Some Diagnosis and Therapy Techniques in Rainbow Trout (Oncorhynchus Mykiss) Furunculosis,
in Intensive System Bred

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Abstract
Clinical and paraclinical investigations (morpho-pathologic, bacterioscopic, bacteriologic and sensitiveness test to antibodies) done on 20 fish (rainbow trout), dead or in agony state, taken from a furunculosis pesthole in Salmonidae and proceeded from an intensive exploitation unit of trout, in Cluj county, put in evidence the following aspects: 1. Clinical exam, in breeding basins, it appears individuals (5 %) which have presented locomotor troubles, listless and untidy swimming in water surface, lateral swimming anorexia, and on tegument it is observed diffuse hemorrhagic zones, lost of fish scale and necrosis of fins. It appears also secondary infections with fungi of Saprolegnia genus under the form of a white-dirty, downy film. From the 20 trout corpses were isolated 2 bacterial strains, from anterior kidney and from raclage in fin basis, with congestive lesions. Bacteria isolated are developing only after an incubation at 20 – 25 °C and does not grow up to 37 °C, and to microscopic exam directly from sauce, the mobility is absent. Classic biochemical tests put in evidence the following positive biochemic properties: cataloze, oxidaze, indolal and the presence of hemolytic activity. Biochemical properties testing on API20E gallery also puts in evidence properties as: positive gelatinosis and sucraze fermentation. Based on bacterial strains development isolated only to 20 - 25 °C and the above mentioned biochemic properties, respectively the absence of pigment, bacterial strains isolated are appointed in Aeromonas genus, Aeromonas salmonicida species, achromogenes subspecies. By sensitiveness test in different medicine substances, using antibiogram technique, bacterial strains isolated from pesthole are sensitive in a decreasing order to: nalidixic acid, oxitetracycline, florfenicol and euroflaxin and resistant to ampicyline, amoxiclav and colistin.

Keywords: furunculosis, inseminations, Oncorhynchus mykiss

1. Introduction
Clinical and paraclinical investigations (morpho-pathologic, bacterioscopic, bacteriologic and sensitiveness test to antibodies) done on 20 fish (rainbow trout), dead or in agony state, taken from a furunculosis pesthole in Salmonidae and proceeded from an intensive exploitation unit of trout, in Cluj county, put in evidence the following aspects: 1. Clinical exam, in breeding basins, it appears individuals (5 %) which have presented locomotor troubles, listless and untidy swimming in water surface, lateral swimming anorexia, and on tegument it is observed diffuse hemorrhagic zones, lost of fish scale and necrosis of fins.

2. Materials and methods
Investigations regarding possibilities of diagnosis and treatment in a pesthole suspect of salmonidae furunculosis, were effected in a piscicole unit in
Cluj county, specialized in rainbow trout intensive breeding (Oncorhynchus mykiss) for consumption. The piscicole unit has in possession a total surface of 0.82 ha, from which water surface 0.18 ha, distributed in 73 basins. Water alimentation source is constituted by accumulation lake Gilau. Unit is equipped with administrative buildings, incubation house fodder warehouse, fodder kitchen, sheds, storage spaces, etc.

It were done clinical investigations on fish populations in basin following individuals which presented general signs of disease. Also, from exploitation basins were taken 20 fishes (recently dead or in agonic state), which presented on body surface, tegument lesions (hemorrhagic zones, tumefaction, abscesses and ulcers for bacterioscopy, bacteriology and sensitiveness testing to antibodies and chemiotherapics of strains which have diagnosis.

For bacteriology exam smears were done from pathologic material taken from zones with cutaneous lesions and colored by Gram method. Bacteriology exam is following isolation and identification of bacteria from pathologic material, harvested in the cutaneous lesions level, responsible of disease state producing. It were effected inseminations on culture solid mediums in Petri plates: agar with infusion of hard, brain (BHI), tryptose soya – agar (TSA) or agar with 5 % plus blood sheep. Plates inseminated are incubated at laboratory temperature 48 hours. Morphologic characters exam were effected on smears from Gram method colored culture. In the same time, from bacterial colonies obtained, biochemic tests were effected, for some biochemic properties determination, as catalase test, oxidase test and API 20E multi-test system.

Sensitiveness test to antibodies and chemiotherapics of strains obtained, was realized by using diffusiometric method and antibiogram, and results interpretation was done with the help of a graduated ruler for inhibition zones measurement (in mm.).

3. Results and discussion

Investigations effected on fish populations in basins (clinic and of laboratory), respective pathologic samples taken (20 recently dead fishes or in agonic state)[1,2], put in evidence the following aspects:

1. Clinical exam
To an attentive macroscopic exam, in some basins were observed fishes which have presented on tegument, in different body zones, hemorrhagic lesions (stains, echymosis) but also denuded zones of tegument. In certain individuals, it appears, in lateral zones of body or on back, tumefactions, abscesses and ulcers, even necrosis of fins (cut off aspect!). Also mycelium superinfections are present in ulcerous lesion zone, with Saprolegnia genus fungi under the form of a fine down with cotton aspect [3,4].

2. Necropsy exam
As a result of necropsy effected on dead fish samples, suspects of furunculosis, it was observed the following anatomic – pathological table

- the presence of some tegumentary hemorrhagic lesions under the form of spots and echymosis localized to fins basis, in dorsal zone, on abdomen and branchia, and also different denuded zones of tegument
- in some individuals it appears, supplementary, abscesses with a fluctuant content, ulcerous and necrosis zones of fins, these one appearing as cut off.
- It is present the secondary infection with Saprolegnia genus fungi under the form of a fine down with cotton aspect
- It appears also hemorrhagic lesions in viscera level, especially in liver, kidney and pyloric appendix

3. Bacterioscopy exam
Bacterioscopy exam of smears, done from pathologic material taken from cutaneous lesions and colored by Gram method, puts in evidence in microscopic field, the presence of a polymorph bacterial microflora represented by cocobacillus, right or easy courved bacillus, Gram negatives. Based on morphological characters, bacterial microflora presented in prepared samples is appointed in Aeromonas genus [5].

4. Bacteriology exam
As a result of bacteriology exam effected from a number of 20 trout corpses ill with, on ordinary culture mediums (sauce, agar, agar with sheep blood) and special mediums of differentiate (Mac Conkey and Istrate-Meitert) it were isolated 2 strains of Aeromonas salmonicida, achromogenes subspecies, from 2 corpses, in anterior kidney and
in raclage done in basis of abdominal fins which presented congestion and ecchymosis [6].

To bacteriology exam done on ordinary mediums of sauce and agar it were put in evidence the following cultural characters: in sauce – intense turbidity without formations of surface (film, ring, membrane), white-grey homogenizable storage, quantitative moderate.

On agar it appear colonies of average size (1 – 3 mm), demi-opac with unregulated borders, smooth and glowing surface, without pigments.

On agar with sheep blood, the 2 bacterial strains are developing under the form of some average colonies (1 – 3 mm), round, with regulated borders, easy bulging profile, smooth and glowing surface, surrounded by a large zone of complete hemolysis.

On mediums of differentiate (Mac Conkey and Istrate-Meitert) there are developing under the form of some average size colonies, with morphological characters mentioned above, negative lactoses.

To doing the differentiation of Aeromonas salmonicida species facing to those of Aeromonas hydrophila, it was done the differentiate incubation to 20 - 25°C and to 37°C. The strains that we have isolated have not developed to 37°C after 24 – 48 hours of incubation.

The exam of some biochemical traits which are used currently as rapid tests of diagnosis, appears positive to catalase, oxidase and indol tests and negative to sulphured hydrogen.

Biochemical traits exam on API 20E gallery puts in evidence that gelatinose test and sucrose fermentation is positive.

5. As a result of sensitivity test of bacterial strains found, to different medicine substances, by antibiogram technique (diffusiorimetric method) it is ascertained that Aeromonas salmonicida achronomogenes var. strains, isolated from disease pesthole are sensitive to the following medicine products:

- nalidixic acid, diffusion beach of 32 mm
- oxythetracycline, diffusion beach of 25 mm
- fluorphenicol, diffusion beach of 23 mm
- eurofloxacin, diffusion beach of 18 mm.

We mention the fact that Aeromonas salmonicida, achronomogenes var. strains found as a result of sensitiveness test to antibodies by diffusiometric method, are resistant to: ampiciline, amoxiciline, colistin (picture 1.)

Figure 1. Antibiogram realized to sensitiveness screening selection of Aeromonas salmonida achronomogenes var. strain.
4. Conclusions

Clinical and paraclinical investigations (morpho-pathologic, bacterioscopic, bacteriologic and sensitiveness test to antibodies) done on 20 fish (rainbow trout), dead or in agony state, taken from a furunculosis pesthole in Salmonidae and proceeded from an intensive exploitation unit of trout, in Cluj county, put in evidence the following aspects:

- To clinical exam, in breeding basins, it appears individuals (5 %) which have presented locomotor troubles, listless and untidy swimming in water surface, lateral swimming anorexia, and on tegument it is observed diffuse hemorrhagic zones, lost of fish scale and necrosis of fins. It appears also secondary infections with fungi of Saprolegnia genus under the form of a white-dirty, downy film.

- From the 20 trout corpses were isolated 2 bacterial strains, from anterior kidney and from raclage in fin basis, with congestive lesions.

- Bacteria isolated are developing only after an incubation at 20 – 25°C and does not grow up to 37°C, and to microscopic exam directly from sauce, the mobility is absent.

- Classic biochemical tests put in evidence the following positive biochemic properties: catalaze, oxidaze, indole and the presence of hemolytic activity. Biochemical properties testing on API20E gallery also puts in evidence properties as: positive gelatinousis and sucraze fermentation.

- Based on bacterial strains development isolated only to 20 - 25°C and the above mentioned biochemic properties, respectively the absence of pigment, bacterial strains isolated are appointed in Aeromonas genus, Aeromonas salmonicida species, achromogenes subspecies.

- By sensitiveness test in different medicine substances, using antibiogram technique, bacterial strains isolated from pesthole are sensitive in a decreasing order to: nalidixic acid, oxitetracycline, florfenicol and eurofloxacin and resistant to ampicilin, amoxiclav and colistin [7].

References


