirculating system. The triggering factor of the bacteriosis was the captivity stress, fish becoming vulnerable when germs attacked in the water, potential pathogens [4].

The present study was carried out as set against the proliferation of pathogenic bacteria in a recirculating system where there was species *Onchorhynchus mykiss* and it aimed at testing several antibiotics in order to identify the most efficient treatment.

*Corresponding author: angelica.docan@ugal.ro
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MATERIAL AND METHODS

Fish biomass and the growing conditions

Fish biomass used in this study was represented by *Oncorhynchus mykiss* specimens aged twelve months raised into a recirculating system of the pilot aquaculture station from the Aquaculture, Environment Science and Cadastre Department. Total biomass was 67263 g, randomly distributed in the four growth units.

The pathological and bacteriological examination

The fish with evident signs of disease underwent a complete pathologic examination: clinical, macroscopic and microscopic. The bacteriological examination was carried out in stages according to the following protocol:

- The transfer of the bacteria from the affected areas (where they were found) on nutritive agar. In order to isolate the bacteria, the seeding was carried out within the in tegumentary ulcer, kidneys, blood and intestinal tracts, according to classic methods.
- Incubation at 25ºC, 48 h.
- The isolation of the bacteria for the pure cultures through reseeding on nutritive agar, incubated in the same conditions.
- The bacterioscopic examination carried out on bacterial smears from pure colonies, Gram stained, using immersion objective. Preparing and reading antibiogram according to the Kirby-Bauer diffusion qualitative method.

RESULTS AND DISCUSSIONS

Clinical manifestations. Anatom-Pathological modifications

During the experiment, the temperature of the technological water increased up to 26ºC, which is above upper limit for rainbow trout [1]. When the temperature of the technological water surpassed 22ºC, the fish became lethargic, without reactions, they presented inappetence and stopped feeding.

Among others, there were exemplars with signs of clinical disease, with evident lesions:

- Hemorrhagic ulcers at tegument surface, surrounded by a whitish epidermis area which, due to congestive reaction, merged, discovering the muscles. The apparition of furuncles indicates the chronicization of the infection [5].
- Fins bruising.
- Hemorrhages around the mouth, resulting in necrosis of the mandible.

Photo 1. Rainbow trout with clinical signs of disease

When the affected exemplars were dissected, modifications at the internal organs level were observed, sign of a bacterial invasion: bruising of the liver, intestinal and pyloric appendix bleedings and swellings, muscles petechial hemorrhages, splenomegaly,

congestion of the kidneys with evident degenerative processes.

Photo. 2. Bruising of the lever, intestinal bleeding

Photo. 3 Swelling and bleeding pyloric appendix, bleeding petechia

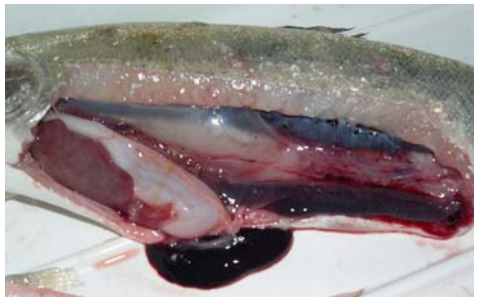

Photo. 4. Splenomegaly, congested kidneys

Photo. 5. Inflamed and hemorrhagic intestine

These modifications emphasized some bloodstream disturbances, dystrophic and inflammatory processes. The petechial hemorrhages on the visceral surface as well as the liquefaction of the kidneys are the result of the bacterial proteolytic enzymes action [6].

The main factor which influences the appearance of the furunculosis is the increase of the technological water temperature [6]; the temperature of 15-20°C is correlated with the intensification of clinical signs of the furunculosis as a direct result of thermal stress [10] and with a rapid development/proliferation of the *Aeromonas salmonicida salmonicida* bacteria [3], [8].

Identification of etiological agent. Diagnostic

The results of the clinical examination, as well as of the anatomical lesions conducted to furunculosis clinical suspicion. To confirm the diagnostic, we have associated the clinical examination with bacteriological examination in order to identify the etiological agent of the disease.

The bacterioscopic examination carried out on Gram stained bacterial smears underlined a mix micro-flora which, after isolation and identification, were confirmed to be *Aeromonas* and *Bacillus* strains. The interpretation of smears led to the identification of some negative (red colored), grouped, rod-shaped, not motile bacteria (without cilia)), with length between 0,8 – 1,2 μ, which led to their categorization into *Aeromonas* genus.

The main criteria for taxonomic categorization were: positive reaction to oxidase test-distinctive genus feature, lack of motility – distinctive feature for the species differentiation and brown pigment synthesis– characteristics of the S*almonicida* subspecies.

The bacteriological and bacterioscopic exams conducted to the identification of the illness pathogenous agent, *Aeromonas salmonicida salmonicida*, which, in association with clinical and anatomo-pathological examinations confirmed the diagnosis as ***furunculosis***.

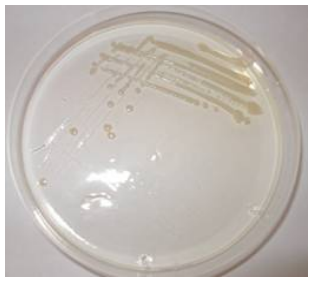

Photo. 6 The morphology of bacterial cultures

Photo. 7 Brown pigmentation of colonies

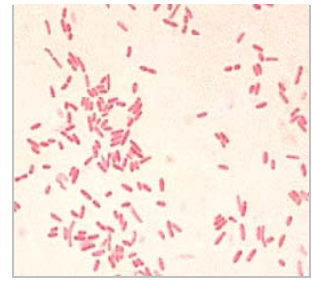

Photo. 8 *Aeromonas salmonicida* (10 oc x 100 ob, Gram stain)

Treatment scheme

After isolation of the *Aeromonas salmonicida* from the kidneys, blood, abscesses, in order to apply the most efficient treatment scheme, the sensibility of different antibiotics was tested through antibiogram technique, which is recommended by the literature since, in time, *A. salmonicida* grows resistant to certain antibiotics [5].

The interpretation of antibiograms after 24 hours has outlined the appearance of a confluent bacteria colonies and inhibition of bacterial growth around the micro-tablets, as it follows:

- *chloramphenicol*: 3,5 – 4 mm diameter of inhibition area
- *kanamycin*: 1,5 – 2 mm diameter of inhibition area
- *streptomycin*: 1,5 mm diameter of inhibition area

Thus, germs of *Aeromonas salmonicida* isolated from the contaminated trout exemplars manifested a "multi-steep"-like antibioresistance, being sensitive to chloramphenicol, kanamycin, streptomycin and resistance to penicillin, ampicillin, tetracycline, oxacillin and erythromycin

Since rainbow trout refused the medicated feed, the antibiotics were intramuscularly injected (kanamycin and streptomycin) or through baths directly into the tank (chloramphenicol) in this, last case, separating the tank by recirculating system.

Photo. 9 Sensitivity of the *A. salmonicida* isolated from kidney

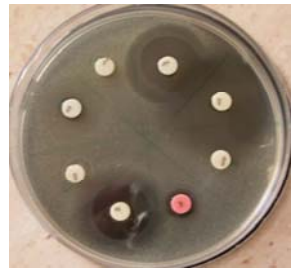

Photo. 10 Sensitivity of the *A. salmonicida* isolated from blood

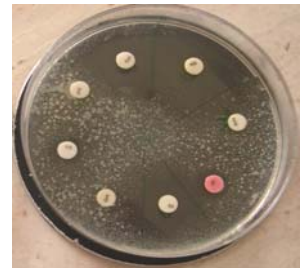

Photo. 11 Sensitivity of the *A. salmonicida* isolated from abscess

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Table 1 Treatment strategy on *Onchorhynchus mykiss* using different antibiotics

Experimental group	Antibiotics	Management	Dose
B1- control group	Without treatment	-	-
B2	Kanamycin (250 mg active substance/ml)	intramuscular injections	100 mg/kg fish/day
B3	Streptomycin (200 mg active substance/ml)	intramuscular injections	100 mg/kg fish/day
B4	Chloramphenicol	Bathing 6 h	900 mg/l water/day

After 7 days of treatment, the best results were obtained for the trout exemplars in B4 (87% survival) which, as opposed to exemplars in B2 and B3 (42% survival), were not stressed by specific operations of intramuscular shots. The mortality was of 90% in the case of the control variant.

CONCLUSIONS

When stress factors have an acute and prolonged influence, the compensatory abilities of the organism could be limited leading to an increase of fish sensibility to infections, acute physiological disturbances, and even high mortality rates.

The stress caused by the increase of the technological water temperature from the recirculating system represented the main factor for the appearance of the bacteriosis, influencing the clinical expression of the infection. The association of the anatomo-pathologic and bacteriological clinical examinations, as well as the presence of *Aeromonas salmonicida*, led to the diagnosis of furunculosis. Sensibility tests through the antibiogram tests showed that *Aeromonas salmonicida ssp. salmonicida* manifested a "multi-steep"-like antibiotic resistance, being sensitive to chloramphenicol, kanamycin and streptomycin. Following the application of the treatment scheme, the best results were obtained in the case of baths with chloramphenicol for a period of 7 days.

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