Investigation on the Incidence, Diagnostic, Possibilities and Design of Prophylactic Treatment in an Outbreak of Bacterial Haemorrhagic Septicemia of Carp

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Abstract

The investigations carried out on a sample of 53 breeders (31 females and 22 males), in the aquaculture farming system of Suatu village, County of Cluj, concerning the incidence of bacterial hemorrhagic septicemia, its diagnosis and prophylactic-therapeutic possibilities. The results highlight an incidence of bacteriosis differentiated according to the category of breeders, 54.8% in females and 36.3% in males. Necropsy examination carried out on 3 specimens of suspected bacteriosis, highlights the presence of hemorrhagic skin lesions, which are dotted or diffuse, respectively musculo-cutaneous necrosis and ulcers. Regarding the internal injuries, opening of the body cavity, revealed consistency changes and septicemia form in major internal organs, as well as the presence of a sanguinolent appearance liquid. The bacterioscopic examination of smears from the pathologic material (skin surface and internal samples) colored with the Gram method, revealed bacterial microflora, represented by coccobacillus and straight bacilli or slightly curved, both Gram negative. Furthermore, the bacteriological (culture) examination performed on usual and selective culture media, distinguished the isolation of pure culture bacteria belonging to the genus *Aeromonas*. The exam of biochemical traits, with the aid of API 20 E multitest determines that the isolated strains of *Aeromonas* spp. belong to the species *Aeromonas hydrophila*, indicted in the emergence and evolution of the bacterial hemorrhagic septicemia. Testing strains sensitivity to antibiotics and chemotherapy (through antibiogram level), reflects the isolated bacteria's values of sensitivity in decreasing order: amoxicillin, oxytetracycline, florfenicol and enrofloxacin. However, reduced sensitivity was recorded to tetracycline and ampicillin and resistance to erythromycin.

Keywords: carp, diagnosis, investigations, prophylactic treatment

INTRODUCTION

As previously stated, the term of bacterial hemorrhagic septicemia was already discovered since 1933 (Snieszko and Bullock, 1976) and it is known by various synonyms such as red-mouth disease, infectious dropsy, rubella disease or red-pest. The disease was described by several authors

since then (Bohai *et al.*, 1993, Joseph and Carnahan, 1994, Austin and Austin, 2007) and considered as opportunistic pathogens. The same pattern was found as well in Romanian lakes affecting carp breeders (Bud *et al.*, 2001, Dăscălescu and Costea, 1995, Negrea, 2007). The aim of the investigations was to assess the incidence, the possibilities of

diagnostic and design of prophylactic treatment in the case of an outbreak of bacterial hemorrhagic septicemia in carp breeders, raised in a traditional system. Thus, the focused on the incidence of the disease based on the clinical diagnosis and laboratory techniques for the pathogen detection and also on the prophylactic and therapeutic conduct which can be applied in the outbreak.

MATERIALS AND METHODS

Investigations were conducted in Cluj county, in an aquaculture farming system from Suatu, specialized in raising carp in an extensive manner, on a group of 53 breeders (31 females and 22 males). The aquaculture farming system has a total surface of 50 ha and it is structured in 3 lakes: Suatu, with a surface of 30 ha, Bercheș with a surface of 12 ha and Aruncuta with a surface of 8 ha. Among the existing species we can mention the common carp (*Cyprinus carpio*) crucian carp (*Carassius gibelio*) and other species such as the rudd (*Scardinius erythrophthalmus*) and the roach (*Rutilus rutilus*), etc.

Among the breeders diagnosed with anatomic and clinical lesions with external bleeding, a number of 3 were badly affected. On these samples

bacterioscopic and bacteriological examinations were performed, in order to clearly establish etiological diagnosis, within the laboratories belonging to the Ihtiopathology Department, Faculty of Animal Science and Biotechnologies, Cluj Napoca.

RESULTS AND DISCUSSIONS

The results of the investigations carried out on 53 breeders (31 females and 22 males), reveals that the incidence of the bacterial hemorrhagic septicemia occurs differently based on gender (Table 1).

In this breeders group it seems that the incidence of the disease is higher in the female breeders (54.8 %) compared to the values in the male breeders at 36.3 %. Same results are visually highlighted in Fig.1.

The morphopathology examinations at skin and visceral levels, reveals the presence of numerous bleeding spots at the base of the fins and on the body surface as well. Same evolution of the infections were described previously by Austin B. and Austin D.A. in 2007, on other freshwater fish. As a result of the hemorrhagic necrotic processes, the tissue necrosis occurs after a period of time.

Tab.1. The incidence of the bacterial hemorrhagic septicemia of carp

No.	Breeder group	No.	Positive	Positive %
1	Females	31	17	54,8
2	Males	22	8	36,3
Total		53	25	47,1

Fig. 1. SHB incidence variation based on gender

This was noticed as white-gray color spots which favours limitation and detachment with the emergence of ulcerative lesions. This status can evolve and affect the muscles as well. It should be noted the fact that these ulcerative traumas are facilitating the invasion and fast development of pathogenic fungus from *Saprolegnia* genus. There are visible as well other areas of necrosis, more or less spreaded at the level of the fins, including the caudal one. Beside the external lesions, during necropsy, we noticed haemorrhagic and/or haemorrhagic- necrotic changes at the level of the internal organs, with the presence of a sero-sanguineous liquid in the body cavity, in organs such as spleen, kidney, liver, both increased in volume and the presence of yellowish gray outbreaks (necrotic processes).

The bacterioscopic examination of the smears made on the pathological samples harvested from the level of the skin lesions and stained by the

Gram method – described by Dăscălescu P, Costea M (1995) - highlights in the microscopic field (ob.100x) bacterial microflora, represented by the coccobacilli and straight or slightly curved bacilli, Gram-negative.

The bacteriological examination (cultured samples) conducted on the pathological samples harvested from three breeders severely affected, performed on culture mediums (broth agar, blood agar) allowed the isolation of a pure culture of micro-organisms of the genus *Aeromonas*.

Following the examination of the biochemical samples with a API 20 E multitest system we encountered the following positive reactions to: ADH, INDOL, GELATINAZA, GLU, MAN, SAC, AMY, ARA, on the tested strains.

The results of the biochemical samples obtained after the usage of the API 20 E multitest showed that the isolated strains of *Aeromonas* spp. belong to the *Aeromonas hydrophila* species,

Fig. 2. Congestive and hemorrhagic lesions in muscles and internal organs

Fig. 3. *A. hydrophila* morphology in a carp skin sample, Gram stain

group I, as it can be observed in. Fig 5. Similar findings were revealed previously by Joseph SW, and Carnahan AM (1994).

After testing the sensitivity of the *Aeromonas hydrophila* strains challenged with different

drugs by the antibiogram method (diffusimetric method) we noticed that the isolated strains of *Aeromonas hydrophila* from the analysed outbreak were sensitive, in an increasing order to the following antibiotics: amoxicillin, florfenicol,

Fig 4 (a, b). *Aeromonas hydrophila*, on agar and serum culture revealing round, smooth and shiny colonies

Fig 5. Positive biochemic reactions revealed by the multitest API 20 E system

Fig.6. *A. hydrophila* sensibility test through antibiogram technique

enrofloxacin, oxitetracyclin. The sensitivity of the bacterial strains was reduced under tetracyclin while other strains were resistant to erythromycin and ampicillin.

CONCLUSIONS

The incidence of bacteriosis presented varied values depending on breeder's gender group, 54.8% in females and 36.3% in males.

The necropsy performed on 3 specimens with supposed bacteriosis revealed that the presence of hemorrhagic lesions on skin (diffuse or dotted) and necrosis of skin and muscles.

The bacterioscopic examination of smears from the pathologic material (skin surface and internal samples) and colored with the Gram method, revealed bacterial microflora, represented by coccobacillus and straight bacilli or slightly curved, both Gram negative.

The bacteriologic examination performed on normal and selective media revealed as well the isolation of the *Aeromonas* germs. The examination of biochemical traits, with the multitest system API 20 E showed that isolated germs of *Aeromonas* spp belong actually to the *Aeromonas hydrophila* strain, incriminated for the appearance and evolution of the outbreak of bacterial hemorrhagic septicemia.

Testing the sensitivity to antibiotics and chemotherapy of the diagnosed strains reflects the followings: a) values of sensitivity for isolated bacteria were in ascending order: amoxicillin, oxytetracycline, florfenicol and enrofloxacin, b) reduced sensitivity to tetracycline and c) resistant to ampicillin and erythromycin.

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