

YERSINIOSIS OUTBREAK IN RAINBOW TROUT (*ONCORHYNCHUS MYKIS*) AT A FISH FARM FROM NORTHERN ROMANIA

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ABSTRACT - Yersiniosis (Enteric diseases – red mouth) is a septicemic infecto-contagious disease of salmonides, produced by *Yersinia ruckeri*, young rainbow trout being the most susceptible to infection. The disease appears as an exogenous or endogenous infection, influenced by favouring factors. The infection sources are represented by sick and asymptomatic carrier fishes that eliminate *Yersinia* through faeces, contaminating water and fodder. At the beginning of the disease outbreak, which is the subject of the present scientific paper, sudden water warming (15-17°C) in the spring of 2008 was incriminated. The bacteriological examination carried out on the anterior kidney and heart blood from trout suspected of yersiniosis had as result the isolation of some *Yersinia ruckeri* bacteriological strains, confirming the suspected diagnosis, based on lesions (high degree anaemia of oral mucosa and branchia, haemorrhagia on tongue mucosa, exophthalmia and ocular haemorrhagia). The identification of the causative agent was based on morphological aspects (gram-negative, motile and rod-shaped) and distinctive metabolic characters, tested by using API galleries. Antibiogramas pointed out the *Y. ruckeri* strain susceptibility to

Oxytetracycline, Flumequine, Trimethoprim and Ceftiofur and a medium susceptibility to Amoxicillin and Enrofloxacin.

Key words: trout, yersiniosis, red mouth

REZUMAT – Un episod de yersinioză la păstrăvul curcubeu (*Oncorhynchus mykis*) într-o fermă piscicolă din nordul țării. Yersinioza (boala entrică – gura roșie) este o boală infecto-contagioasă septicemică a salmonidelor, determinată de *Yersinia ruckeri*, cei mai receptivi la infecție fiind păstrăvii curcubeu tineri. Boala apare ca infecție exogenă sau endogenă, sub influența factorilor favorizanți. Sursele de infecție sunt reprezentate de peștii bolnavi și purtători asimptomatici, care elimină yersinii prin fecale, contaminând apa și furajele. În declanșarea episodului de boală, care constituie subiectul prezentei lucrări, a fost incriminată încălzirea bruscă a apei (15-17°C) în primăvara anului 2008. Examele bacteriologice efectuate din rinichiul anterior și sângele din cord la păstrăvii suspecți de yersinioza au avut ca rezultat izolarea de tulpini bacteriene aparținând speciei *Yersinia ruckeri*, confirmându-se diagnosticul suspectat pe baza leziunilor

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(anemia pronunțată a mucoasei bucale și a branhiilor, hemoragii pe mucoasa linguală, exoftalmie, hemoragii oculare). Identificarea agentului causal s-a bazat pe caracterele morfologice (bacilli gram negativi, mobili) și caracterele biochimice distinctive, testate cu ajutorul galeriilor API. Antibiogramele efectuate au evidențiat sensibilitatea tulpinilor de *Y. ruckeri* față de Oxitetraciclină, Flumequin, Trimetoprim și Ceftiofur și o sensibilitate moderată față de Amoxicilină și Enrofloxacin.

Cuvinte cheie: păstrăv, yersinioză, gura roșie

INTRODUCTION

Yersiniosis was diagnosed and described by many authors, for the first time in USA in 1950 and then in Italy and other European countries that practice trout breeding under intensive system. The economic losses are due to the mortality, which can reach up to 95%.

In Romania, the first episode was reported in 1997, after being described several cases in different age rainbow trout samples (Popescu et al., 2008).

The infection sources are represented by sick and asymptomatic carrier fishes that eliminate *Yersinia* by faeces, contaminating water and fodder.

The disease has an acute evolution and appears to be consecutive to the tissue penetration by opportunistic germs as a consequence of immunosuppression, induced by heat discomfort, stress, handling, overcrowding, or after infection with the virulent *Yersinia*

eliminated from sick fish (Guguianu and Miron, 2002).

The symptoms and lesions are generally nonspecific, found in most septicaemia infections: anorexia, apathy, melanosis, exophthalmia, haemorrhagic diathesis, ascites, splenomegalia, etc. (Baudouy, 1981). The constant lesions, which raise suspicion of yersiniosis are pale gills, haemorrhagia on tongue mucosa and eye haemorrhagia (Austin and Austin, 2007).

The confirmation of diagnosis is done by isolating and identifying the causal agent, using classical methods, miniaturized multitest systems (API ID 32 GN) and / or rapid serological tests with agglutinant antibodies (Douglas et al., 1991; Dăscălescu and Costea, 1995). Controlling this disease is mainly achieved by means of organizational methods, because in acute forms, the treatment is ineffective. When the evolution is slower, we may use the antibiotherapy based on the results of antibiograma (Ghittino, 1985).

MATERIALS AND METHODS

The anatomopathological and bacteriological investigations for diagnosis were carried out on 21 samples of sick trout, with sizes between 8 and 14 cm, observing the working protocol, specific of each investigation type.

Inoculations were carried out from kidneys and heart on TSA agar (Trypticase soy agar) poured in Petri dishes. The inoculated plates were incubated at room temperature (22-25 °C) for 48 hours.

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The identification of the isolated strains was based on the corroboration of culture and morphological aspects in Gram stained smears, with metabolic characters tested by using API galleries, oxidase strips for the reaction of some reagents (hydrogen peroxide and methyl red 0.2%).

The susceptibility of *Yersinia ruckeri* strains to antibiotics and chemioterapics was tested by antibiograma, diffusion method, using the MÜLLER HINTON agar and microcomprimats of Oxytetracycline, Amoxicillin, Enrofloxacin, Flumequine, Trimethoprim and Ceftiofur.

RESULTS AND DISCUSSION

At the external fish examination, an emphasized lesion polymorphism was noticed, consisting in melanosis, congestion and petechiae on body sides, skin ulcers, caudal fin breaking up, exophthalmia and ocular haemorrhagia and vertebral column deforming (*Figures 1, 2 and 3*).

The constant lesions found in all the investigated fishes were great paleness of branchia and oral mucosa, tongue petechiae and ocular haemorrhagia (*Figure 4*).



Fig. 1 - Trout samples – Assembly view



Fig. 2 - Trout samples with exophthalmia and punctiform cutaneous haemorrhagia



Fig. 3 – Trout samples with superficial and deep ulcers in various locations



Fig. 4 – Trout samples with petechiae on tongue mucosa, ocular haemorrhage and anaemia

The internal lesions were represented by the lack of food content and gaseous distension of the digestive tract, splenomegaly and haemorrhagia in the perivisceral fatty tissue (in a small number of cases).

Inoculations were followed by obtaining pure cultures, made of small, uniform, white – grey colour “S” type colonies.

Smear microscopic examination carried out in cultures have shown Gram negative, straight, slightly curved, medium sizes, with no

specific groups of bacillus and cocobacillus.

Morphology of isolated germs, negative reaction to oxidase test and positive to catalasis test were selection criteria for API 20E galleries (identification system of enterobacteriaceae), for investigating the enzymatic equipment of isolated strains.

The metabolic profile of tested strains by using the 20 miniaturized biochemical tests is shown in *Figures 5 and 6*.

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Fig. 5 - Profile obtained after 24 hours of strain incubation, using replicated colonies from TSA medium

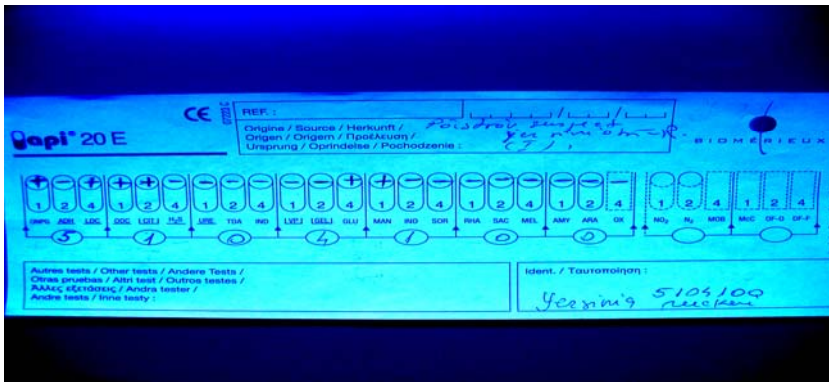


Fig. 6 - Sheet with test results

Legend:

- ONPG=ortho-nitrophenyl- β -galactoside,ortho-nitrophenyl galactopyranoside;
- ADH=arginine-dehydrolase;
- LDC=lysine-decarboxylase;
- ODH=ornitin-decarboxylase;
- CIT=citrate using; U=urease;
- TDA=tryptophan-desaminase;
- IND=indole;
- VP=Voges-Proskauer;
- GEL=gelatinase;
- GLU= glucose fermentation;
- MAN= manitose fermentation;
- INO= inositol fermentation;
- SOR= sorbitol fermentation;
- RHA= rhamnose fermentation;
- SAC= sucrose;
- MEL= melibiose fermentation;
- AMI= amigdalina fermentation;
- ARA= arabinose fermentation;
- OX=cytochrom-oxidase.

In the result sheet, the tests were split in groups of three and corresponded to a certain value: 1, 2 or 4. Summing up the values corresponding to the positive reactions of each group, we obtained the figures combination: 5,104,100.

In the API 20 E Profile Index, because this numerical profile corresponded to *Hafnia alvei* and *Yersinia ruckeri* species, we performed for differentiation the supplementary test of xylose fermentation. The incapacity of tested strains of fermenting xylose justifies, according to data from literature, their framing in *Yersinia ruckeri* species.

Antibiogramas pointed out the susceptibility of *Y. ruckeri* strains to Oxytetracycline, Flumequine, Trimethoprim and Ceftiofur and a medium susceptibility to Amoxicillin and Enrofloxacin.

CONCLUSIONS

An episode of yersiniosis was signalled in rainbow trout from the region of Moldavia.

Lesions found in all the examined fishes were the great paleness of branchia and oral mucosa, tongue petechiae and ocular haemorrhagia.

The diagnosis was specified by isolating *Y. ruckeri* species from anterior kidney and heart.

The biochemical identification of *Y. ruckeri* species was performed by using API 20 E galleries and fermentation test of xylose.

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