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# Red Mouth Disease in Rainbow Trout *(Oncorhynchus mykiss)* - a Case Report on Lake Trout Farm from Bicaz, Romania

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**Abstract.** Yersiniosis or red mouth disease is a contagious infectious disease of salmonids caused by the bacterium Yersinia ruckeri, which lead to significant economic losses in trout aquaculture worldwide. Sources of infection are the sick fish and asymptomatic carriers, which eliminates yersiniosis faeces, causing contamination of water and feed.

Triggers the disease was in March, the surge in water temperature values of 15-17$^0$ C. The fish affected were the younger with low immune system, the sudden departure of the winter period. Histological examinations, immunohistochemical and bacteriological made from liver, spleen, kidney and blood of the trout heart yersiniosis suspects resulted in the identification of the species bacterium Yersinia ruckeri strain, confirming the suspected diagnosis based on clinical investigations (pronounced anemia of mouth and gills mucosa, dark color of skin, bleeding on the lingual mucosa, protruding eye bleeding).

Bacteriological confirmation was done by identifying the causative agent, based on morphological characters (gram negative bacilli, mobile) and the distinctive biochemical characters tested using the API rapid tests.

Histological examinations revealed colonies of bacteria in liver, spleen and kidneys were confirmed to be colonies of Yersinia ruckeri by immunohistochemistry with anti-Yersinia ruckeri.

Antibiogram revealed sensitivity of Yersinia ruckeri at: Oxytetracycline, Flumequin, Trimethoprim and Ceftiofur, and moderate susceptibility to Amoxicillin and Enrofloxacin.

**Keywords:** trout, yersiniosis, immunohistochemistry, diagnosis.

## INTRODUCTION

Etiologic agent of red mouth disease is the bacterium *Yersinia ruckeri*, very virulent bacteriosis from fam. *Enterobacteriaceae*, originally isolated in 1950 from rainbow trout (*Oncorhynchus mykiss*) in the Hagerman Valley of Idaho, USA, by R. Rucker (quoted Ghittino P., 1985). Now bacteria are found in populations of salmonids in North America, Australia, South Africa and Europe. Economic losses caused by this disease are translated by high mortality, up to 95%.

In Romania first case of Yersiniosis was reported in 1997 after several cases were described in rainbow trout of different ages (Popescu et al., 2008). Sources of infection of fish were sick and asymptomatic carriers that eliminate *Yersinia* faeces, contaminating food and water (Bullock et Cipriano, 2004, Ghittino 1985).

Developed acute illness due to sudden temperature change in March-April, causing thermal discomfort in trout held in net-cages. Following the immunosuppression caused by heat stress, overcrowding, there was a penetration of the skin with opportunistic or virulent

strains of *Yersinia ruckeri* removed from sick fish (Guguianu and Miron, 2002; Guguianu, 2009, Noga 1999).

Clinical symptoms are nonspecific, common for the most septicaemic infections: anorexia, decreased appetite, apathy, swimming on the surface of the water, hipermelanosis, bleeding diathesis, splenomegaly. (Avci, H., S.S. Birincioglu, 2005, Boudony, 1981).

Yersiniosis suspicion was given by the black skin, pale gills, hemorrhages and bleeding point on the lingual mucosa and eye (Austin and Austin, 2007, Tobback, E. 2007).

## MATERIAL AND METHOD

The research was performed on 21 rainbow trout (*Oncorhynchus mykiss*) patients, ranging in size from 8-15 cm, which were performed bacteriological investigations, histology and immunohistochemistry.

Sowings were made from kidney and heart agar TSA (Trypticase soy agar) poured into Petri plates. Seeded plates were incubated at room temperature (22-25°C) for 48 hours.

Identification of isolates was based on cross-cultural aspects and morphology in Gram stained smears with metabolic properties tested using API galleries, bands and oxidase reaction of reagents (hydrogen peroxide, methyl red 0.2 ‰).

Susceptibility testing of strains of *Yersinia ruckeri* to antibiotics and chemotherapy was performed by antibiotic, diffusimetrical method, using the MÜLLER HINTON and microcomprimate of Oxytetracycline, Amoxicillin, Enrofloxacin, Flumequin, Trimethoprim and Ceftiofur.

Samples taken from liver, spleen, kidneys were fixed in formaldehyde 10% aqueous solution, included in paraffin, sectioned and stained by the method Hematoxiline - Eosin (HE) and DAB - Pap hematoxylin for immunohistochemistry. Immunohistochemical examinations were carried out in lab Faculty of Veterinary Medicine of Bologna, Italy.

## RESULTS AND DISCUSSIONS

External examination of the whole fish was found increased lesion polymorphism, consisting hipermelanosis, congestion and petechiae on the body sides, fragmentation caudal fin, protruding and bleeding eye, scoliosis (one case). Lesions consistently present in all fish investigated were growing pale gills and mouth, petechial hemorrhages lingual and eye (Fig. 1, Fig. 2, Fig. 3).

Bacteriological examinations carried out in anterior kidney and blood of the trout heart yersiniosis suspects resulted isolation of bacterial strains of the species *Yersinia ruckeri* respecting working protocol specific to each type of investigation (Dascalescu 1995).

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At necropsy it was observed that the internal injuries were the lack of food content and gaseous distension of the tube, splenomegaly and bleeding perivisceral fine fat (6 cases).

Sowings made resulted in obtaining pure cultures of colonies formed type, “S” small, uniform white-gray.

Microscopic examination of smears of cultures revealed Gram-negative bacilli and coco bacilli, straight and slightly curved, medium size, without a group characteristic.

Morphology of germs isolated, negative reaction to test and oxidase positive catalase test were the criteria for choosing the API 20E galleries (enterobacteriacea identification system) for inventory of equipment enzyme isolates.

Metabolic profile of the 20 isolates tested using miniaturized biochemical tests is shown in Fig. 4 and Fig. 5.

The statement of results, tests are divided into groups of three and have a value of 1, 2 or 4. Summing the appropriate values in each group positive reactions was obtained combination of numbers, 5104100.

Since the API 20 E analytical catalog number corresponds to the profile of the species *Hafnia alvei*, and *Yersinia ruckeri*, the tie was made extra xylose fermentation test. Inability to ferment xylose strains tested, justified, according to data from literature, their classification in the species *Yersinia ruckeri* (Austin and Austin 2007).

Antibiogram made *Yersinia ruckeri* strains showed sensitivity towards Oxytetracycline Flumequin, Trimethoprim and Ceftiofur and moderate susceptibility to amoxicillin and enorfloxacin.

Histological examinations of fragments from the liver confirmed the presence of bacteria located in small groups against a vacuolare dystrophies and areas of necrosis (Fig. 6, Fig. 7, Fig. 8, Fig. 9).

The kidney were noted at histological examination of bacterial colonies around urinary tubules compress. Around bacterial colonies were observed melano-macrophages centers (MMC) and erythrocyte mass, which demonstrated inflammation at this level (Fig. 10, Fig. 11).

Immunohistochemical method was used to highlight the bacteria *Yersinia ruckeri* with brown color was a new technique used in Romania to identify *Yersinia* bacteria in fish, using polyclonal anti-*Yersinia ruckeri* applied on sections included in paraffin after a preliminary dewaxing. Thus, were identified by comparison with controls brown bacterial colonies in the liver, spleen and kidney (Fig. 12, Fig. 13 Fig. 14 Fig. 15, Fig. 16, Fig. 17) (Lazăr 2009).

## CONCLUSIONS

- The diagnosis consisting of Yersioniosis of the trout can be suspected on the base of the septicemic evolution, of the oral haemorrhagic lesions and of the profound anemia estate. Confirmatory diagnosis requires isolation of causal agent of internal organs, anterior kidney binding (equivalent to mammalian bone marrow).
- Biochemical identification of *Yersinia ruckeri* species is easily achieved using the API 20 E galleries.
- Given the behavior of different strains of *Yersinia ruckeri* to antimicrobial substances tested, recommended the establishment of antibiotic on the basis of antibiogram.
- Immunohistochemical examination has a significant role in determining with certainty the diagnosis of yersiniosis in trout.

Fig. 1. Dark color of the skin in the dorsal region

Fig. 2. Exophthalmia and red spots in mouth cavity

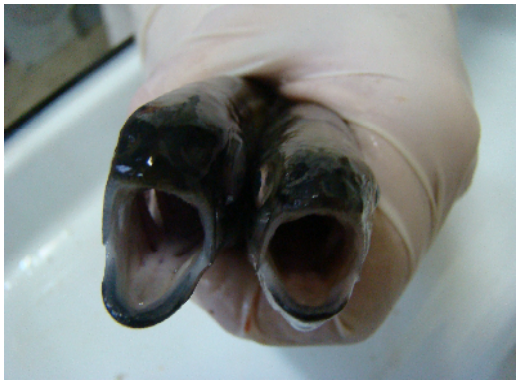

**Fig. 3.** Colurless of the buccal mucous

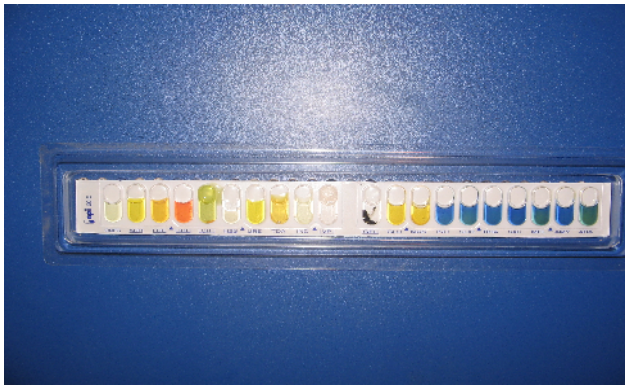

**Fig. 4.** Profile obtained after 24 hours of incubation of Yersinia ruckeri strain, using colonies given from the TSA surroundings

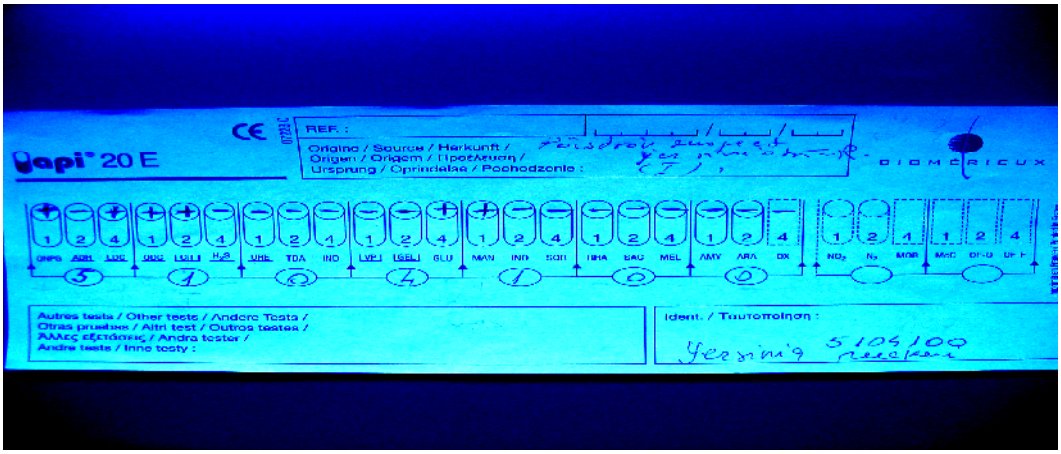


**Fig. 5.** API 20E Tests results

***Legend**: ONPG = orto-nitrofenil-galactopiranozidasis; ADH=arginin-dehidrolasis; LDC=lizin-decarboxilasis; ODH=ornitin-decarboxilasis; CIT= using citrates; U=ureasis; TDA=triptophan-desaminasis; IND=indol; VP=Voges-Proskauer; GEL= gelatinosis; GLU= fermentation glucoses MAN= fermentation manitosis; INO= inositolisis fermentation; SOR= sorbitol fermentation; RHA= rhamnosiosis fermentation; SAC= zacharosis fermentation; MEL= melibiosis fermentation; AMI=amigdalinosis fermentation; ARA=arabinosis fermentation; OX=citocrom-oxidasis.*

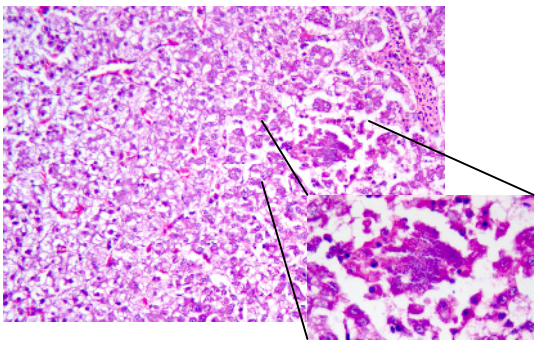

**Fig. 6.** Section through liver. Pronounced vascular dystrophy having necrotic centre's rich in bacteria. Col. HE, x100

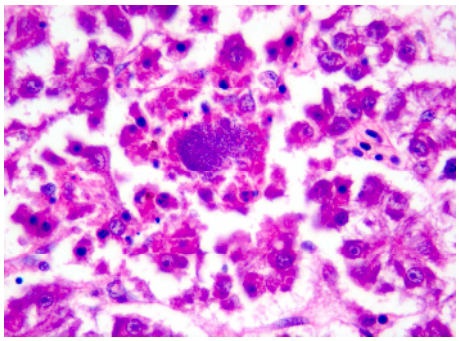

**Fig. 7.** Bacterial colonies in the liver. Col. HE, x 630

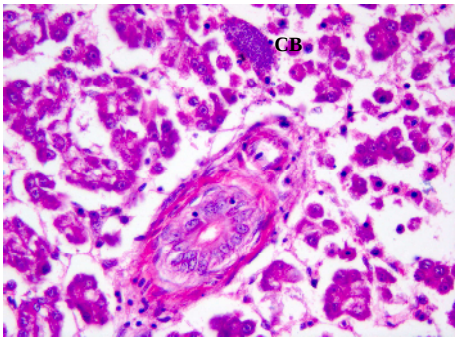


Fig. 8. Bacterial colony (CB) in the biliary duct proximity.
Col. HE, x400

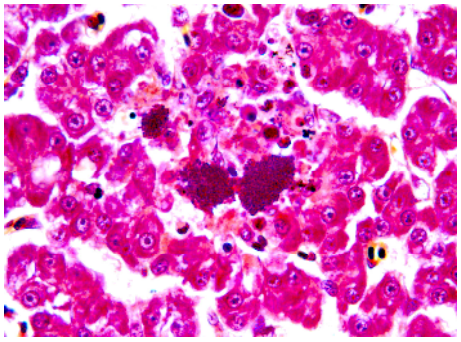

Fig. 9. Liver. Specifically red color of the negative Gram bacteria.
Col. Gram, x 400

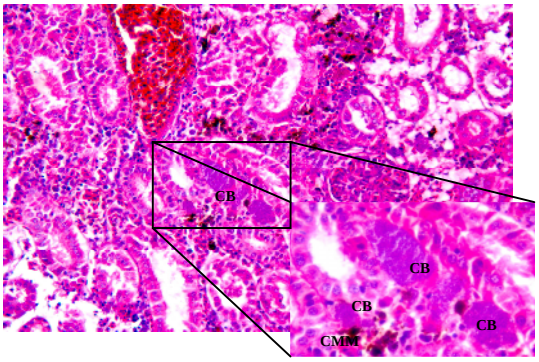


Fig. 10. Flat urinary tubes surrounded by the bacterial colonies (CB). Melanin-macrophages centers (CMM).
Col. HE, x 200

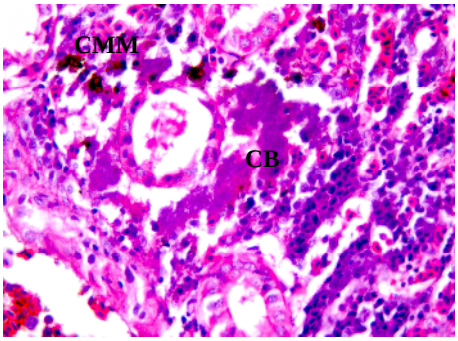


Fig. 11. Kidneys. Urinary tube surrounded by bacterial agglomeration (CB). Melano-macrophagic centers (MMC). Red blood cells. Col. HE, x 400

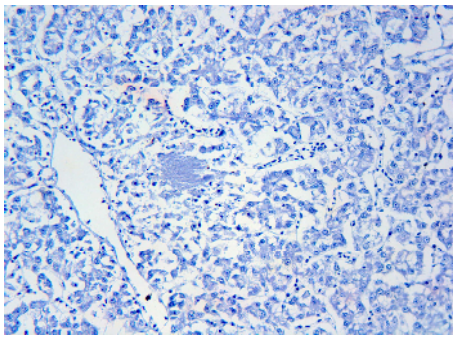

Fig. 12. Liver. Witness lot.
Col. Hematoxilina Papanicolau, x 100

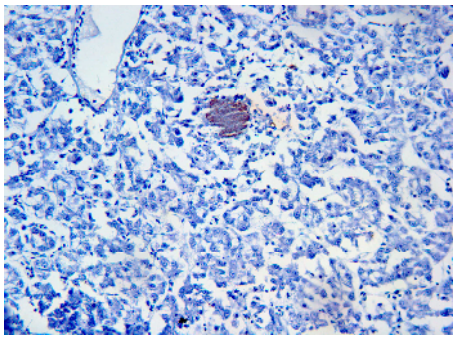

Fig. 13. Liver. Brown color of *Yersinia ruckeri* colony.
Col. Hematoxilina Papanicolau, x 200

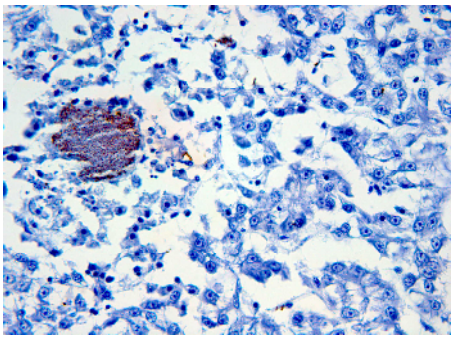

Fig. 14. Liver. Brown color in *Yersinia ruckeri* colony.
Col. Hematoxilina Papanicolau, x 400

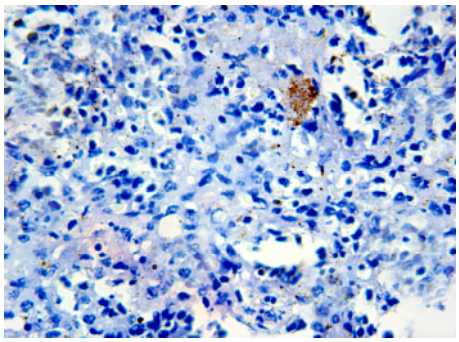

Fig. 15. Spleen. Brown colonies of *Yersinia ruckeri*.
Col. Hematoxilina Papanicolau, x 630

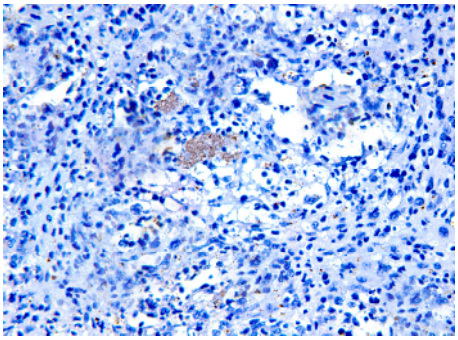

**Fig. 16.** Spleen. Brown colonies of *Yersinia ruckeri*
Col. Hematoxilina Papanicolau, x 200

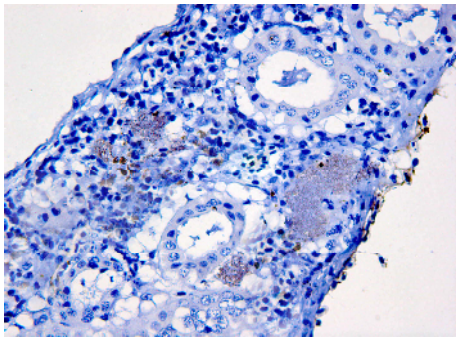

**Fig. 17.** Kidney. Brown colonies of *Yersinia ruckeri*
Col. Hematoxilina Papanicolau, x 400

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