

## PRESENCE OF BACTERIAL DISEASES OF FISH IN THE SERBIA DURING THE PERIOD 2005-2010.

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### PRISUSTVO BAKTERIJSKIH BOLESTI RIBA NA PODRUČJU SRBIJE U PERIODU OD 2005. DO 2010. GODINE

#### **Abstrakt**

U petogodišnjem istraživanju prisustva bakterijskih bolesti riba u Srbiji, ispitano je 28 ribnjaka na kojima se uzgaja kalifornijska pastrmka (*Oncorhynchus mykiss*) i 15 ribnjaka u kojima se uzgajaju šaran (*Cyprinus carpio*) i srodne vrste. Tokom istraživanja fenotipski smo identifikovali 120 izolata iz 2320 uzoraka riba, i utvrdili prisustvo jersinioze, furunkuloze, renibakterioze, eritrodermatitisa i septikemije izazvane pokretnim aeromonadama. Najčešće su detektovane infekcije uzrokovane sa *Yersinia ruckeri*, *Aeromonas hydrophila* i *A. salmonicida*, ali je utvrđeno i prisustvo infekcija uzrokovanih sa *Pseudomonas putida*, *Flavobacterium psychrophilum* i *Pseudomonas fluorescens* su također osnovane. Takođe, po prvi put je utvrđena pojava *Janthinobacterium lividum* infekcije i oboljenja crvenih pega.

**Ključne reči:** bolesti riba, bakterijske bolesti

#### INTRODUCTION

The disease is the primary limiting factor in aquaculture and may significantly affect the economic and socio-economic development in many countries (Subasinghe and Bernoth, 2000). The appearance and development of fish diseases is a consequence of the interaction of pathogen, host and environment. The relatively small number of pathogenic bacteria is responsible for major economic losses in cultured fish (Toranzo et al., 2005). Antibacterial therapy is usually administered through healing foods. Environmental contamination in the vicinity of ponds occurs mostly through feces and uneaten food (Hirsch et al., 1999). In addition to the release of active substances in aquatic ecosystems, the usual therapeutic interventions in aquaculture can lead to antibiotic resistance in bacterial pathogens of fish, but also in other bacteria present in the environment

(Alderman and Hastings, 1998). Many diseases affecting farmed fish also represent a threat to natural fish populations (Thoesen, 1994). Any systematic study of fish populations will certainly lead to establishing the presence of infectious disease. Whether this is indeed a newly diagnosed disease or disease has not previously observed, remains unclear. New pathogens can take the place of existing (Austin and Austin, 2007). Therefore, the ponds need regular control in order to establish the presence of pathogens. The aim of this study was to determine the presence of bacterial fish diseases in Serbia during the 2005-2010 in 28 trout and 15 carp farms.

## MATERIAL AND METHODS

Samples for bacteriological examination were collected from 28 trout and 15 carp farms. As a material for testing parenchymatous organs, skin and gills of clinically diseased fish were used. A total of 2320 samples of altered organs of fish were examined bacteriologically. Sampling was carried out through repeated visits to fish farms in situations in which it was necessary to solve existing health problems. Fish for examinations were transported to the laboratory and dissected for bacteriological testing. In all fish, external and internal examination was performed. Gills and body surface were examined microscopically for the presence of *Flavobacterium* sp. Then, the surface of the body was swabbed with 70% ethyl alcohol to prevent contamination. Liver, kidney and spleen were inoculated in trypticase soy agar (TSA), blood agar (BA), furunculosis agar, Mueller-Hinton agar (MH), Rimler-Shotts agar (RS), KDM-2 agar and brilliant green agar (BG), depending on the origin of fish. Samples from external lesions were inoculated on cytophaga agar and trypticase soy agar. After incubation at 20-25°C for 4 days or at 10-15°C for 10 days, isolated bacteria were subcultivated in order to test the purity of isolates. Pure culture of isolated colonies were biochemically characterized using API 20E, API 20NE (Biomerieux), and following biochemical tests were performed: Gram stain, cytochrome oxidase, catalase, beta-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H<sub>2</sub>S production, urease, tryptophan deaminase, indole production, Voges-Proskauer, gelatinase, fermentation of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, amygdalin, methyl red, arabinose, lactose, esculin, xylose, mobility, and oxidative / fermentative test. The isolates were identified to the genus or species level based on standard bacterial taxonomic procedures (Krieg and Holt, 1984, Holt et al, 1994; Austin and Austin, 2007). The isolates were stored in broth with the addition of 15-20% glycerol at -80°C. For confirmatory identification of *Renibacterium salmoninarum*, direct immunofluorescence (FAT) and PCR were used.

## RESULTS AND DISCUSSION

During the five-year study of presence of bacterial fish diseases in the Republic of Serbia, carried out in a total of 43 fish farms, following diseases were founded:

**Yersiniosis** - yersiniosis is a bacterial disease caused by gram-negative bacteria *Yersinia ruckeri*. The disease is widespread and occurs in most countries where salmonids are intensively cultivated. In Serbia, the disease was first diagnosed in 1987 (Ocvirk et al., 1987). In the period 2005 - 2010, yersiniosis was present on majority of examined trout farms in a one-year old rainbow trout. In addition to nonspecific symptoms (dark pigmentation, exophthalmia, ocular hemorrhages), subcutaneous bleeding in the

mouth were present. The internal lesions were represented by petechial hemorrhage in pyloric caeca and perivisceral fatty tissue, edema of the kidney and spleen, as well as the absence of food from the intestines, which were filled with yellow, mucous content. *Yersinia ruckeri* was isolated from the spleen and kidney of diseased fish after incubation in aerobic atmosphere for 48 hours at 20°C. However, despite these cases, where the disease was caused by highly virulent "Hagerman" strain, with severe clinical and major morbidity (40%), in a few trout ponds we have isolated low virulent strains of *Yersinia ruckeri*, without the presence of specific clinical symptoms, and mortality did not exceed the technological limit.

**Carp erythrodermatitis** - Carp erythrodermatitis is a subacute to chronic skin disease that occurs at temperatures from 4 to 30°C (Pol et al. 1980). The etiologic agent of Carp Erythrodermatitis was isolated and described as an atypical, achromogenic variant of *A. salmonicida* (Bootsma et al. 1977; Bootsma and Blommaert 1978). The disease was first diagnosed in the former Yugoslavia in 1977 (Bootsma et al., 1977). In our study, disease was found in young carp in a number of fish farms. Chronic form of erythrodermatitis was diagnosed in carp yearlings during the spring period. At low temperatures in winter and spring disease develops slowly and runs with considerable losses. In summer, acute form of carp erythrodermatitis was present in a number of carp farms. Clinical signs consisted of deep ulcers accompanied by peripheral necrosis which was present in the epidermis and extended into the underlying musculature, hemorrhagic inflammation at the base of the fins, and slight to extreme exophthalmia. The symptoms were limited to external involvement only, and no pathologic signs were apparent on gross examination of the internal organs. Achromogenic atypical *Aeromonas salmonicida subsp. nova* was consistently isolated on trypticase soy agar containing 0.01% Coomassie brilliant blue from samples taken from the periphery of small ulcers.

**Furunculosis** - contagious disease caused by gram-negative bacteria *Aeromonas salmonicida subsp. salmonicida*. The first case of the disease in Serbia reported in 1972 (Snoj and Brglez, 1972). The disease is present in European countries with intensive Salmonids cultivation, and also in North America, South Africa and Japan. Improvements of cultivation technology and adequate health care have led to a reduction in mortality and incidence of clinically overt disease, although the disease is still present. In our study, furunculosis with characteristic clinical symptoms was detected in four examined trout pond. In one case the disease was diagnosed in grayling. *Aeromonas salmonicida subsp. salmonicida* was isolated from the lesions, spleen and kidney of diseased fish on TSA and furunculosis agar, after incubation under aerobic conditions for a 48<sup>h</sup> at 20°C.

**Bacterial kidney disease** - Bacterial Kidney Disease (BKD) is a systemic infection of salmonids caused by the gram-positive diplobacillus *Renibacterium salmoninarum*. The disease has a wide geographic range. In our study, during the period 2005 - 2010, five cases of BKD were diagnosed. In all cases the disease was detected in a one-year old rainbow trout. Infected fish showed pale gills, exophthalmia, and abdominal distension. Turbid fluid was present in abdominal and pericardial cavities, and creamy-white granulomatous lesions were present in the kidney and, less frequently, in the spleen and the liver. Cumulative mortality was around 35%. Direct immunofluorescence test and PCR showed the presence of *R. salmoninarum*, a bacterium was isolated from the kidney of diseased trouts in KDM-2 agar.

***Janthinobacterium lividum* infection** - *Janthinobacterium lividum* is a Gram-negative bacteria, belonging to the family Oxalobacteraceae. Typical and atypical forms of

*J. lividum* is considered as normal microflora of water and soil (Sneath, 1984). However, several cases of the disease of rainbow trout caused by the bacteria, with a mortality rate about 30% was reported (Austin et al., 1992, Austin et al. 2003), and cases of human infection with fatal outcome were described (Patijanasoontorn et al., 1992). During december 2007 increased mortality (20%) in one-month old trout weighting 0.5 - 1.0 g, with lethargy, pale gills, dark skin pigmentation and abdominal enlargement was found. Pathoanatomical examination revealed oedema of kidney, liver and spleen ischemia, and presence of moderate amounts of clear liquid. Based on morphological, physiological and biochemical characteristics, isolated bacteria were identified as *Janthinobacterium lividum*.

**Red mark syndrome (RMS)** is a chronic and typically non-lethal skin condition affecting rainbow trout (*Oncorhynchus mykiss*) (Verner-Jeffreys et al., 2006). The condition is characterised by single to multiple skin lesions, typically found on the flanks. These lesions can affect carcass quality, which downgrades the product and lowers its market value. Morbidity ranges up to 80% and the disease affects fishes from 15cm in length to brood stock size (Pond, 2007). Results of field investigations indicated the condition had an infectious aetiology, but the cause could not be definitively proved (Ferguson et al., 2006; Verner-Jeffreys et al., 2008). Red mark syndrome was determined at a one trout farm in 2008, at two trout farms in 2009, and in four trout farms in 2010. The affected fish showed with a range of lesions of differing severity. Clinical signs include appearance of bright red, raised; circumscribed, ulcerated and indurated lesions in the skin that usually range up to 3 cm or more in diameter. Microscopically, the disease was characterized as a subchronic, focal, non-suppurative dermatitis with extensive lymphocytic infiltration. However, despite extensive analysis, including mycology, bacteriology and virology, no single potential disease agent was isolated from affected fish.

**Motile aeromonad septicemia** - Bacterial infections caused by motile members of the genus *Aeromonas*, are among the most common and troublesome diseases of fish raised in ponds and recirculating systems. The widespread distribution of these bacteria in the aquatic environment and the stress induced by intensive culture practices predisposes fish to infections. In our study, motile aeromonad septicemia in carp (*Cyprinus carpio*), grass carp (*Carassius carassius*), grass carp (*Chenopharyngodon idella*) channel catfish (*Ictalurus punctatus*) and rainbow trout (*Oncorhynchus mykiss*) was present in a number of fish farms. In addition, sporadic cases of infection with *Pseudomonas putida*, *Flavobacterium psychrophilum*, and *Pseudomonas fluorescens* in these species was detected.

## CONCLUSIONS

The most commonly reported bacterial fish diseases in Europe are vibriosis, pasteurellosis, yersiniosis, furunculosis, flexibacteriosis, columnaris disease, motile aeromonad septicemia, pseudomonas infections, streptococcosis, mycobacteriosis. Most of these diseases were detected in this survey. The occurrence of diseases that previously did not report warns that the measures taken to protect the health of the fish are not enough. We need to invest additional effort in order to maintain the health status of fish populations through the use effective biosecurity measures.

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