

## THE USE OF CONJUGATED EFFECT OF THE BACTERICIDAL TREATMENT FOR YERSINIOSIS IN RAINBOW TROUT

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### Abstract

Enteric red mouth disease (ERM) or yersiniosis, is an infectious and contagious disease, that is wildly spread across the world. In Romania, trout culture is facing this serious disease that causes a high mortality among fish population, varying between 10 and 60%.

The etiologic agent of the disease is the virulent bacterium *Yersinia ruckeri*, from the Enterobacteriaceae family. This bacterium is considered strictly pathogenic, although it can survive a few months in water and sediments. The sources of infection can be the ill fish, carriers of the pathogen, that spread the bacteria in the water through their excrements of the poorly maintained ponds, water and sediment, farm equipment, bird excrements, some mammals. The predominant factors leading to yersiniosis disease are poor fish rearing conditions (accumulations out of the accepted limits for organic substances, nitrates, oxygen deficit, presence of periphyton etc.) combined with adaptive stress. When the poor conditions lead to an immunity loss of the fish and the disease sets in.

Purpose of this paper is to find new treatments that act simultaneous internally on the pathogen inside the trout through administration of antibiotics in food, but also externally by destroying the residual bacteria found in mud and periphyton, through administration of non-toxic disinfectants, which does not cause side effects to the antibiotic medication.

The work method consists in applying the same antibiotic treatment in the two ponds (B2, B3) under identical growth conditions and density, selected from the same batch of trout yersiniosis, with the difference that in the B3 pond, oral administration of medication was cumulated with long term peracetic acid baths.

The diagnosis of Yersiniosis was based on the identification of the first symptoms of the disease and was confirmed by microbiological examination and the treatment was based by sensitivity tests which decided the type of administered antibiotic.

Results were quantified by monitoring mortalities in the two ponds and shows that in the B3 pond where the trout was treated with antibiotic and disinfectant, the recorded mortality is 3.18 % lower.

The current experiment, using an antibiotic treatment administered orally, combined with a general disinfectant, administered in the water, peracetic acid, reduced the rainbow trouts mortality caused by Yersiniosis.

**Key words:** Yersiniosis, treatment, antibiotic, peracetic acid

### INTRODUCTION

In human nutrition, fish plays an important and unique role by contributing with specific nutrients, proteins, unsaturated fatty acids (fish-specific), minerals, micronutrients and vitamins to the diet.

The global demand for protein consumption is on the rise, as a result of demographic growth. As a result, the aquaculture sector registers the fastest development and has a production potential of approximately 50% of the global fish consumption [3].

In our country, salmon culture is one of the branches with increasing development. Among the salmonids species profitable for farming, rainbow trout (*Oncorhynchus mykiss*

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Walbaum, 1792) stands out. The rearing density of trout and the environmental factors variations can generate the disease state. Yersiniosis, or red mouth disease (ERM), is an infectious and contagious disease that causes high mortality amongst trout, reaching values between 10 and 60% [5]. This septicemic disease has as etiological factor a virulent bacteria from the Enterobacteriaceae family, Gram-negative rod-shaped, facultative anaerobically organism [2].

Yersiniosis can manifest itself through unspecific clinical symptoms, common to septicemic infections, such as decreased appetite, apathetic swimming or on the water surface (due to gills dysfunction), exhaustion, hypermelanosis, haemorrhage and splenomegaly [4].

The usually evolution shows a pathognomonic symptomatology, presented by oral haemorrhage, unilateral or bilateral exophthalmia, subcutaneous haemorrhage at the base of pectoral and pelvic fins, abdominal bloating with yellow liquid accumulation in intestines, inflamed anus.

Fish dissection shows haemorrhages in liver, pyloric appendages, the intestine filled with viscous-yellowish fluid specific to gastroenteritis.

The disease can be transmitted by sick fish, which can spread the bacteria in water through their excrements [7]. Other carrying sources of the pathogen can be poorly maintained ponds, water and sediments, contaminated farm equipment, birds excrements, mammals and human [5].

*Yersinia ruckeri* bacterium is considered strictly pathogenic, although it can survive a few months in water and sediments. The biofilm that forms on submerged surfaces is the result of bacteria colony multiplication, which adheres with the flagella on the substrate. Recent studies demonstrated that biofilm formation represents an important characteristic for bacteria survival on the surfaces and in the sediments from aquatic environments.

The presence of periphyton (composed of phytoperiphyton, zooperiphyton and bacterioperiphyton) in ponds, although it represents a nutrient rich source of food for trout, it increases the risk of fish contamination with *Yersinia ruckeri*.

The purpose of this thesis is to find new solutions to complete treatment of yersiniosis in ponds that can not be emptied, through the use of an orally administered antibiotic treatment, combined with a general disinfectant with bactericidal properties – peracetic acid [1], in order to increase the biocidal effect of the treatment meant to reduce mortality in trout infected with *Yersinia ruckeri*.

## MATERIALS AND METHODS

The object of the study is represented by 2 rainbow trout populations, affected by yersiniosis, from 2 identical ponds belonging to a trout farm from Neamț county.

The observations were made during a 16 days period between 08.27 and 09.11.2018. The biological material, having an age of 14 months with an average length of 21 cm and an average live weight of 105 g, was reared with a density of 1000 kg / 330 m<sup>3</sup>, in identical conditions for both ponds B2 and B3. The ponds have a stone paved bottom and a cement slope. Periphyton developed on the perimeter of both ponds.

Rainbow trout was fed with a ration representing 1% of its weight, with pelleted feed Aller and the size of the pellets was 4,5 mm. Water chemistry was within the allowable variation limits for mountain waters, and dissolved oxygen in water, during the entire experiment, varied between 7-9 mg/l. The antibiotic treatment was carried out with the same dosage and for the same period of time (08.31-09.09.2018) in both ponds. In pond B3, antibiotic was administered alongside a disinfectant (peracetic acid 15% concentration, under the name Divosan Forte), with a daily dosage of 750 ml/ 100m<sup>3</sup> water, for 7 days, between 08.31. and 09.06.2018.

The complete examination included the parasitological examination, performed on a wet smears, mounted on glass slide with a cover slip, examined under a Oxion Euromex microscope, equipped with Ccmex3.0 MP camera and under Olympus SZ 61 stereomicroscope.

## RESULTS AND DISCUSSIONS

The result of the parasitological examination was negative.

Only the symptoms of bacterial disease presented in photos 1, 2, 3 and 4, were observed.

The disease started asymptomatic in ponds during the summer of 2018, in an acute form with high mortality, suddenly appearing, and pathognomonic symptoms was obvious starting with 27<sup>th</sup> July.

The cephalic haemorrhages (oral and intraocular, fig. 1 and 2) were relevant together with abdominal distension, haemorrhages at the base of pair fins and inflamed anus (fig. 3), liver and pyloric appendages haemorrhages, splenomegaly (fig. 4) and the presence of yellow liquid in intestines, accompanied by haemorrhages at the level of intestinal villi, symptom specific to enteritis.



Fig. 1 Oral haemorrhage



Fig. 2 Intraocular haemorrhage



Fig. 3 Haemorrhages at the base of pair fins and inflamed anus



Fig. 4 Liver and pyloric appendages haemorrhages

Because of high mortality registered in the given ponds, samples were sent to the Institute for Diagnosis and Animal Health Bucharest for bacteriological examination.

Due to the delay in establishing the treatment, the seriously ill trout became unable to consume food, not having access to the medicines from fodder, died without the chance of being treated, fact that explained the high mortality registered in the 2 ponds.

The experiment was analyzed in a larger time frame from 27.08. to 11.09.2018 than the treatment period (31.08-09.09.2018) and the results are presented in table 1.

The antibiotic treatment administered in fodder, prepared after the method presented by Noga E.J. [6], started from 31.08.2018 for a 10 days period and concluded on 09.09.2018. The active substance used for treating was enrofloxacin (Enrodem 50 with 500 mg/g hydrosoluble powder), and the treatment dosage was 50,6 mg/kg fish per day.

Table 1 The evolution of rainbow trout mortality, in ponds B2 and B3, during the experiment

No.	Date of sampling specimens of dead fish	Mortality registered in B2 pond (number of specimens)	Mortality registered in B3 pond (number of specimens)
1.	27.08.2018	65	68
2.	28.08.2018	128	135
3.	29.08.2018	110	102
4.	30.08.2018	103	101
5.	31.08.2018	97	99
6.	01.09.2018	95	92
7.	02.09.2018	90	89
8.	03.09.2018	88	86
9.	04.09.2018	74	78
10.	05.09.2018	65	31
11.	06.09.2018	27	23
12.	07.09.2018	26	20
13.	08.09.2018	20	18
14.	09.09.2018	18	9
15.	10.09.2018	9	7
16.	11.09.2018	8	2

The antibiotic was decided upon the microbiological examination with antibiogram. The sensitivity test registered the best values for enrofloxacin and flumequine. Peracetic acid treatment started simultaneous with the antibiotic treatment, and lasted 7 days, ending on 06.09.2018.

The use of internal treatment combined with external treatment lead to a decrease in trout mortality by 3,18% registered in pond B3, according to fig. 5.

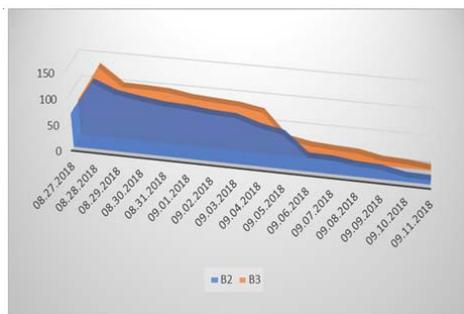


Fig. 5 Fish mortalities in B2,B3 for the treatments period

By using peracetic acid, with proven bactericidal effects, the potential pathogens from water were destroyed the bacterial biofilm.

Due to the advanced disease stage, the results do not have maximum relevance, because most specimens that were in a critical stage of disease stopped eating, thus they did not have access to the antibiotic treatment present in fodder.

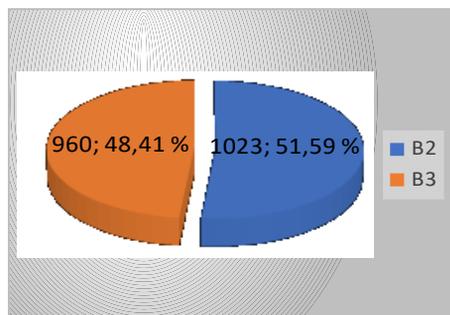


Fig. 6 Rainbow trout mortality in B2 and B3 ponds

The registered results can vary greatly if the disease is diagnosed early and the treatment is applied immediately so that fish can consume the fodder which has the antibiotic treatment.

*mykiss*, Walbaum), 2009, Thesis submitted in fulfillment of the requirements for the degree of Doctor in Veterinary Sciences (PhD), Faculty of Veterinary Medicine, Ghent University.

## CONCLUSIONS

In pond B3, the oral treatment with antibiotic combined with the external treatment represented by long term baths (prolonged immersion) with peracetic acid, lead to the decrease of mortality by 3,18%, compared to B2 pond.

It is recommended to monitor the disease recurrence when administering the treatment in the 2 studied situations.

The experiments must be resumed in the incipient stages of the disease when the fish have access to the treatment administered in fodder.

The obtained results, even if the differences are not great, open a new perspective in the use of the combined treatment for trout culture.

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