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Case Report

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Staphylococcus warneri and *Shewanella putrefaciens* co-infection in siberian sturgeon (*Acipenser baerii*) and hybrid sturgeon (*Huso huso x Acipenser baerii*)

Abstract

The present study describes the first case of *Staphylococcus warneri* and *Shewanella putrefaciens* co-infection in Siberian sturgeons (*Acipenser baerii*) and hybrid sturgeons (*Huso huso* x *Acipenser baerii*). On a sturgeon farm with recirculation aquaculture systems in North Bulgaria, an average daily mortality of 30 fish has occurred. The main pathological findings consisted in ulcerations on the skin, multiple haemorrhages on the ventral part of the body, yellow-tinted muscles with multiple haemorrhages, hyperemic spleen, yellow ochre coloured liver with frail consistency and surface petechial haemorrhages. Mesenteric blood vessels were hyperemic, and intestines were filled with bloody liquid content. Pure bacterial cultures of *Staphylococcus warneri* were isolated from liver and spleen, and *Shewanella putrefaciens*- from intestines. Histopathological examination was done. Antimicrobial susceptibility pattern of the isolates was determined.

Keywords: *Staphylococcus warneri*, *Shewanella putrefaciens*, siberian sturgeon, hybrid sturgeon, co-infection

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Introduction

Fish are in continuous contact with microorganisms in the water and sediment, which influences the microbial species diversity on their skin, gills and alimentary tract.[1] Although all microorganisms are part of the normal aquatic environment, some opportunistic pathogens cause bacterial diseases in fish with high mortality rates.[2] Examples are several Gram-positive and Gram-negative bacteria including *Staphylococcus warneri* (*S. warneri*) and *Shewanella putrefaciens* (*S. putrefaciens*).[3]

From pond water samples at a fish farm, Newaj-Fyzul et al.,[4] isolated Gram-positive bacteria identified as *Staphylococcus* spp. In the belief of Musharrafieh et al.,[5] *S. warneri* is a part of the normal fish microflora, but Gil et al.,[6] were the first to describe a disease on rainbow trouts caused by *S. warneri*, which makes it an opportunistic pathogen. Thereafter, Metin et al.,[7] also reported *S. warneri* infection in rainbow trouts.

S. putrefaciens is a Gram-negative facultatively anaerobic bacterium from the *Shewanellaceae* family. It is usually isolated from sea water, sediment and marine fish.[8,9] The first outbreak of disease caused by *S. putrefaciens* was described by Saeed et al.,[10] Kozinska & Pekala[8] and Pekala et al.,[11] also reported *S. putrefaciens* infection in fish.

The present study describes the first case of *S. warneri* and *S. putrefaciens* co-infection in Siberian sturgeons (*Acipenser baerii*) and hybrid sturgeons (*Huso huso* x *Acipenser baerii*).

Case presentation

On a sturgeon farm with recirculation aquaculture systems in North Bulgaria, an average daily mortality of 30 fish has occurred in September 2015 among fish weighing 200-800 g. In this study, a total of 10 fish were used (5 Siberian and 5 hybrid sturgeons).

Gross anatomy findings consisted in ulcerations, 1 to 1.5cm in diameter (Figure 1) on the skin and multiple haemorrhages on the ventral part of the body. After dissection of the abdominal cavity, yellow-tinted muscles with multiple haemorrhages were observed (Figure 2). The spleen was hyperemic. The liver was of yellow ochre colour, frail consistency and surface petechial haemorrhages. Small gray-yellowish nodules were detected in some areas. Mesenteric blood vessels were hyperemic, and intestines were filled with bloody liquid content.

Figure 1 Ulcerations of the skin (arrows).

The specimens for histopathological examination were fixed in 10% neutral formalin and processed by routine histology techniques.[12,13] Cross sections (4 μm) were stained with haematoxylin-eosin (H/E).

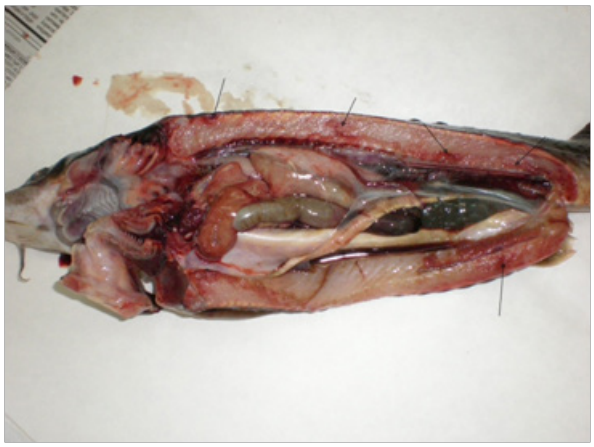

Figure 2 Multiple haemorrhages on the affected muscles (arrows).

For microbiology examination, samples from skin lesions, liver, spleen and intestines were collected and cultured on tryptic soy agar (Fluca, India) supplemented with 5% defibrinated ovine blood (TSBA), MacConkey agar (NCIPD, Bulgaria) and GSP agar (Merck, Germany). Plates were incubated aerobically at 37°C for 24-48 h. In addition, the samples were cultured on blood agar with 2% NaCl at room temperature for 48 h. Isolates were further identified by using BioLog Gen III microplates identification system (BioLog, USA) according to the manufacturer's instructions. Antimicrobial susceptibility pattern of the isolates was determined by agar diffusion test according to CLSI[14] with the following antimicrobial discs: penicillin 10E, ceftazidime 30µg, amoxicillin/clavulanic acid 20/10µg, erythromycin 15µg, enrofloxacin 5µg, chloramphenicol 30µg, flumequine 30µg and tetracycline 30µg. Disks were supplied by Oxoid (UK) and NCIPD (Bulgaria).

Pure bacterial cultures were isolated from liver, spleen and intestines. Primary identification of the liver and spleen isolates revealed Gram-positive cocci in clusters, catalase-positive and oxidase negative. Results showed species affiliation to *Staphylococcus warneri* with a probability of 0.980 in two independent trials. The biochemical profile of the isolates based on positive reactions is presented in Table 1.

Table 1 Utilization of BioLog Gen III microplate substrates by *Staphylococcus warneri*

Substrates					
Sugars	**Polyvalent alcohols**	**Hexose-PO4's**	**Amino acids**	**Hexose acids**	**Carboxylic acids, testers, and fatty acids**
D-Maltose	D-Mannitol	D-Fructose-6-PO4	L-Arginine	Pectin	Methyl Pyruvate
D-Trehalose	D-Arabitol		L-Aspartic Acid	D-Gluconic Acid	D-Lactic Acid Methyl Ester
Sucrose	Glycerol		L-Glutamic Acid		L-Lactic Acid
D-Turanose			L-Histidine		α-Keto-Glutaric Acid
B-Methyl-D-Glucoside			L-Serine		L-Malic Acid
α-D-Glucose					Acetoacetic Acid
D-Fructose					Acetic Acid
					Formic Acid

From intestines, Gram staining showed Gram-negative straight and slightly curved rods that gave strong positive reactions in catalase and oxidase tests. Furthermore, the colonies were brown pigmented and lactose-negative on MacConkey agar. The isolate was defined as *Shewanella putrefaciens* B with BioLog Gen III system with a high probability of 0.989 in two subsequent trials. The positive biochemical reactions are given in Table 2.

Table 2 Utilization of BioLog Gen III microplate substrates by *Shewanella putrefaciens*

Substrates		
Sugars	**Amino acids**	**Carboxylic acids, testers, and fatty acids**
D-Maltose	Gelatin	Methyl Pyruvate
Sucrose		D-Lactic Acid Methyl Ester
Inosine		L-Lactic Acid

Antimicrobial susceptibility pattern of the isolates is shown in Table 3. *S. warneri* showed resistance to penicillin, tetracycline and intermediate susceptibility to the third generation cephalosporin ceftazidime. *S. putrefaciens* was susceptible to all tested antimicrobials.

Table 3 *Staphylococcus warneri* and *Shewanella putrefaciens* B antimicrobial susceptibility pattern (S, susceptibility; I, intermediate susceptibility; R, resistance)

Antimicrobial agents	***Staphylococcus warneri***	***Shewanella putrefaciens***
Penicillin	R	R
Amoxicillin/ clavulanic acid	S	S
Ceftazidime	I	S
Erythromycin	S	S
Enrofloxacin	S	S
Chloramphenicol	S	S
Flumequine	S	S
Tetracycline	R	S

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The histopathological exam showed dystrophy of renal parenchyma and catarrhal enteritis of intestines. The major part of epithelial cells were detached from the basement membrane and shed into the intestinal lumen (Figure 3). The most obvious changes were detected in the liver. Multiple small clear lacunae (vacuoles) were seen in the cytoplasm of liver cells: lipid droplets extracted during the processing of the specimen (Figure 4). The nuclei of many cells were of the reduced size, angular and intensively stained (karyopyknosis). The chromatin of other cells was gathered into small clusters around the nuclear membrane (hyperchromatosis of the nuclear membrane). At some areas dystrophic processes have evolved into necrobiotic in single liver cells or in cell groups. Consequently, the nuclei of affected cells exhibited a various stage of karyolysis or were completely lysed, and cell boundaries have become indistinct. Vascular hyperaemia was established in the connective tissue stroma.

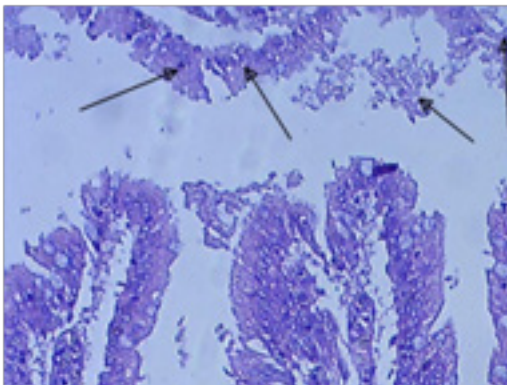

Figure 3 Catarrhal enteritis. Numerous epithelial cells are detached from the basement membrane and shed into the lumen of the intestine (arrows). H/E, magnification 10×.

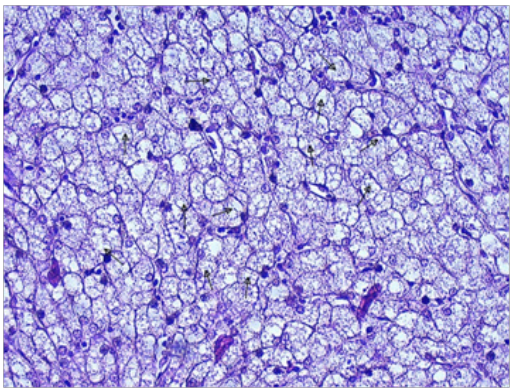

Figure 4 Fatty liver dystrophy. In the cytoplasm of liver cells, multiple small clear lacunae (vacuoles) could be seen, representing lipid droplets extracted during the processing of the specimen (arrows). H/E, magnification 20×.

Discussion

The Siberian sturgeon (*Acipenser baerii*) is sensitive to various diseases as White sturgeon iridovirus (WSIV), White sturgeon herpesviruses-1, 2 (WSHV-1, 2), Shovenose sturgeon iridovirus (SSIV), White sturgeon adenovirus (WSAV),[15] vibriosis, pasteurellosis,[16] aeromonosis,[17] yersiniosis.[18] This is the first study describing the co-infection caused by *S. warneri* and *S. putrefaciens* in Siberian sturgeons (*Acipenser baerii*) and hybrid sturgeons (*Huso huso* x *Acipenser baerii*).

Two cases of *S. warneri* infection in fish are reported. Gil et al.,[6] were the first to describe a disease on rainbow trouts caused by *S. warneri*. Affected trouts demonstrated fin ulcerations, exophthalmia, distended abdomens as a result of accumulated ascitic fluid and liver discoloration. A similar condition in rainbow trouts provoked by *S. warneri* is also reported by Metin et al.[7] The clinical signs included lethargy, anorexia, erosive lesions on anal fins' base, haemorrhages in the eyes, mouth and the anus, anal prolapse and dark pigmentation. The necropsy revealed liver haemorrhages, splenomegaly, enlarged kidneys and yellow exudate in the intestine, liver discoloration, haemorrhages and lysis of muscles. Saeed et al.,[10] reported high death rates in rabbitfish (*Siganus rivulatus*) reared in net cages in the Red Sea. The diseased fish exhibited discoloration, haemorrhagic necroses on the body and the mouth, frayed fins and exophthalmia. In Poland, Kozinska & Pekala[8] isolated a variety of bacteria from the internal organs of carps and trouts exhibiting lethargy, skin discoloration and death, but the predominant species was *S. putrefaciens*. According to the authors *S. putrefaciens* is an opportunistic pathogen for fish causing illness at certain conditions as stress or in fish with compromised immune defense. Recently, Pekala et al.,[11] reported a disease on carps and trouts with gill necrosis or skin ulcerations, cause by *S. putrefaciens*. Gross anatomy findings consisted in haemorrhages, oedematous kidneys and enlarged spleen. The findings of these authors are comparable with ours.

Metin et al.,[7] affirmed that staphylococcosis was an economic problem in fish farms and recommended observation of veterinary sanitary measures. Moreover, *S. warneri* and *S. putrefaciens* can cause infections in human.[19,20] The described pathological, histological and microbiological findings in this case clearly demonstrated the pathogenic potential of *S. warneri* and *S. putrefaciens* in Siberian sturgeons and hybrid sturgeons. The most obvious changes were established in the liver and intestine where *S. warneri* and *S. putrefaciens* were respectively isolated from. We deem that the present work will add to the clinical and laboratory experience.

Acknowledgments

None.

Conflicts of interest

We have no conflict of interest to declare.

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