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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

MARINE VIBRIOS ASSOCIATED WITH DISEASED SEA BASS (*Dicentrarchus labrax*) IN TURKEY

Jale Korun[1*], Gülşen Timur[2]

[1] Department of Fish Diseases, Faculty of Fisheries, Akdeniz University, Antalya-Turkey

[2] Department of Fish Diseases, Faculty of Fisheries, Istanbul University, Istanbul-Turkey

Abstract: This study describes the aetiological agents of vibriosis outbreaks in cultured sea bass, *Dicentrarchus labrax*, L. from four different farms in the Aegean region of Turkey. Diseased fish were characterized by lethargie, exophthalmus, ulcers and skin lesions, haemorrhagies in the internal organs, ascites, paleness of kidney and liver. Morphological, physiological and biochemical tests were used to determine the phenotypic properties of pure cultures of isolated colonies in the samples taken from the internal organs and blood. Aquarapid-Va diagnostic kits and Mono-Va agglutination kit were also included in the study. The principal histological change was massive deposition of haemosiderin in the melano-macrophage centers in the spleen tissue.

Keywords: European sea bass (*Dicentrarchus labrax* L.), *L. anguillarum*, *V. ordalii*, *V. harveyi*, diagnostic kits, histology

[*] **Correspondence to:** Dr. Jale KORUN, Department of Fish Diseases, Faculty of Fisheries, Akdeniz University, 07058, Kampus, Antalya-TURKEY.
Tel: (+90 242) 310 20 18, Fax: (+90 242) 226 20 13

E-mail: jalekorun@yahoo.com

Introduction

Vibriosis is an important disease in some commercially important fish species including cultured European sea bass (*Dicentrarchus labrax*), gilt-head seabream (*Sparus aurata*), silver sea bream (*Sparus sabra*), snarpsnout sea bream (*Puntazzo puntazzo*), red porgy (*Pagrus pagrus*), sole (*Solea senegalensis*), turbot (*Scophthalmus maximus*) and Atlantic salmon (*Salmo salar*). The outbreaks have been reported in several countries including China, Greece, Spain, Venezuela, Israel and Turkey (Korun and Gokoglu, 2007; Yiagnisis et al., 2007; Demircan and Candan, 2006; Tanrikul et al., 2004; Villamil et al., 2003; Zorrilla et al., 2003a; Zorrilla et al., 2003b; Li et al., 1999; Cagırgan and Yurekliturk, 1996; Colorni et al., 1981). Generally, the term 'vibriosis' has been used to define *Listonella* (*Vibrio*) *anguillarum* infections (Ghittino et al., 2003). However, other members of the genus *Vibrio*, e.g. *V. alginolyticus*, *V. ordalii*, *V. salmonicida*, *V. harveyi* and *V. parahaemolyticus*, were also isolated from diseased fish (Toranzo et al., 2005; Austin and Austin, 1999).

The purpose of this study was to identify the aetiological agents of vibriosis outbreaks in the European sea bass cultured in four different farms located in the Aegean region of Turkey and examine the histological changes produced by naturally acquired infections of *L. anguillarum*, *V. ordalii* and *V. harveyi* in the moribund fish.

Materials and Methods

Two epizootics (E1 and E2) at four different sea bass farms (F1, F2, F3 and F4) in the Aegean region of Turkey were observed in January and May 2003 when the water temperature ranged between 19˚C to 22˚C. The daily mortalities in these farms were 10%, 15%, 9% and 12%, respectively. Affected fish (25 fish) were obtained from marine cages at F2, F3, F4; in the case of F1, moribund fish were taken from indoor tanks. Before microbiological isolation, the fish were anaesthetized with 1.5 ml 2-phenoxyethanol (Fluka, Switzerland) per 1 L sea water. Samples taken from kidney, spleen, liver and blood, and streaked onto tryptic soy agar (TSA-S) (Merck, Germany) supplemented with 2% NaCl and brain hearth infusion agar (BHIA-S) (Merck) supplemented with 2% NaCl. The inoculated media were incubated at 22˚C for 48 h. Aquarapid-Va (*Listonella* (*Vibrio*) *anguillarum* from BIONOR A/S, Skien, Norway) was used following the manufacturer's recommendations. Samples of kidney tissue (0.5±0.1 g) were immediately tested with the Aquarapid-Va kit. The sensivity and specificity of the test kit were evaluated according to the formulation reported by Rønning (1994). The Mono-Va agglutination kit was used to confirm the identification of the bacterial strains isolated from the diseased fish. Plates were incubated at 22 ˚C and colonies were isolated after 48 h on the media TSA-S and BHIA-S and further characterized by morphological, biochemical and enzymatic tests as previously described (Austin and Austin, 1999; Stavric and Buchanan, 1995; Baumann and Furniss, 1994). Antibiotic susceptibility test was performed using the disk diffusion method on Mueller-Hinton agar (Merck) described by Barry and Thornsberry (1985) and Bauer et al. (1966), except that 1% NaCl was used to prepare the media. Eigth or 10 colonies from BHIA-S incubated for 24 h at 22˚C, were suspended in 2 ml of sterile 0.9% NaCl saline to a density equal to McFarland Opacity Standard No 0.5 (approximately cell density $1.5x10^8$/ml). The bacterial suspension was inoculated onto Mueller-Hinton salt agar. Antibiotic discs (Oxoid, England) containing the folowing antibiotics: ampicillin 10 µg (AMP 10), c. sulphonamides 300 µg (S3 300), flumequine 30 µg (UB 30), kanamycin 30 µg (K 30), novobiocin 15 µg (NVB 15), oxolinic acid 2 µg (OA 2), penicilin G 10 iu (10 iu=6µg), tetracycline 30 µg (TE 30) and trimethoprime 5 µg (W 5) were dispensed on the surface of the medium and incubated for 24 h at 22˚C. After this incubation, the diameters (in millimeters) of the complete inhibition zones of visible bacterial growth around each disc were measured and the results were recorded as resistant or susceptible according to the interpretive limits of the Clinical and Laboratory Standards Institute (NCCLS, 2003). Excised pieces of tissues from skin, muscle, gills, spleen, liver, kidney, stomach and intestine were fixed in 10% buffered formalin solution containing 1% NaCl within 48 h after necropsy. The tissues were dehydrated in an ethanol series, infiltrated and embedded in parafin wax and sectioned on a microtome at 5 µm. Tissue sections (5 µm thick) were stained with Meyer's haematoxylin and eosin as described by Bullock (1989) and Coolidge and Howard (1979).

Results and Discussion

European sea bass (*D. labrax*) is one of the most economically important fish species in the Mediterranean region (Varsamos et al., 2006; Afonso et al., 2005). Vibriosis is reported among the bacterial diseases affecting sea bass farming (Toranzo et al., 2005; Pujalte et al., 2003; Zorrilla et al., 2003b; Botella et al., 2002; Dos-Santos et al., 2001; Pedersen et al., 1997; Bakrouf et al., 1995; Santos et al., 1995; Myhr et al., 1991; Dec et al., 1990).

Vibriosis is characterized by dark skin, pale gills, haemorrhagic areas near the mouth and on the base of fins, exopthalmia, corneal opacity and ulcers on the skin surface. Internally, moribund fish show severe anaemia and have haemorrhage in the abdominal fat, kidney and liver (Toranzo et al., 2005; Zorrilla et al., 2003a; Austin and Austin, 1999; Le Breton, 1999; Company et al., 1999; Alvarez et al., 1998; Balebona et al., 1998; Cagırgan and Yurekliturk, 1996).

The clinical features of moribund sea bass affected by *Vibrio* outbreaks are given in Table 1. Necropsy findings of these fish included haemorrhage in the liver, air bladder and intestinal wall, pale kidney and liver, ascites in the body cavity, yellowish-bloody fluid in the intestine, enlarged spleen and empty stomach. These results were similar to clinical and necropsy findings reported by the above mentioned authors. However, in this study, the corneal opacity in the diseased fish affected with the different *Vibrio* species was not observed.

Vibrio is one of the most important bacterial genera in aquaculture (Vandenberghe et al., 2003). Some species such as *L. anguillarum*, *V. harveyi*, *V. alginolyticus* and *V. ordalii* have been characterized as fish pathogens (Company et al., 1999; Balebona et al., 1998; Lee et al., 1996) as well as crustaceans and bivalve molluscs species (Alvarez et al., 1998; Bolinches et al., 1986; Bowser et al., 1981), some species e.g. *V. alginolyticus* have been reported as probionts (Gomez-Gil et al., 2000). Members of *Vibrio* genus are fermentative, motile, oxidase positive and sensitive to 0/129 Vibriostat test (150 μg) (Austin and Austin, 1999; Baumann and Furniss, 1994; Baumann and Schubert, 1984). Among *L. anguillarum* isolates, total of 23 serotypes (O1-O23, European serotype designation) were reported (Pedersen et al., 1999). However, only serotype O1, O2 and also O3 have been associated with losses of fish throughout the world (Toranzo et al., 2005). *V. ordalii* has been formerly classified as *L. anguillarum* biotype 2 (Schieve and Crosa, 1981). However, Actis et al. (1999) reported that *V. ordalii* had different properties such as phenotypic and genotypic characters. *L. anguillarum*, a Gram-negative, facultatively rod-shaped bacterium, gives positive reaction to the arginine dihydrolase test and uses citrate. This species is ONPG-positive. *V. ordalii* strains are arginine dihydrolase negative and do not use citrate. The strains are also ONPG-negative. *V. harveyi* strains are Gram-negative motile rods that are oxidase positive, Voges-Proskauer negative, lysine decarboxylase and ornithine decarboxylase positive, ONPG-negative and urease negative (Gauger and Gomez-Chiarri, 2002; Austin and Austin, 1999; Balebona et al., 1998; Alsina and Blanch, 1994).

The phenotypic characteristics of *Vibrio* species isolated from moribund sea bass are shown in Table 2. The isolated strains had similar phenotypic properties to other *Vibrio* species reported by Austin and Austin (1999), Balebona et al. (1998), Baumann and Furniss (1994) and Baumann and Schubert (1984). Therefore, the isolated strains were identified as *L. anguillarum*, *V. ordalii* and *V. harveyi*.

Traditional bacteriology is appropriate for the detection of common and easily cultured pathogens, however, these methods can be time-consuming (Adams, 2004). The development of diagnostic procedures applicable under field conditions has improved the accuracy and the time required for diagnosis of fish species (Gonzalez et al., 2004). BIONOR AS has developed different systems for example Aquarapid kit and Mono agglutination kit for the detection of fish pathogens. Aquarapid kits reduce the diagnosis and identification times of bacterial pathogens and their resultant diseases (Magariños et al., 1996). In field studies, the Aquarapid-Va kit produced positive results for *L. anguillarum* and *V. ordalii* and negative for diseased fish infected with *V. harveyi*. The sensivity and specifity of the kit were 1.0 and 1.0, respectively. Therefore, as Rønning (1994) suggested, the Aquarapid-Va kit was able to identify *L. anguillarum* and *V. ordalii*. The *L. anguillarum* strain agglutinated in the Mono-Va agglutination test kit for *L. anguillarum*.

No agglutination was observed between the test and control reagents of this kit when the kit was applied to other members of the *Vibrionaceae* family such as *V. ordalii*, *V. harveyi* in agreement with the findings of Romalde et al. (1995).

The most effective chemicals to vibriosis treatment were ampicillin, flumequine, furazolidone, oxolinic acid, sulphamethazine and nitrofurantoin (Soffientine et al., 1999; Balebona et al., 1998). The isolated *Vibrio* species in our study were sensitive to six of the nine antibiotics tested: flumequine, kanamycin, novobiocin, oxolinic acid, penicilin G and tetracycline. *V. ordalii* strains were sensitive to ampicillin whereas it was not observed in the other *Vibrio* strains. However, all the isolates were resistant to c. sulphonamides and trimethoprime.

Table 1. Clinical features of affected sea bass

Gross sings	**Disase outbreak number**			
	F1[a] (E1)	**F2[b] (E2)**	**F3[c] (E2)**	**F4[d] (E2)**
Lethargy	+	+	-	-
Eratic swimming behaviour	-	-	+	+
Anorexia	+	+	+	+
Exophthalmos	+	-	+	+
Darkening of the body colour	+	+	+	+
Loss of scales	+	+	+	+
Pale gills	+	+	+	+
Haemorrhage on body surface, head, jaws and gills	+	+	+	+
Ulcers on skin	-	+	+	+
Skin lesions	-	+	+	+

a: infected with *V. ordalii*; **b:** *L. anguillarum*; **c-d:** *V. harveyi*; **E1:** January 2003; **E2:** May 2003.

Examination of tissue sections from moribund fish (230-250 g) infected with *V. ordalii* showed following changes. The kidney was severely affected, displaying necrosis in renal tubules. In the spleen, massive deposition of haemosiderin in the melano-macrophage centres was observed. In addition to these findings, vacuoler degeneration in the liver and haemorrhage in the pericardium (Figure 1) were found. However, there were no histological changes in the intestinal mucosa of the infected fish.

The histology of diseased fish (85-140 g) from which *L. anguillarum* was isolated showed severe depletion of haemopoietic cells, deposition of haemosiderin in the spleen and peritubular vacuolar degeneration or liquefactive necrosis in the renal tubules. Haemorrhage and cellular inflammatory infiltration were observed in the necrotic lateral musculature under the haemorrhagic skin lesions (Figure 2). Haemorrhage was also seen in liver (Figure 3), gills and the propria mucosa of the skin.

The histological changes in moribund fish (2-5 g) infected with *V. harveyi* from two farms showed similar changes observed in the other affected fish infected with other *Vibrio* spp. consisted that liquefactive necrosis in the renal tubules and interrenal haemopoietic tissue and depletion of haemopoietic tissue in the kidney and necrosis in the gill filamantens. In addition to these findings, intestinal mucosa membrane was found necrotic and sloughed into the lumens.

Table 2. Results of phenotypic characterization of *Vibrio* strains from infected sea bass

Tests	E1 (January 2003)	E2 (May 2003)		
	F1[a]	F2[b]	F3[c]	F4[d]
Gram stain	-	-	-	-
Cell morphology	R	R	R	R
Catalase	+	+	+	+
Oxidase	+	+	+	+
Motility	+	+	+	+
O/F (glucose)	F	F	F	F
Gas from glucose	-	-	-	-
Swarming	-	-	+	+
Luminescence	-	-	-	-
Voges-Proskauer	-	+	-	-
Methyl-Red	-	-	+	+
Indole production	-	+	+	+
ADH	-	+	-	-
LDC	-	-	+	+
ODC	-	-	+	+
Amylase	-	+	-	-
Gelatinase	+	+	V	+
Urease	-	-	-	-
Growth at:				
4˚C	-	-	-	-
22˚C	+	+	+	+
37˚C	-	+	+	+
40˚C	-	-	-	-
Growth in:				
0% NaCl	-	-	-	-
3% NaCl	+	+	+	+
6% NaCl	-	+	+	+
8% NaCl	-	-	-	-
Acid production from:				
D-glucose	+	+	+	+
L-arabinose	-	-	-	-
myo-inositol	-	-	-	-
Lactose	-	-	-	-
D-mannitol	-	+	+	+
Sucrose	+	+	+	+
Nitrate reduction	-	+	+	+
Citrate utilization	-	+	-	-
H_2S production	-	-	-	-
Haemolysis of sheep erythrocytes	-	+	-	-
TCBS, growth	+	+	+	+
TCBS, sucrose fermentation	Y	Y	Y	Y
β-galactosidase (ONPG)	-	+	-	-
Sensivity to:				
O/129 (10 µg/disk)	S	S	S	S
O/129 (150 µg/disk)	S	S	S	S

Symbols: (+): positive, (-): negative, V: variable result, (F): fermantative, (Y): yellow coloured colonies, (S): sensitive, (R): rod, (ADH): arginine dihydrolase, (LDH): lysine decarboxylase, (ODH): ornithine decarboxylase, (ONPG): o-nitrophenyl-β-D-galactopyranoside, (O/129): 2, 4-diamino-6,7-diisopropylpteridine phosphate, (TCBS): thiosulphate citrate bile salt sucrose, a: *V. ordalii*; b: *L. anguillarum*; c-d-: *V. harveyi*.

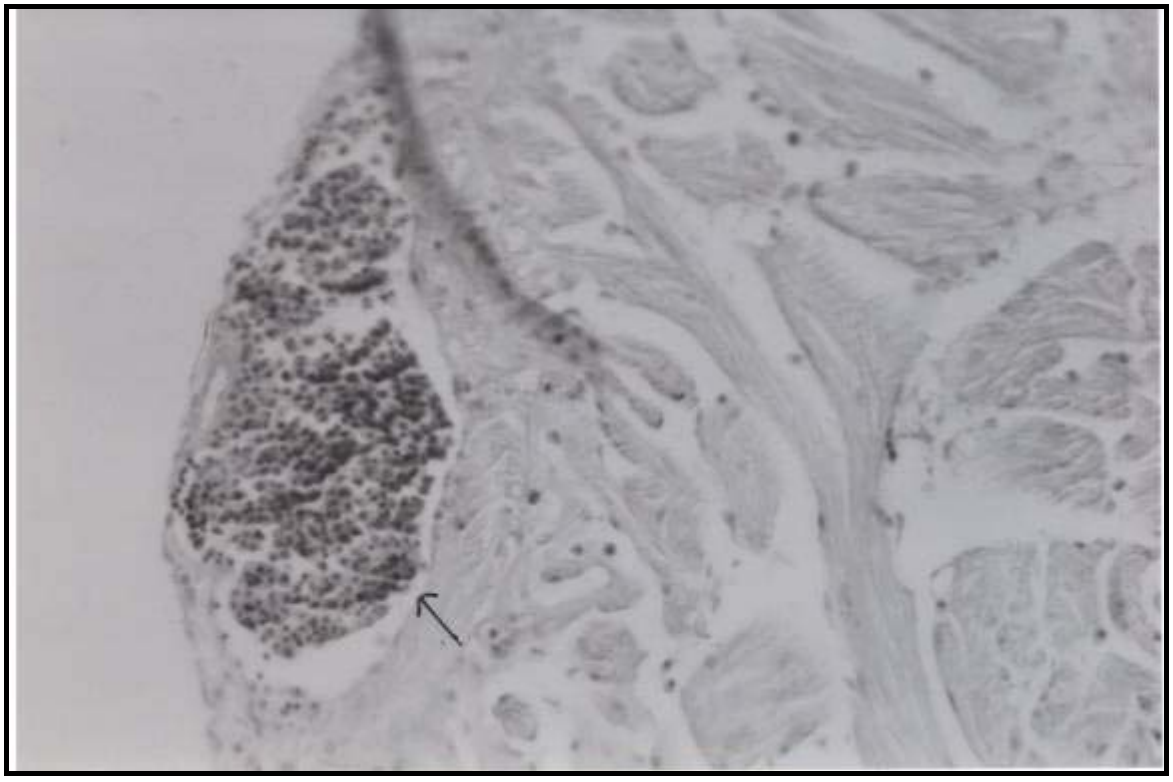

Fig. 1. Haemorrhage (arrowed) in the pericardium of fish infected with *V. ordalii*, Haemotoxylin and Eosin x 500.

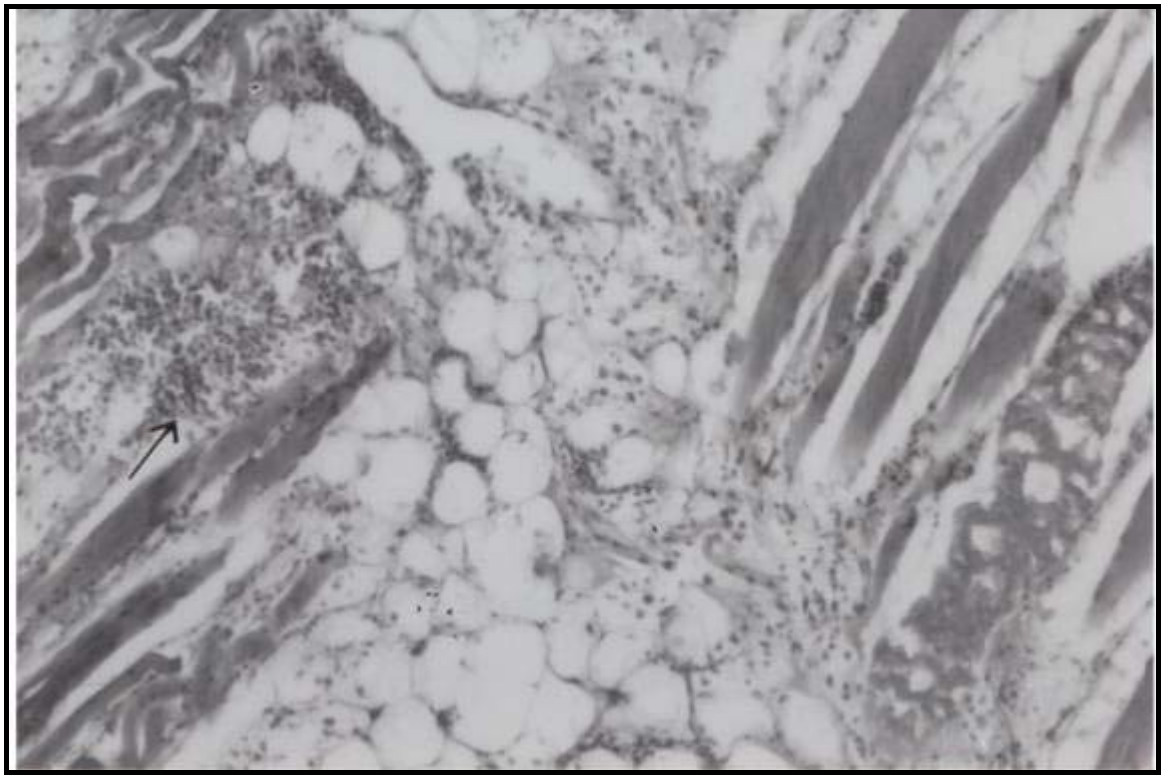

Fig. 2. Haemorrhages (arrowed) and cellular infiltration in the necrotic body muscles of fish infected with *L. anguillarum*, Haematoxylin and Eosin x 250.

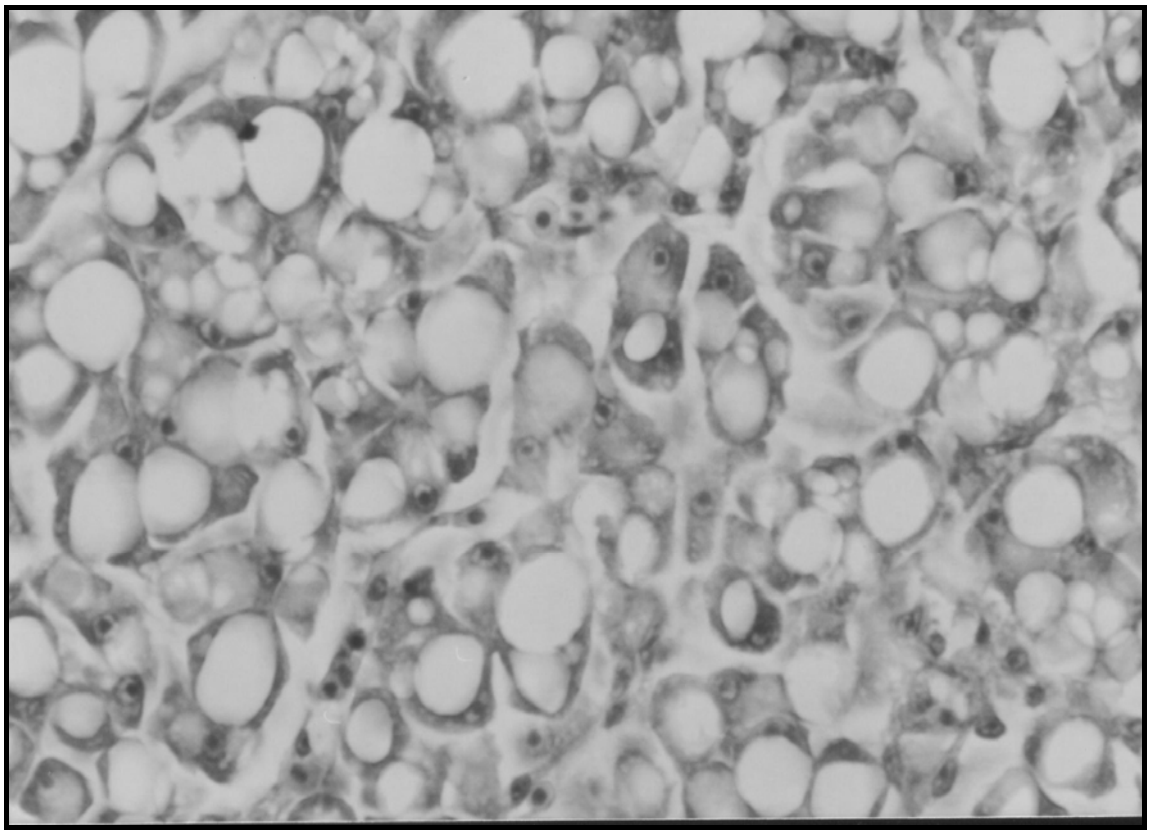

Fig. 3. Haemorrhages and vacuoler degeneration in the liver of fish infected with *L. anguillarum*, Haematoxylin and Eosin x 1000.

The histopathology of *Vibrio* spp. infected fish bore similarities to that observed in sea bass by other workers (Stephens et al., 2006; Agius and Roberts, 2003; Tendencia, 2002; Ransom et al., 1984) with the most striking similarities such as large reduced areas of haemopoietic tissue and deposition of haemosiderin in the melano-macrophage centres in the spleen, vacuoler degeneration or necrosis of tubule epithelium and haemorrhages in some glomerulus in the kidney, or liquefactive necrosis in renal and interrenal haemopoietic tissues. However, intestinal mucous membrane was found necrotic and sloughed into the lumens only in juvenile fish infected with *V. harveyi* and also there were no depositions of haemosiderin in the liver of the moribund fish affected with *V. ordalii*, *L. anguillarum* and *V. harveyi*.

In controlling of vibriosis, vaccine is an effective protective measurement (Myhr et al., 1991). Fish in marine aquaculture are usually vaccinated against vibriosis sourced from *L. anguilarum* (Angelidis, 2006; Le Breton, 1999; Pedersen et al., 1997). In many countries, vaccines procedures have induced number of disease outbreaks from this species (Pedersen et al., 1997; Myhr et al., 1991). However, this case has affected that other *Vibrio* species caused infections in fish (Pedersen et al., 1997; Myhr et al., 1991). Futhermore, it was reported that different *Vibrio* species were isolated from the vaccinated fish (Myhr et al., 1991). In Turkey, vibriosis vaccines have been introduced since 1990s (Cagırgan, 2004) and employed containing antigen preparations of *L. anguillarum* serotypes I & II and *V. ordalii*. The above mentioned explanations may be an answer why different *Vibrio* species isolated in this study.

Conclusion

In conclusion, the aetiological agents of vibriosis outbreaks in the European sea bass cultured in four different farms located in the Aegean region of Turkey were identified and the histological changes produced by naturally acquired infections of *L. anguillarum*, *V. ordalii* and *V. harveyi* in the moribund fish were examined in this study.

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References

Actis, L. A., Tolmasky, M. E., Crosa, J. H. (1999). Vibriosis *in Woo, P. T. K., Bruno, D. W., ed., Fish Diseases and Disorders, vol. 3.,* CAB Intern., Publ., United Kingdom.

Adams, A. (2004). Immunodiagnostic in aquaculture. *Bulletin of the European Association of Fish Pathologists*, **24**(1): 33-37.

Afonso, A., Games, S., da Silva, J., Marques, F., Henrique, M. (2005). Side effects in sea bass (*Dicentrarchus labrax*, L.) due to intraperitoneal vaccination against vibriosis and pasteurellosis, *Fish & Shellfish Immunology*, **19**: 1-16.

Agius, C., and Roberts, R. J. (2003). Melano-macrophage centres and their role in fish pathology, *Journal of Fish Diseases*, **26**: 499-509.

Alsina, M. and Blanch, A. R. (1994). Improvement and update of set of keys for biochemical identification of enviromental *Vibrio* species. *Journal of Applied Bacteriology*, **77**: 719-721.

Alvarez, J. D., Austin, B., Alvarez, A. M. and Reyes, H. (1998). *Vibrio harveyi*: a pathogen of penaeid shrimps and fish in Venezuela. *Journal of Fish Diseases*, **21**: 313-316.

Angelidis, P. (2006). Immersion booster vaccination effect on sea bass (*Dicentrarchus labrax* L.), *Journal of Animal Physiology and Animal Nutrition*, **90**: 46-49.

Austin, B., and Austin, D. A. (1999). *Fish Pathogens Disease in Farmed and Wild Fish*, 3rd ed. (revised), Praxis Publ., Chiester, UK.

Bakhrouf, A., Ouada, H. B. and Oueslati, R. (1995). Sea bass *Dicentrarchus labrax* vibriosis treatment in a pisciculture area in Monastir, Tunisia, *Marine Life*, **5(2)**: 47-54.

Balebona, M. C., Zorrilla, I., Moriñigo, M. A. and Borrego, J. J. (1998). Survey of bacterial pathologies affecting farmed gilt-head sea bream (*Sparus aurata*, L.) in south-western Spain from 1990 to 1996. *Aquaculture*, **166**: 19-35.

Barry, A. L. and Thornsberry, C. (1985). Susceptibility tests: diffusion test procedures. *Manual of Clinical Microbiology*, 4 th ed., Washington.

Bauer, A. W., Kirby, W. M., Sherris, J. C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disc method. *American journal of Clinical Pathology*, **45**: 493-496.

Baumann, P. and Furniss, A. L. (1994). Vibrionaceae, *in Hensyl W. R., ed, Bergey's Manual of Determinative Bacteriology*, Williamd and Wilkins, Baltimore.

Baumann, P. and Schubert, R. H. W. (1984). Family II. Vibrionaceae, Veron 1965, *in Krieg, N. R.., ed, Bergey's Manual of Systematic Bacteriology*, Williamd and Wilkins, Baltimore.

Bolinches, J., Toranzo, A. E., Silva, A., and Barja, J. L. (1986). Vibriosis as the main causative factor of heavy mortalities in the oyster culture industry in Nortwestern Spain. *Bulletin of the European Association of Fish Pathologists*, **6**: 1-4.

Bowser, P., Rosenmark, R. and Reiner, C. R. (1981). A preliminary report of vibriosis in cultured American lobsters, *Homarus americanus*. *Journal of Invertebrate Pathology*, **36**: 80-85.

Botella, S., Pujalte, M., Macián, J. and Garay, E. (2002). Amplified fragment length polymorphism (AFLP) and biochemical typing of *Photobacterium damselae* subsp. *damselae*. *Journal Applied of Microbiology*, **93(4)**: 681-688.

Bullock, A. M. (1989). Laboratory Methods, *in Roberts, R. J., Fish Pathology*, Bailliere Tindall, London, UK.

Cagırgan, H. (2004). Vaccine development in sea bass fry (*Dicentrarchus labrax* L. 1758) against vibriosis. *E. U. Journal of Fisheries & Aquatic Sciences*, **21(3-4)**: 271-274.

Cagırgan, H. and Yurekliturk, O. (1996). A research on the diagnosis and treatment of cultured sea bream (*Sparus aurata* L.) and sea bass (*Dicentrarchus labrax*). *The*

Journal of Centre Veterinary Control and Research Institute, **21(35)**: 113-122.

Colorni, A., Paperna, I. and Gordin, H. (1981). Bacterial infections in gilt-head sea bream *Sparus aurata* cultured at Elat, *Aquaculture*, **23**: 257-267.

Coolidge, B. J. and Howard, R. M. (1979). *Animal Histology Procedures*, National Institute of Health, Maryland, USA.

Company, R., Sitjo-Bobadilla, A., Pujalte, M. J., Garay, E., Alvarez-Pellitero, P. and Pérez-Sánchez, J. (1999). Bacterial and parasitic pathogens in cultured common dentex, *Dentex dentex*, *Journal of Fish Diseases*, **22**: 299-309.

Dec, C., Angelidis, P. and Baudin Laurencin, F. (1990). Effects of oral vaccination against vibriosis in turbot, *Scophthalmus maximus* (L.) and sea bass, *Dicentrarchus labrax* (L.). *Journal of Fish Diseases*, **13(5)**: 369-376.

Demircan, D., and Candan, A. (2006). Identification of *Vibrio anguillarum* by PCR (*rpoN* Gene) associated with vibriosis in marine fish in Turkey. *Turkish Journal of Veterinary and Animal Sciences*, **30**: 305-310.

Dos Santos, N. M. S., Taverne-Thiele, J. J., Barnes, A. C., Ellis, A. E., and Rombout, J. H. W.M. (2001). Kinetics of juvenile sea bass (*Dicentrachus labrax*, L.) systemic and mucosal antibody secreting cell response to different antigens (*Photobacterium damselae* subsp. *piscicida*, *Vibrio anguillarum* and DNP), *Fish & Shellfish Immunology*, **11**: 317-331.

Gauger, E. J., and Gómez-Chiarri, M. (2002). 16 S ribosomal DNA sequencing confirms the synonymy of *Vibrio harveyi* and *Vibrio charchariae*. *Diseases of Aquatic Organisms*, **52**: 39-46.

Ghittino, C., Latini, M., Agnetti, F., Panzieri, C., Lauro, L., Ciappelloni, R. and Petracca, G. (2003). Emerging pathologies in aquaculture: effects on production and food safety. *Veterinary Research Communications*, **27(1)**: 471-479.

Gomez-Gil, B., Roque, A., Turnbull, J. F. (2000). The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. *Aquaculture*, **191**: 259-270.

Gonzalez, S. F., Osorio, C. R., Santos, Y. (2004). Evaluation of the Aquarapid-Va, Aquaeia-Va and dot blot assays for the detection of *Vibrio anguillarum* in fish tissues. *Journal of Fish Diseases*, **27**: 617-621.

Korun, J. and Gokoglu, M. (2007). *Listonella anguillarum* isolated from hatchery-cultured red porgy *Pagrus pagrus* in Turkey. *Journal of Animal and Veterinary Advances*, **6(6)**: 823-827.

Lee, K. K., Yu, S. R., Chen, F. R., Yang, T. I., Liu, P. C. (1996). Virulence of *Vibrio alginolyticus* isolated from diseased tiger prawn, *Penaeus monodon*. *Current Microbiology*, **32**: 229-231.

Li, J., Yie, J., Foo, W. T., Ling, J. M., Xu, H., Woo, N. Y. S., 1999. Antibiotic resistance and plasmid profiles of Vibrio isolates from cultured silver sea bream, *Sparus sabra*. *Marine Pollution Bulletin*, **39(1-12)**: 245-249.

Breton, Le A. D. (1999). Mediterranean finfish pathologies: present status and new developments in prophylactic methods. *Bulletin of the European Association of Fish Pathologists*, **19(6)**: 250-253.

Magariños, B., Toranzo, A. E. and Romalde, J. L. (1996). Phenotypic and pathobiological characteristics of *Pasteurella piscicida*. *Annual Review of Fish Disease*, **6**: 41-64.

Myhr, E., Larsen, J. L., Lillehaug, A., Gudding, R., Heum, M. and Håstein, T. (1991). Characterization of *Vibrio anguillarum* and closely related species isolated from farmed fish in Norway. *Applied and Enviromental Microbiology*, **57(9)**: 2750-2757.

NCCLS, (2003). *Performance standards for antimicrobial disc susceptibility tests: approved standard, National Committee for Clinical Laboratory Standard of Antimicrobial Susceptibility*, *Document M2-AS*, Pennsylvania, USA.

Pedersen, K., Dalsgaard, I. and Larsen, J. L. (1997). *Vibrio damsela* associated with diseased fish in Denmark. *Applied and Enviromental Microbiology*, **63(9)**: 3711-3715.

Pedersen, K., Austin, B., Austin, D. A. and Larsen, J. L. (1999). Vibriosis associated with mortality in cultured plaice *Pleuronectes platessa* fry. *Acta Veterinary Scandinavia*, **40**: 263-270.

Pujalte, M. J., Sitjà-Bobadilla, A., Maclán, M. C., Belloch, C., Álvarez-Pellitero, P., Pérez-Sánchez, J. Uruburu, F. and Garay, E. (2003). Virulence and molecular typing of *Vibrio harveyi* strains isolated from cultured dentex, gilthead sea bream and european sea bass. *Systematic Applied Microbiology*, **26(2)**: 284-292.

Ransom, D. P., Lannan, C. N., Rohovec, J. S. And Fryer, J. L. (1984). Comparison of histopathology caused by *Vibrio anguillarum* and *Vibrio ordalii* in three species of Pacific salmon, *Journal of Fish Diseases*, **7**: 107-1115.

Romalde, J. L., Magariños, B., Fouz, B., Bandin, I., Núñez, S. and Toranzo, A. E. (1995). Evaluation of Bionor Mono kits for rapid detection of bacterial fish pathogens. *Diseases Aquatic Organisms*, **21**: 117-119.

Rønning, O. (1994). *Evaluation of Bionor aquarapid test kits, SINTEF Industrial Chemistry*, *STF 27*, *F 944026*.

Santos, Y., Pazos, F., Bandin, I. and Toranzo, A. E. (1995). Analysis of antigens present in the extracellular products and cell surface of *Vibrio anguillarum* serotypes 01, 02, and 03. *Applied and Enviromental Microbiology*, **61(7)**: 2493-2498.

Schieve, M.H. and Crosa, J.H. (1981). Molecular characterization of *Vibrio anguillarum* biotype 2, *Canadian Journal of Microbiology*, **27**: 1011–1018.

Soffientino, B., Gwaltney, T., Nelson, D. R., Specker, J. L., Manuel, M. and Gómez-Chiarri, M. (1999). Infectious necroziting enteritis and mortality caused by *Vibrio carchariae* in summer flounder *Paralichthys dentatus* during intensive culture. *Diseases Aquatic Organisms*, **38**: 201-210.

Stavric, S. and Buchanan, B. (1995). The isolation and enumaration of *Vibrio vulnificus* from fish and seafoods. *Laboratory Procedure MFLP-73, Health Protection Branch*, Ottowa, Canada.

Stephens, F. J., Raidal, S. R., Buller, N. and Jones, B. (2006). Infection with *Photobacterium damselae* subspecies *damselae* and *Vibrio harveyi* in snapper, *Pagrus auratus* with bloat. *Austrilian Veterinary Journal*, **84(5)**: 173-177.

Tanrikul, T. T., Cagırgan, H., Toksen, E. (2004). Identification of isolated *Vibrio* sp. from sea bass (*Dicentrarchus labrax* L. 1758) using API 20 E system. *Ege University Faculty of Fisheries Journal of Fisheries and Aquatic Sciences*, **21**: 243-247.

Tendencia, E. A. (2002). *Vibrio harveyi* isolated from cage-cultured sea bass *Lates calcarifer* Bloch in the Philippines. *Aquaculture Research*, **33**: 455-458.

Toranzo, A. E., Magariños, B. and Romalde, J. L. (2005). A review of the main bacterial fish diseases in mariculture systems. *Aquaculture*, **246**: 37-61.

Vandenberghe, J., Thompson, F. L., Gomez-Gil, B., Swings, J. (2003). Phenotypic diversity amongst *Vibrio* isolates from marine aquaculture systems. *Aquaculture*, **219**: 9-20.

Varsamos, S., Flik, G., Pepin, J. F., Wendelaar Banga, S. E. and Brevil, G. (2006). Husbandry stress during early life stages affects the stres response and health status of juvenile sea bass, *Dicentrarchus labrax*. *Fish & Shellfish Immunology*, **20**: 82-96.

Villamil, L., Figueras, A., Toranzo, A. E., Planas, M., Novoa, B. (2003). Isolation of highly pathogenic *Vibrio pelagus* strain associated with mass mortalities of turbot, *Scophthalmus maximus* (L.), larvae. *Journal of Fish Diseases*, **26**: 293-303.

Yiagnisis, M., Vatsos, I. N., Kyriakou, C., Alexis, M. (2007). First report of *Vibrio anguillarum* isolation from diseased big scale sand smelt, *Atherina boyeri* Risso 1810, in Limnos, Greece. *Bulletin of the European Association of Fish Pathologists*, **27(2)**: 61-69.

Zorrilla, I., Arijo, S., Chabrillon, M., Diaz, P., Martinez-Manzanares, E., Balebona, M. and Moriñigo, M. A. (2003a). *Vibrio* species isolated from diseased farmed sole, *Solea senegalensis* (Kaup) and evaluation of the potential virulence role of their ex-

tracellular products. *Journal of Fish Diseases*, **26**: 103-108.

Zorrilla, I., Moriñigo, M. A., Castro, D., Balebona, M. C., Borrego, J. J. (2003b). Intraspecific characterization of *Vibrio alginolyticus* isolates recovered from cultured fish in Spain. *Journal of Applied Microbiology*, **95**: 1106-1116.