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A STUDY ON BACTERIAL HAEMORRHAGIC SEPTICEMIA IN FARMED YOUNG RUSSIAN STURGEON IN TURKEY (*Acipencer gueldenstaedtii*)

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ABSTRACT

Bacterial haemorrhagic septicemia outbreaks occurred in young farmed Russian sturgeon (*Acipencer gueldenstaedtii*) in 2002-2003 associated with *Aeromonas hydrophila* alone or mixed infection with *Flavobacterium hydatis*. These bacteria induced low mortalities among the fish reared in concrete ponds at Sapanca Freshwater Fish Culture and Research Station, Istanbul University, Fisheries Faculty. Clinically, affected fish, 1-2 years old (100-380 g in weight) showed haemorrhages on the skin of ventral side of head, at the base of fins and around ganoid scales. Affected fish gills generally were congested, haemorrhagic and necrotic. Some affected fish gill filaments showed epithelial cell hyperplasia, necrosis and sloughed of the necrotics cells.

Key Words: *Aeromonas hydrophila, Flavobacterium hydatis*, Russian sturgeon, haemorrhagic septicemia.

ÖZET

TÜRKİYE'DE GENÇ KÜLTÜR RUS MERSİN BALIKLARINDA (*Acipencer gueldenstaedtii*) GÖRÜLEN BAKTERİYEL HEMORAJİK SEPTİSEMİ ÜZERİNDE BİR ÇALIŞMA

İstanbul Üniversitesi Su Ürünleri Fakültesi Sapanca İçsu Balıkları Üretim ve Araştırma İstasyonu'nda beton havuzlarda yetiştiriciliği yapılan genç Rus mersin balıklarında (*Acipencer gueldenstaedtii*) 2002-2003 yıllarında *Aeromonas hydrophila* tek başına veya *Flavobacterium hydatis* ile karma enfeksiyon şeklinde bakteriyel hemorajik septisemi oluşturarak balıklarda düşük mortaliteye neden olmuştur. Klinik olarak hasta balıklarda (100-380gr) deri renginde koyulaşma, yüzgeç diplerinde ve ganoid pulların çevresinde hemorajiler görülmüştür. İç organlarda hiperemi ve hemoraji yaygın bir şekilde görülürken solungaçların genellikle hiperemik, hemorajik ve nekrotik olduğu tespit edilmiştir. Bazı hasta balıkların solungaç filamentlerinde epitelyal hücre hiperplazisi, nekroz ve nekrotik hücrelerde dökülme görülmüştür.

Anahtar Kelimeler: *Aeromonas hydrophila, Flavobacterium hydatis*, Rus mersini, hemorajik septisemi.

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INTRODUCTION

Despite the extensive information about the bacterial disease in most teleost fishes, a few data exist on bacterial diseases of sturgeon. A motile aeromonad septicemia caused by *Aeromonas hydrophila* frequently induced considerable losses in Persian sturgeon (*Acipenser persicus*) fingerlings in northern Iran (Soltani and Kalbassi, 2001) and also in the Harrison river sturgeon in Canada (Raverty and Nikl, 1999). *Yersinia ruckeri* was found responsible for 10% mortalities in the young (15-30 g) cultured sturgeon (*Acipenser baerii*) in south western of France (Vuillaume *et al.*, 1987). Bauer *et al.,* (2002) reported that *Flavobacterium johnsoniae* was isolated from diseased farmed young sturgeons (3-4 g) in Russia and it was also isolated from 6-7 years old diseased farmed Russian sturgeon with low mortality in Turkey reported by Karataş et al., 2010. An outbreak of *Pseudomonas fluorescens* was reported in young (10g in size) farmed Siberian sturgeon (*A. baerii*) with high mortality (40%) in northern Italy (Brunetti *et al.*, 2006).

The aim of this study was to characterize pathogens responsible for the outbreaks of disease in young farmed Russian sturgeon (1-2 years old) reared from fertilized eggs which were imported from Krasnodar Russia in 2001. The result of bacteriological examinations and details of the histopathology of the disease are presented here.

MATERIAL AND METHODS

Fish

Ten affected fish (100-380g) obtained from concrete ponds of Istanbul University, Fisheries Faculty Research Station in Sapanca in 2002-2003, was sampled for bacteriology and histopathology. Water temperature at the time of sampling varied from between 20 and 22ºC.

Bacteriology

Bacteriological samples of liver, kidney and spleen were streaked onto Tryptic Soy Agar (TSA) plates and plates were incubated at 22ºC for 48h. The morphological and physiological characteristics of bacterial colonies from each plates were determined by conventional biochemical and physiological tests (Austin and Austin, 1987) and bacteria identified following Bergey's Manual (Holt *et.al.*, 1994).

Histopathology

Samples of tissues from gut, kidney, spleen, liver heart and gills were processed for histopathology by fixing in 10% buffered formalin, and processed for paraffin embedding. Histological sections (5µm) were stained with haematoxyline and eosin and examined by light microscopy (Bullock, 1978).

RESULTS

Affected fish (100-380g) generally showed darkening of the skin, with haemorrhages on the ventral side of the head skin, at the base of fins and around the ganoid scales (Figure 1). Considerable level of mortalities occurred in fish stocks.

Figure 1. Hyperemia and haemorrhages on the ventral side of the head skin, at the base of fins and around the ganoid scales in the affected fish.
Şekil 1. Hasta balıklarda başın alt yüzünde, yüzgeç diplerinde ve ganoid pulların etrafında hiperemi ve hemoraji

Internally, the most obvious findings were congestion of the visceral organs with haemorrhages over the viscera (Figure 2). In addition to these findings, black colouration of the liver was also observed in some affected fish.

Two Gram-negative bacteria cultured from visceral organs following incubation of kidney, spleen and liver swabs at 22ºC for 48h on TSA. These bacteria were identified as *A. hydrophila* and *Flavobacterium hydatis*. *A. hydrophila* produced cream coloured colonies consist of Gram negative, motile and short (0.5-0.8 x 1.5-3.5µm) rods, were not sensitive vibriostat (O/129); cytochrome oxidase; and catalase positive, fermentative by O/F glucose test. *F. hydatis* produced yellow coloured colonies consist of long rods (0.5x 8-10µm), showed gliding movements, catalase positive, but not oxidase positive, fermentative by O/F glucose test. A few long, slender thread-like cells were seen in the young cultures of the *F. hydatis* in Gram stained preparates (Figure 3). The morphological, physiological and biochemical features of the isolates were shown in Table 1.

Figure 2. Congested visceral organs, with haemorhages over the visera.
Şekil 2. Hiperemik visceral organlar ile visceral organlarda hemorajiler

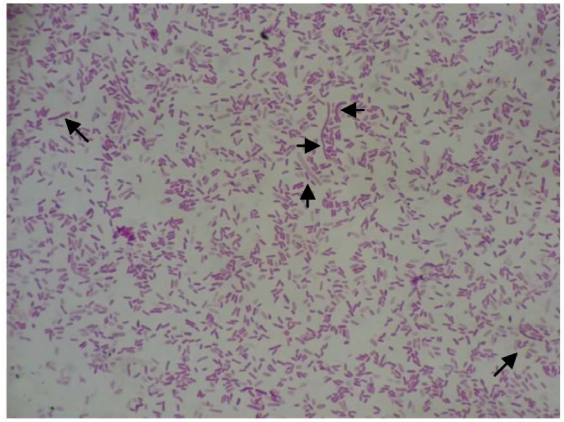

Figure 3. A few long, slender thread-like cells (arrowed) in young cultures of *Flavobacterium hydatis* (Gram x1000).

Şekil 3. *Flavobacterium hydatis*'in genç kültürlerinde bir kaç ince uzun iplik benzeri (okla gösterilmiştir) bakteri hücreleri (Gram x1000).

Table 1. The morphological, physiological and biochemical features of isolated bacteria.
Tablo 1. İzole edilen bakterilerin morfolojik, fizyolojik ve biyokimyasal özellikleri

	Aeromonas hydrophila	*Flavobacterium hydatis*
Gram staining	–	–
Motility	+	+*
Cytochrome Oxidase	+	–
Oxidative-Fermentative Metabolism	+/+	+/+
Voges Proskauer reaction	–	V
Methyle red test	V	–
Indole	V	V
Citrate	+	–
Arabinose	–	V
Galactose	–	+
Mannose	+	+
Lactose	–	–
Fructose	+	–
Maltose	+	+
Sucrose	+	+
Glucose	+	+
Mannitol	–	–
Xylose	–	V
Arginine dihydrolase	+	+
Lysine decarboxylase	–	–
Ornithine decarboxylase	–	–
Growth 37°C	+	+
%0 (w/v) NaCl	+	+
%4 (w/v) NaCl	–	–
%7 (w/v) NaCl	–	–
0/129	R	S
ONPG (β-Galaktosidase)	+	+

*: gliding motility; V: variable; R: resistance; S: sensitive

Histologically, diseased sturgeons showed depletion of haemopoietic elements both in the renal (Fig. 4a) and spleenic haemopoietic tissue. The remaining cells of spleen were usually necrotic. The kidneys showed peritubuler and glomeruler oedema, considerable tubular degeneration and necrosis (Figs 4b). Multifocal large liquefactive necrotic areas and haemorrhages were found in the affected liver and focal necrosis is found in the cardiac muscle. Multifocal melanomacrophage centers and liquefactive necrotic areas were seen in some affected liver (Fig. 4c) sections. The intestinal (Fig. 4d) and gastric mucous membrane was usually necrotic and sloughed into the lumen. Affected fish gills with *A. hydrophila* were generally congested and haemorrhagic (Fig. 4e). Epithelial hyperplasia, sloughed epithelial cells and mucus were observed in the gill filaments of affected fish with *F. hydatis* (Fig. 4f).

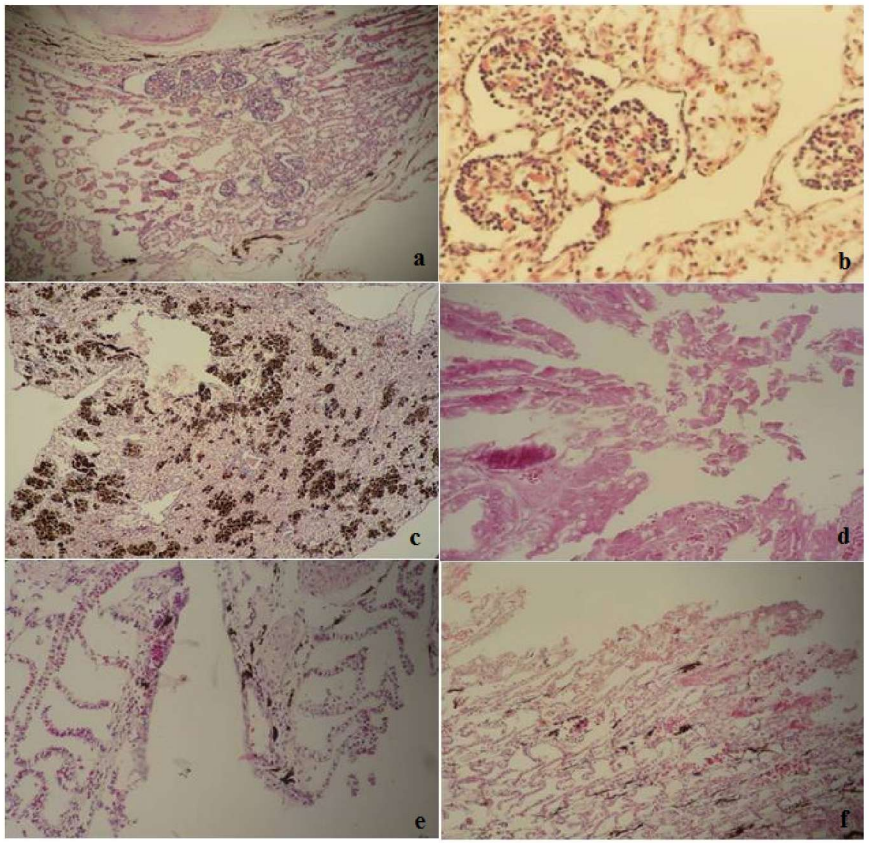

Figure 4. Histological sections of affected fish tissues (a) depletion of haemopoietic elements, degenerative and necrotic tubules in kidney (H&E, X100), (b) periglomeruler oedema and haemorrhagie in the affected glomerulus (H&E, X200), (c) multifocal melanomacrophage centers in the affected liver (H&E, x100), (d) necrotic mucosa membrane and sloughed into the lumen of the affected intestine (H&E, x200), (e) congested and haemorrhagic gill filaments (H&E, x200), (f) and epithelial hyperplasia, haemorrhages of the necrotic gill filaments tips (H&E, x100).

Şekil 4. Hasta balık dokularının histolojik kesitleri (a) böbrekte hemopoietik dokuda azalma, dejeneratif ve nekrotik tübüller (H&E, X100), (b) glomeruluslarda periglomerular ödem ve hemoraji (H&E, X200), (c) karaciğerde multifocal melanomakrofaj odakları (H&E, x100), (d) bağırsakta nekrotik mukoza membranında lümene dökülme (H&E, x200), (e) solungaç filamentlerinde hiperemi ve hemoraji (H&E, x200), (f)nekrotik solungaç filament uçlarında epitelyal hiperplazi ve hemorajiler (H&E, x100).

DISCUSSION

For the most diseases investigation of sturgeon, regardless of species, a bacterial agent has been identified as the definitive cause of mortality in many countries (Vuillaume *et al.*, 1987; Raverty and Nikl, 1999; Francis-Floyd, 2000; Soltani and Kalbassi, 2001; Brunetti *et al.*, 2006).

Francis-Floyd (2000) has been reported that most bacterial agents isolated from most sturgeon disease cases which were predisposed stress factor in their history, were examined University of Florida; identified as opportunistic pathogen bacteria namely, *A. hydrophila*, *Pseudomonas* spp. and *A. sobria.* In this study, similarly we also identified opportunistic pathogen bacteria namely *A. hydrophila* and *F. hydatis* as definitive causes of mortality of Russian Sturgeon reared in the large concrete ponds at low level of dissolved oxygen (<4mg/l) with organic loads, particularly when the accumulations are on the bottom of ponds.

The affected fish had showed darkening in colour with haemorrhages on the ventral side of the head skin, and at the base of fins and around the large ganoid scales and congested visceral organs, with haemorrhages over the viscera, mortality, as described in other teleost fish infected with *A. hydrophila* (Richards and Roberts, 1978, Soltani and Kalbassi, 2001; Guvener and Timur, 2005).

F. hydatis isolates were consist of 8-10µm long rods as described other workers (Strohl and Tait, 1978; Austin and Austin, 1987, Bernardet *et al.*, 1996) but we observed a few long, slender thread-like cells in young cultures. *F. hydatis* exhibits swarming and gliding motility under certain conditions, and the structure of its cell wall is similar to that of *F. johnsoniae* (Bernardet *et al.*, 1996, Karataş *et al.*, 2010). Their micromorphology, gliding motility and the ability of the acid production from glucose, cytochorome oxidase negative and fermentative (F) metabolism character had made them distinct from other *Flavobacterium* spp. (Bernardet *et al.*, 1996, Austin and Austin, 1999). For the reason *F. johnsoniae* should have given positive cytochrome oxidase reaction we differentiated our isolate as *F. hydatis.*

Depletion of haemopoietic elements in the renal and splenic haemopoietic tissue, considerable tubular degeneration and necrosis in the kidney, multifocal necrotic areas and haemorrhages in the liver, in the gills and necrotic intestinal and gastric mucous membrane were also similar histopathological findings were described in other teleost fish with motile aeromonad septicemia (Richards and Roberts, 1978; Güvener and Timur, 2005). In addition to these findings in the affected fish with *F. hydatis*; hyperplasia of the gill epithelia, necrose and sloughed of epithelial cells were observed beside the haemorrhagie as described in the bacterial gill diseases induced by flavobacteria (Noga, 1999). Diffuse melanomacrophage centers were observed in some affected liver sections similar to the kidney which that normally do not observe in the teleost fish liver. These diffuse melanomacrophage centers should be turned the liver colour to black.

Finally, this is the first report of the haemorrhagic septicaemia induced by *A. hydrophila* associated with or without *F. hydatis*, as a secondary pathogen in farmed Russian sturgeon in Turkey.

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