

Investigations Concerning Possibilities of Diagnosis and Treatment in One Pesthole of Bacterial Haemorrhagic Septicemia in Carp (*Cyprinus Carpio*)

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Abstract

Investigations were done on 7 alive samples in agonic or death stage, taken from an extensive exploitation pond, in north-east Transylvanian zone, with an water surface of 0,2 ha and a total fish quantity of about 200 kg, second summer carp of an average weight of 300 g/individual. As a result of clinical, bacterioscopy and bacteriology and also necropsy laboratory exam it were put in evidence some aspects. So, clinical exam has revealed, by the presence of integument hemorrhagic ulcerous lesions, also the presence of some crustaceae ectoparasites, *Argulus foliaceus* species, which have made easier the disease spreading in whole effective. Necropsy exam puts in evidence, anatomy-clinic, the presence of integument hemorrhagic lesions under a point or diffuse form, but also ulcers and muscle – cutaneous necrosis. As a following of bacterioscopy exam of smears done from pathologic material taken in cutaneous lesions and colored by Gram method it was ascertained the presence of a bacterial polymorph flora, of bacillus and cocobacillus type, Gram negative. Bacteriologic exam (cultural), realized on ordinary culture mediums (sauce, water) and selective mediums (agar with blood) isolates in pure culture germs from *Aeromonas* kind, basis on morphological and cultural characters, which are framed in *Aeromonas* genus patterns described by speciality literature. As a following of biochemical characters exam, with the help of multitest API20E system it was established that *Aeromonas* spp. strains isolated, belong to hydrophila group 1 species, species which is responsible of bacterial hemorrhagic septicemia performing in carp. By testing the sensitiveness to antibodies and chemotherapy in strains which have been found as diagnosis (diffusiometric method of antibiogram), it was ascertained that group 1 *Aeromonas hydrophila* strains, isolated from disease pesthole, were in decreasing order sensitive to : Eurofloxocin, Florfenicol (Floron) and Oxitetracycline and resistant to Ampiciline, Amoxicilin, Eritromicin.

Keywords: bacteriologic, *Cyprinus Carpio*, pesthole

1. Introduction

Investigations were done on 7 alive samples in agonic or death stage, taken from an extensive exploitation pond, in north-east Transylvanian zone, with an water surface of 0.2 ha and a total fish quantity of about 200 kg, second summer carp of an average weight of 300 g/individual. As a result of clinical, bacterioscopy and bacteriology

and also necropsy laboratory exam it were put in evidence some aspects.

2. Materials and methods

Investigations were done in a carp extensive exploitation nursery in north-east of Transylvania. Total water surface is of 0.2 ha, with a total volume of 2840 mc water. Alimentation with water of this arrangement is done through a water plug, placed on Crasna river course. Total quantity of fish approximated in basin is of 200 kg carp of

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second summer, with an average of weight of 300 g/ individual. From basin fish samples were taken which have presented troubles of balance and removal, in which through an anatomic clinical exam, were observed cutaneous hemorrhagic ulcers lesions. Some affected individuals were in agony stage and from these a number of 7 individuals were transported in ichtiopathology discipline laboratory, where it were done the following exams:

- necropsy exam
- bacterioscopy exam
- bacteriology exam
- testing of sensitiveness to antibodies and chemioterapics of strains
- treatment

Necropsy exam was done in integument level and by body cavity opening for putting in evidence the cutaneous lesion table and intern organs modifications.

Bacterioscopy exam was done on smears obtained from pathologic material taken from cutaneous lesion zones, colored by Gram method.

Bacteriology exam (cultural) has followed isolation of bacterial source involved in disease producing. Pathologic material samples were taken in cutaneous lesion level and in intern organ (hart, kidney) using tampons and sterile Pasteur pipette. It were done inseminations in ordinary culture mediums (sauce and agar), and the test-tubes with mediums were incubated in laboratory temperature (20 – 25⁰ C, during some days (3 – 4 days).

Concomitantly it were done also inseminations, on special mediums (agar with blood) for researching of hemolytic activity.

Test-tubes in which was observed the development of micro-organisms (bacteria) were use for doing the replications (doing the subcultures), on selective mediums (Istrate – Meitert) and on multitesting systems (API20E) for a more accurate identification of genus and species affiliation.

Testing of strain sensitiveness to antibodies and chemioterapics was don by diffusimetric method of antibiogram. The method principle is based on property of antibodies to diffuse into cultural solid mediums, realizing different concentration gradients, which decrease gradually from center to diffusion zone edge.

Between medicine substances with antibody action the following were used: Europloxacin, Floron, Oxitetracycline, Ampiciline, Amoxiciline and Eritromicine.

After etiologic diagnosis establishment it was initiated a prophylactic therapeutic protocol which consists in:

- a. disinfection on water surface with quick lime of 100 kg/ha dose
- b. therapy with antibodies in concordance to antibiogram effected by using Eurofloxacin 20 g / 10 kg granule feed during 7 days, 2 ration / day.

3. Results and discussion

Researches done on a number of 7 samples (fish) alive, in agonic state or dead, with hemorrhagic lesions and cutaneous ulcers, taken from a carp breeding pond in extensive system [1], have revealed the following aspects:

A. Anatomic clinical exam

To a macroscopic attentive examination, by the presence of balance and removal troubles, it is observed the presence of some parasites fixed on integument, fin. Microscopic prepared effected from these zones and examined with objective 4x to microscope, put in evidence the presence of crustaceus *Argulus foliaceus*. By their hematophag nutrition mode, in cutaneous picking point, the open gates for pathogenic bacterial microflora, including *Aeromonas hydrophyla* species [2].

B. Necropsy exam

As a result of necropsy exam it is observed in integument level, in different body zones, the presence of some hemorrhagic and ulcerous muscle – cutaneous lesions (superficial and profound), well determined or diffuse.

In some individuals it appears hemorrhagic lesions also in branchial level, eyeball, buccal cavity [3].

To the body cavity opening it appears intern organs modifications as: multiple hemorrhages, present in sanguinolent liquid in body cavity; hypertrophy and splenic and renal congestion; multiple intestinal hemorrhages.

C. Bacterioscopy exam

As a result of bacterioscopy exam on smears which were effected from pathologic

material taken from cutaneous lesions and colored by Gram method, it is put in evidence the presence of a polymorph bacterial microflora, represented by cocobacillus and right or easy curved bacillus, gram negative. Based on morphologic characters, bacterial microflora is appointed in *Aeromonas* genus.

D. Bacteriology exam

Bacteriology exam (cultural) done from a number of 7 samples on ordinary culture mediums (sauce, agar), agar with blood, has allowed the isolation in pure culture of *Aeromonas* genus germs from 4 samples. In sauce it is observed the apparition of a reduced turbidity at 24 hours after incubation in room temperature, turbidity which becomes more intense at 48 hours of incubation. To tubes opening it is ascertain that these one emanate an unpleasant, pungent smell.

In tubes with agar it is developing relatively slow, after 48 hours, under small – middle colonies form (1 – 3 mm diameter), with regular borders, smooth and glowing surface, white-grey color, opac. In the case of samples rich in germs it could be observed the development of the culture under the form of a continuous layer (sod).

Morphologic and cultural characters on ordinary mediums, mediums with blood and selective mediums, appoint the present pathogenic microflora in described patterns for *Aeromonas* genus.

For an accurate identification of genus affiliation in primary cultures on ordinary mediums, it were done subcultures (replicates) on selective mediums (Istrate – Meitert). In these mediums development of germs *Aeromonas* genus is realized slow, between 24 – 72 hours, under the form of some round colonies of variable sizes, 1 – 3 mm diameter, green – blue color (lactose negative) and smooth and glowing surface [4,5].

As a result of biochemical characters exam, with the help of multitesting API20E system were recorded the following positive reactions for: ADH, INDOL, GELATINASE, use of sugars GLU,MAN, SAC, AMY, ARA, in strains tested. Following biochemical characters exam by API20E method it was established that *Aeromonas* spp. strains isolated belong to: hydrophila *Aeromonas* species group 1.

E. Sensitiveness test of strains to antibodies and chemotherapics

As a result of sensitiveness test to different medicine substances by antibiogram technique (diffuse – metric method), it is ascertained that hydrophila *Aeromonas* strains, group 1 isolated from disease pesthole were sensitive to : 1. Eurofloxacin; 2. Floron and 3. Oxitetracyclin and resistant to 4. Ampicylin, 5. Amoxicylin and 6. Eritromycine. (figure1.)

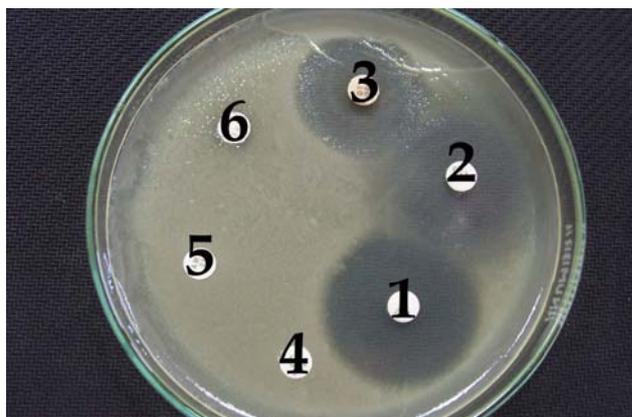


Figure 1. Sensitiveness test to antibodies by antibiogram

F. Prophylaxis, treatment

It was disposed one single disinfection, on water surface with quick lime in 100 kg/ha dose, using about 20 kg quick lime by hand spreading. In the same time, by using Eurofloxacin in carp alimentation in pool, under the form of granule fodder, in 2 daily ration (hours: 8⁰⁰ and 18⁰⁰), it was observed with the beginning in the 3th day post-therapeutic, an improvement of general state : fish are lively, they consume well the fodder and the mortality is significant reduced. Also it is observed a progressive healing of cutaneous wounds which have a final after a certain time period, through a recovery and the healing place has a blue shade.

4. Conclusions

Investigations were done on 7 alive samples in agonic or death stage, taken from an extensive exploitation pond, in north-east Transylvanian zone, with an water surface of 0.2 ha and a total fish quantity of about 200 kg, second summer carp of an average weight of 300 g / individual. As a result of clinical and bacterioscopy and bacteriology necropsy laboratory exam it were put in evidence the following aspects:

- clinical exam has revealed, by the presence of integument hemorrhagic ulcerous lesions, also the presence of some crustaceae ectoparasites, *Angulus foliaceus* species, which have made easier the disease spreading in whole effective.
- Necropsy exam puts in evidence, anatomy-clinic, the presence of integument hemorrhagic lesions under a point or diffuse form, but also ulcers and muscle – cutaneous necrosis
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- As a following of biochemical characters exam with the help of multitest API20E system it was established that *Aeromonas* spp. strains isolated, belong to hydrophila group 1 species, species which is responsible of bacterial hemorrhagic septicemia performing in carp.

By testing the sensitiveness to antibodies and chemotherapy in strains which have been found as diagnosis (diffusiometric method of antibiogram), it was ascertained that group 1 *Aeromonas hydrophila* strains, isolated from disease pesthole, were in decreasing order sensitive to : Eurofloxocin, Florfenicol (Floron) and Oxitetracycline and resistant to Ampiciline, Amoxicilin, Eritromicin.

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