THE FIRST CASE OF LACTOCOCCOSIS IN RAINBOW TROUT IN SERBIA

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INTRODUCTION

Lactococcosis is a significant fish disease caused by the *Lactococcus garvieae* bacteria. It is a systemic hyper-acute infection with the occurrence of widespread hemorrhaging, described for the first time at the end of the 1950s in Japan, where the first cases were diagnosed in rainbow trout. Now, the disease is present in many parts of the world, affecting sea fish and freshwater fish in aquaculture. In Europe, the first outbreak of this disease in rainbow trout was reported in Spain in 1989. After that, *L. garvieae* was isolated in Italy, the UK, France, Portugal, Greece, Spain, Turkey and Bulgaria.

The impact of lactococcosis is particularly significant as losses often occur when fish reach market size. The disease causes significant losses at temperatures above 15°C. The oral route is the main route of of *L. garvieae* transmission, but the gills and eyes are major spots of attachment and the proliferation of *L. garvieae* during infection period.

Infected fish exhibit a variety of clinical signs, such as anorexia, exophthalmia, melanosis, conjunctivitis, erect swimming, severe internal hemorrhage and congestion of blood vessel, peritonitis, abscess of spleen and liver, meningoencephalitis, and bacterial septicemia.

The causative agent, Lactococcus garvieae is one of the most important bacterial fish pathogens indifferent freshwater and marine fish species in many countries, with the highest economic impact in rainbow trout aquaculture.

L. garvieae is a non-motile, non-sporulating, facultatively anaerobic, catalase and cytochrome oxidase negative, Gram-positive coccus. It is a lactic acid bacterium, first isolated from a case of bovine mastitis it the UK, and later from other animal hosts, such as cows, buffalos, pigs, dolphins, water buffalos, cats and dogs. The bacterium was isolated from rivers and sewage waters, vegetables, meat and dairy products.

Also, *L. garvieae* was involved in an increasing number of human clinical cases including infective endocarditis, septicemia, urinary and skin infections, giving rise to the status of an emerging zoonotic pathogen.

Although *L. garvieae* is a well-known fish pathogen, human infections are usually related to a contact with raw fish. It has gained recognition as an emerging zoonotic opportunistic pathogen, with the ingestion of contaminated foodstuffs being a likely route of infection.

Handling and of raw fish is reported as a source or risk factor in the majority of clinical cases. High levels of antibiotic resistance and resistance genes in *L. garvieae* strains should be considered as a potential danger for trout culture as well as for public health

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In this paper, we report the first case of lactococcosis in rainbow trout in Serbia, isolation and characterization of causative agent, *Lactococcus garvieae* from diseased rainbow trout (*Oncorhynchus mykiss*, Walbaum).

MATERIAL and METHODS

A total of 20 rainbow trout (weighing 100-120g) that showed the clinical signs of the disease were collected from a trout aquaculture facility in Central-West Serbia in July,2018. The samples for bacterial isolation were obtained from the kidney, liver, spleen and eye of the diseased fish. They were streaked on blood agar plates containing 10% defibrinated sheep blood (BA), Mueller-Hinton(MH) agar and trypticase-soy agar (TSA) plates, and incubated at 20°C for 48h. Single colonies were restreaked on the same media to obtain pure isolates. Pure colonies were subjected to Gram staining, followed by catalase and oxidase tests. The routine tests for the determination of biochemical characteristics were carried out as previously described. Additionally, the BBL CRYSTAL™ Gram-Positive (GP) Identification (ID) system was used for the biochemical identification of isolated bacterium.

DISCUSSION

This study confirmed that *L. garvieae* was the etiological agent of a hemorrhagic septicemia in farmed rainbow trout andthat the lactococcosis of rainbow trout caused by *L. garvieae* emerged in Serbia. The occurrence could be attributed to the significant increase in water temperature during summer months, since water temperature is described as the most important environmental factor in the development of the L. garvieae infections in trout. In addition, variations in water temperature can affect fish immune response against bacterial infection. Lactococcosis is a limiting problem for rainbow trout culture in many South European countries. After the first occurrence in Spain and Italy, the pathogen and the associated disease has spread rapidly throughout the South Europe, and further to the South-Eastern part of the continent, with disease outbreaks in Greece, Bulgaria and Turkey. In the affected countries, lactococcosis is a major threat to trout culture, especially during awarm period. The rapid spread of the pathogen is a result of the multiple routes of dissemination and transmission of this pathogen. This includes direct spread though the movement of infected fish or asymptomatic carriers and transmission via contaminated water. Since *L. garvieae* have the ability to adapt and survive in many environmental conditions including a wide range of pH, temperatures, salinity concentrations and nutrient sources, the occurrence of the disease in Serbia isa warning for the neighboring countries with trout aquaculture. It is evident that this agent spreads to the new geographic areas, causing the disease with high mortality in susceptible population. The source of outbreak is not known, but since the disease is present in the region and susceptible species are in a river basin which is shared between countries, under adequate conditions, further spread of the causative agent and concomitant disease is inevitable. Due to the ability of *L. garvieae* to colonize multiple, diverse environments, and because it causes infection in a broad range of different hosts, it is reasonable to expect further spread of the bacterium and the disease. Since vaccination is considered the best option to control lactococcosis in rainbow trout, we hope that an appropriate strategy to prevent this infectionon Serbian trout farms will be available in the future.

RESULTS

The disease outbreak affecting rainbow trout weighing 70 - 120 g occurred ata trout aquaculture facility in Zaovine Lake (43°52'46.3"N 19°24'08.4"E) during July, 2018. This outbreak lasted for three weeks, and cumulative mortality attributed to this pathogen was around 40%. The water temperature during the outbreak was consistently higher than 14°C (with highest temperature of 20°C). The infected fish exhibited lethargy, anorexia, dark skin coloration, marked unilateral and bilateral exophthalmos with the presence of generalized hemorrhaging or blood spots in the eye, eyeball disruption and loss of eye or eyes (Fig 1). Macroscopic examination revealed the accumulation of fluid in the body cavity. Skeletal muscle and liver were anemic with congestion. Liver and spleen were enlarged. Hemorrhages were present in liver, adipose tissue, pyloric caeca and muscle. Enlargement of the spleen and liver, and hemorrhagic enteritis with yellow, gelatinous fluid in the lumen of intestine were observed (Fig 2). After 48h incubation, pure cultures of cream-colored, opaque, round and convex colonies were recovered from eye and kidney samples (Fig 3). The bacterial cells were α-hemolytic, non-motile, oxidase-negative, catalase-negative,Gram-positive cocci occurring in pairs and short chains (Fig 4). Based on colony morphology, phenotypic and biochemical characteristics, the isolated bacterium was presumably identified as Lactococcus garvieae (Table 1). Using the BBL CRYSTAL GP ID system, the isolate was identified as L. garvieae ID: 3440571723 (Table 2).

Figure 1. The hemorrhaging in the eye and exophthalmos in diseased rainbow trout caused by lactococcosis

Figure 2. Lactococcosis in rainbow trout - hemorrhages in internal organs, enlargement of the spleen and liver, and yellow, gelatinous fluid in the lumen of intestine

Figure 3. Growth of colonies of *L. garvieae* SRB NIVS-1 strain on a blood agar plate after 24 hours of incubation in aerobic conditions.

Figure 4. Gram stain of *L. garvieae* SRB NIVS-1 strain with clusters and short chains of gram-positive cocci.

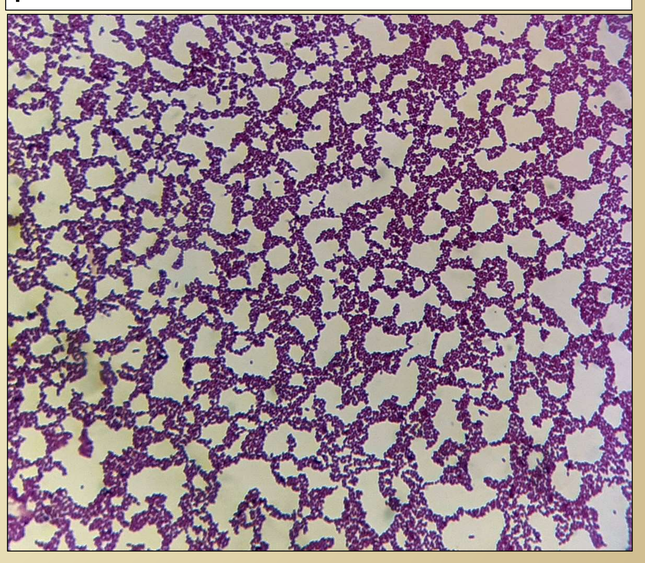

Table 1. Phenotypic characteristic of L.garvieae SRB NIVS-1 strain isolated from diseased rainbow trout.

Phenotypic characteristics	L. garvieae SRB NIVS-1	L. garvieae ATCC 43921
Colony morphology	smooth	smooth
Cell morphology	ovoid-cocci	ovoid-cocci
Gram staining	+	+
Motility	-	-
Production of oxidase	-	-
Production of catalase	-	-
Production of indole	-	-
Production of H_2S	-	-
Citrate utilization	-	-
Methyl red	+	+
Voges Proskauer	+	+
Nitrate reduction	-	-
Starch hydrolysis	-	-
Growth on nutrient agar	+	+
Growth on trypticase soy agar	+	+
Growth on brain heart infusion agar	+	+
Growth on Mueller-Hinton agar	+	+
Growth on MacConkey agar	-	-
Hemolysis in blood agar	α	α

Table 2. The BBL Crystal profile for L. garvieae isolated from diseased rainbow trout.

L-phenylalanine-AMC	+	p-nitrophenyl-β-D-cellobioside	+
L-tryptophan-AMC	+	p-nitrophenyl-α-D-maltoside	+
Trehalose	+	Esculin	+
Sucrose	+	L-valine-AMC	+-
Arabinose	-	pyroglutamic acid-AMC	+-
p-nitrophenyl-β-D-glucoside	+	4MU-N-acetyl-β-D-glucosaminide	+-
p-nitrophenyl-phosphate	-	L-isoleucine-AMC	+
Urea	-	Methyl-α & β-glucoside	+
4MU-β-D-glucoside	+	Maltotriose	+
4MU-α-D-glucoside	+	Fructose	+
L-arginine-AMC	+-	Proline & Leucine-p-nitroanilide	+
Lactose	-	o-nitrophenyl-β-D-galactoside (ONPG) & p-nitrophenyl-α-D-galactoside	-
Mannitol	+	ID: 3440571723 Lactococcus garvieae	
Glycerol	-		

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