First Case Report of *Vibrio harveyi* infection in Salema (*Sarpa salpa*) in a Public Aquarium*

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Abstract

In this study, moribund salema (*Sarpa salpa*) individuals from a public aquarium were investigated to identify the cause of disease. A minor scale loss and partial skin depigmentation were observed in the diseased fish. Internal investigation of fish revealed hyperemia in the liver and the intestines and haemorrhages in the muscle tissues. When the histopathological sections of tissues were examined, it was determined that the bacterial infection affected especially the gill epithelium, the intestinal mucosa, the heart muscle and hemopoietic tissues of the spleen. According to phenotypic and biochemical characteristics as well as SSU rRNA sequencing results, the isolated bacteria was *Vibrio harveyi* and consistently seen all the examined samples of visceral organs including kidney, spleen, liver and blood. However, *Vibrio vulnificus* and *Pseudoalteromonas piscicida* bacteria were also isolated from blood and spleen tissues of one sick fish among others.

Keywords: Vibriosis, *Vibrio harveyi*, salema, *Sarpa salpa*, public aquarium

Bir Deniz Akvaryumunda Salpa Balıklarında (*Sarpa salpa*) *Vibrio harveyi* Enfeksiyonu Olgusu: ilk rapor

Özet

Bu çalışma, bir deniz akvaryumundan alınan hasta salpa balıklarında (*Sarpa salpa*) hastalığa neden olan etken veya etkenleri tanımlamak üzere yapılmıştır. Hasta balıkların vücudunda hafif şiddette deri renginde açılma ile birlikte pul kayıpları gözlenmiştir. İç bakıda, karaciğer ve bağırsak dokularının hiperemik ve kas dokusunun da hemorajik olduğu tespit edilmiştir. Doku kesitleri histopatolojik olarak incelendiğinde, enfeksiyonun; özellikle solungaç epitelyumu, bağırsak mukozası, kalp kası ve dalağın hematopoetik dokusunu etkilediği belirlenmiştir. Fenotipik ve biyokimyasal özellikleri ve SSU rRNA dizileme sonuçlarına göre; incelenen tüm iç organlardan (böbrek, dalak, karaciğer) ve kan dokusundan *Vibrio harveyi* bakterisi izole edilmiştir. Buna ek olarak, incelenen balıklardan sadece birinin kan dokusundan ve dalağından alınan örneklerde *Vibrio vulnificus* ve *Pseudoalteromonas piscicida* bakterileri de izole edilmiştir.

Anahtar kelimeler: Vibriozis, *Vibrio harveyi*, salema, *Sarpa salpa*, deniz akvaryumu

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INTRODUCTION

The salema, *Sarpa salpa* (Linnaeus 1758), is a widely distributed species throughout the Mediterranean, the northeastern Atlantic from southwestern France (Bay of Biscay) to Sierra Leone. The distribution area includes coastal waters of the Azores, Madeira, the Canary Islands and the Cape Verde Islands (Russell, 2014). In Turkey, salema is mostly exhibited in thematic aquaria rather than used for consumption due to its low market value and low esteemed flesh.

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Vibrio harveyi is a well-known bacterium causing disease with high mortality in marine aquaculture and outbreaks have been reported in various fish species including common dentex (*Dentex dentex*) (Company et al., 1999; Pujalte et al., 2003; Turgay and Karataş, 2016), gilthead sea bream (*Sparus aurata*) (Balebona et al., 1998; Haldar et al., 2010; Pujalte et al., 2003) and European sea bass (*Dicentrarchus labrax*) (Pujalte et al., 2003). Moreover, vibriosis caused by *V. harveyi* has also been reported in farmed sole (*Solea senegalensis*) (Zorrilla et al., 2003), cultured wedge sole (*Dicologoglossa cuneat*a) (López et al., 2009), cultured brown spotted grouper (*Epinephelus tauvina*), silvery black porgy (*Acanthopagrus cuvieri*) (Saeed, 1995) and cage-reared grouper (*Epinephelus awoara*) (Qin et al., 2006).

This is the first case report of vibriosis which caused an outbreak in *Sarpa salpa* that held in a public aquarium.

MATERIAL and METHODS

The present study was approved by Istanbul University Local Committee on Animal Research Ethics (Decision no: 29.12.2016).

Case History

This disease outbreak occurred in a thematic public aquarium located in Istanbul/Turkey and the only external symptom observed in fish kept in the quarantine tank was reported as anorexia. Water quality parameters at the time of sampling were as follows: the temperature 23.5°C; the salinity 27 psu; the dissolved oxygen concentration 8.3 mg/L; while other parameters such as un-ionized ammonia (NH_3) 0.001 mg/L, nitrite (NO^-_2) 0.05 mg/L, nitrate (NO^-_3) 40 mg/L and pH 8.1.

Collection and Processing of Tissues

The samples from two individuals were taken from internal organs (liver, spleen, kidney and blood tissue) of moribund salema (between 100-120 g in weight) and streaked onto Marine Agar 2216 (Difco). The plates were incubated at 22°C for 48-72 h. Basic characteristics of the isolates were determined using conventional methods including colony and cell morphology, Gram staining, oxidase activity, motility, oxidation/fermentation (O/F) reaction, growth/appearance on TCBS agar and susceptibility to vibriostatic agent O/129 (10 µg and 150 µg) (Oxoid) (Whitman, 2004). Histopathological examination of the sampled tissues was performed as follows: tissue materials were fixed in 10% buffered formalin, dehydrated in ethanol, xylene series used as a clearing agent and embedded in paraffin wax. Subsequently, tissue sections (5 µm thick) were stained with haematoxylin-eosin (HE) following standard protocol (Culling, 1963) and then they were examined under microscope using the image analysis system NIS-Elements BR Microscope Imaging Software (Nikon Instruments).

DNA extraction, PCR and 16S rRNA gene sequencing

All bacterial isolates that were found to have the same morphological and biochemical characteristics were selected and inoculated into Marine Broth 2216 (Difco) and incubated overnight at 22°C. Total DNA extraction was performed with the GeneJET Genomic DNA Purification Kit (Thermo) according to the manufacturer's instructions and used as template for PCR. An approximately 540 bp long fragment of the 16S rRNA gene was

amplified using the universal bacteria primer set; primer S-20 (5' AGA GTT TGA TCC TGG CTC AG 3') and primer A-18 (5' GWA TTA CCG CGG CKG CTG 3') (Suau et al., 1999). The PCR mixture (50 µl) included 50 ng template DNA (2 µl), 0.4 µM of each primer (2x 2 µl), PCR Master Mix (2X) (Thermo Scientific) (25 µl) and nuclease-free water (Thermo Scientific) (19 µl). The amplification was done using a thermal cycler (Biometra, TPersonal) and a program with the following parameters: initial denaturation at 95°C for 3 min, followed by 30 cycles of amplification (denaturation at 95°C for 30 s, annealing at 56°C for 1 min, extension at 72°C for 1 min) and a final extension step of 72°C for 4 min. PCR products were purified and sequenced bidirectionally by Medsantek (Istanbul, Turkey). Sequence editing and analysis was performed in Bioedit v7.0.0 (Hall, 1999) using the ClustalX 2.1 (Larkin et al., 2007) and BLASTN 2.2.20 algorithm (Zhang et al., 2000). A higher or equal to 99% similarity criterion in 16S rRNA gene sequence was used for identification of the isolates at the species level (Clarridge, 2004). Representative 16S rRNA gene sequences have been deposited in GenBank database under accession numbers MF355397-MF355402.

RESULTS

Externally, a minor scale loss and partial skin depigmentation were observed on diseased fish (Fig. 1A). Internally, the wall of intestines was transparent and hyperemia on the wall was observed in the intestinal submucosa (Fig. 1B).

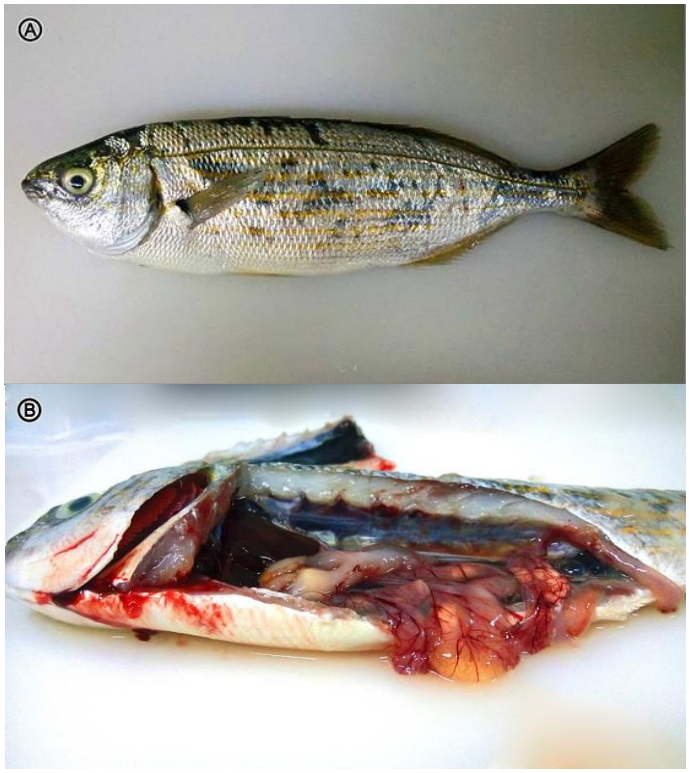

Figure 1. A minor scale loss and partial skin depigmentation (A), transparent intestinal wall and hyperemic intestinal submucosa (B).

Histopathologically, the diseased salema were found to have various degrees of superficial skin lesions with missing epithelial cells. Hyperplasia of secondary gill lamellae along with disruption of gill epithelium were also a common finding of disruption of epithelial layers in all samples. The liver tissues of the examined fish were seen as hyperemic and showing haemorrhagic regions. Multifocal hemosiderin deposits were identified and degeneration of parenchyma cells were determined in the spleen. In addition, hemopoietic tissue of the spleen was greatly reduced in the examined fish. The mucosal epithelial cells were seen as sloughed off into the lumen of the intestines under the microscope (Fig. 2).

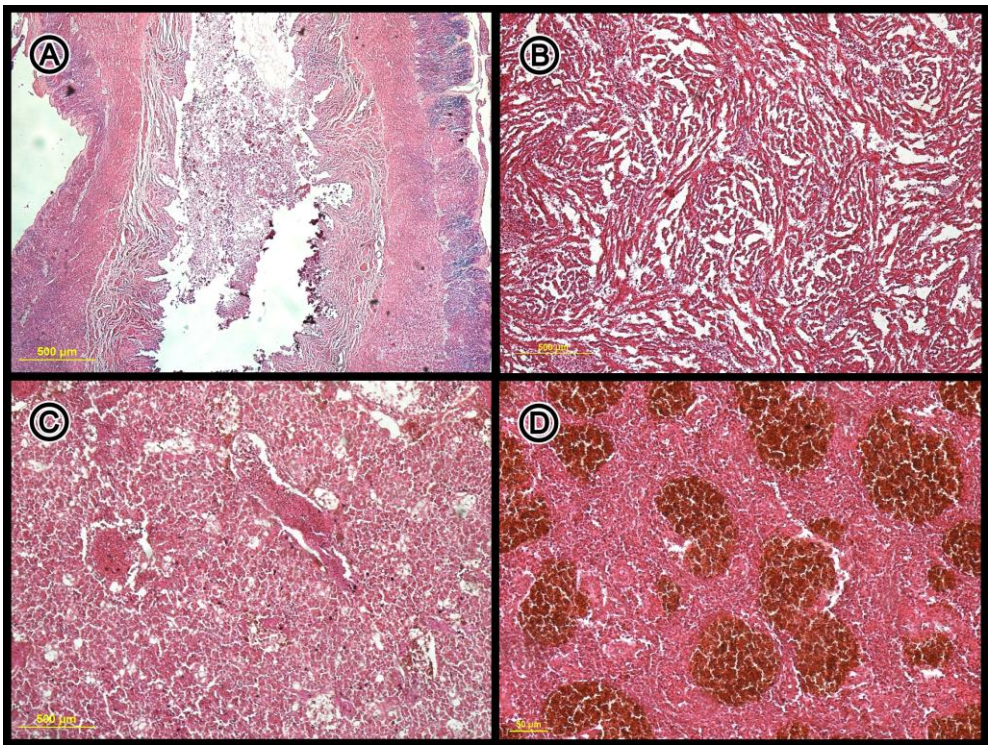

Figure 2. The histopathological changes included hyperplasia of the intestinal mucosa and sloughed mucosal epithelial cells into the lumen of the intestines (4x) (A), lysis of the heart muscle (10x) (B), vacuolar degeneration of the parenchyma cells, hyperaemia and haemorrhage in the liver (10x) (C), multifocal hemosiderin deposits, degeneration of the parenchyma cells and reduced haematopoietic tissue in the spleen (20x) (D).

A total of fourteen pure cultures were obtained from four tissue samples. Three different bacterial species were isolated according to phenotypic and biochemical characteristics (Table 1). Among the isolates, twelve of them obtained from kidney, spleen, liver and blood, were identified as *V. harveyi* according to 16S rRNA gene sequence analysis (acc. no. MF355397-98 and MF355401-02). Only two isolates that had been obtained from blood and the spleen respectively were identified as *V. vulnificus* (acc. no. MF355400) and *Pseudoalteromonas piscicida* (acc. no. MF355399).

Table 1. Morphological and phenotypical characteristics of the isolates

Isolate	*Vibrio harveyi*	*Pseudoalteromonas piscicida*	*Vibrio vulnificus*
Morphology	R	R	R
Gram staining	-	-	-
Motility	+	+	+
Oxidase	+	+	+
Catalase	+	+	+
O/F (glucose)	F	O	F
O/129	S	S	S
Indol	+	+	+
Voges-Proskauer	+	-	+
Methyl red	+	+	+
Nitrate reduction	+	+	+
ONPG	+	-	+
Citrate	+	-	-
Acid production from			
Lactose	+	+	+
Glucose	+	+	+
Sucrose	+	+	+
Growth on			
TCBS	+	-	+
MacConkey	+	-	+

R: rods; +: positive, -: negative; S: sensitive; F: fermentative; O: oxidative

DISCUSSION

V. harveyi is a major pathogen of a wide variety of marine fish including sparids and invertebrates, and the pathogen is considered to have a much more destructive effect in immunocompromised hosts (Austin and Zhang, 2006). Our findings such as hemorrhaging, scale loss and skin depigmentation in addition to haemorrhages in various parts of the body including in the intestines but also hyperemia in visceral organs are typical findings of *V. harveyi* infection in fish. Similar clinical signs were reported in various outbreaks in other sparids such as common dentex (Company et al., 1999; Haldar et al., 2010; Turgay and Karataş, 2016), gilthead sea bream (Pujalte et al., 2003) and non-sparids (Austin and Zhang, 2006). As a matter worth mentioning, we did not observe any eye lesions or corneal opacities that have been reported as a common clinical finding in several disease outbreaks of sparids.

Nowadays, there are many public aquariums around the world, and these aquariums (and in addition zoos) are visited by 700 million people worldwide (Gusset and Dick, 2011). As interest in public aquariums increases, the diversity of exhibited fish is also likely to increase. However, this would for certain create some challenges and a variety of novel disease outbreaks in ornamental fish, which are kept in confined, unnatural and sometimes suboptimal environments. The vast majority of fish in such aquariums is wild caught. Therefore, preventing or reducing disease outbreaks in ornamental fish species,

which could be rare and endangered, will contribute to the conservation of natural stocks and have positive effects on sustainable trade (Tlusty et al., 2013).

Although there are a few parasitic diseases have been described in salema, to our knowledge, there are no previous reports of bacterial disease in this fish species. In this study, *V. harveyi* was isolated from moribund salema in a public aquarium and we believe this to be the first reported case of vibriosis in salema.

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