Hayvancılık Araştırma Dergisi, (1993), 3, 2, 82-83.

THE FIRST ISOLATION OF *Pasteurella piscicida* FROM CULTURED SEA BREAM (*Sparus aurata*) IN TURKEY

Haşmet ÇAĞIRGAN[1]

SUMMARY

In this research the first isolation of *Pasteurella piscicida* in sea bream from Turkey were described. The effected fish showed no exaggerated symptoms in external examination. Most of them was showed loosing of a few scale, darkening of the skin, swiming on the top of the water and sluggishness. However, fish often exhibited hepato-spleno megalie and whitish milier tubercles like nodules on spleen in internal examination. Bacteriological analysis of diseased fish revealed presence of *Pasteurella piscicida* in pure culture in spleen kidney and liver. Bacterium was identified in morphologicaly and biochemicaly. Experimental injections were succeded with inoculation of 3 x 10^5 intra peritonealy to healthy looking sea bream which from not infected farm. The isolate was sensitive to most chemotherapotics in vitro but not effective in vivo.

KEY WORDS : Fish culture, sea bream, (*Sparus aurata*), pasteurellosis, *Pasteurella piscicida.*

ÖZET

Bu araştırmada, Türkiye'de çipura balıklarında *Pasteurella piscicida*'nın ilk defa izolasyonu açıklandı. Hasta balıklarda abartılmış semptomlar yoksa da; balıkların çoğunda bir kaç pulun dökülmesi, deride kararma, suyun üzerinde yüzme ve halsizlik gözlendi. Bununla beraber iç muayenede balıklarda "hepato spleno megali" ve dalakta milier tüberkül'dekine benzer beyazımtırak nodüller görüldü. Bakteriyolojik muayenelerde dalak, böbrek ve karaciğerden saf kültür halinde *Pasteurella piscicida* izole edildi. Bakteri morfolojik ve biyokimyasal olarak identifiye edildi. Sağlıklı görünen ve enfeksiyon olmayan bir çiftlikten sağlanan balıklara 3 x 10^5 bakterinin intraperitoneal olarak verilmesiyle deneysel enfeksiyon meydana getirildi. İzole edilen bakteri in vitro olarak kemoterapotiklerin çoğuna duyarlı bulunurken in vivo olarak etkin değildi.

ANAHTAR KELİMELER : Balık kültürü, çipura (*Sparus aurata*), pasteurellosis, *Pasteurella piscicida*

INTRODUCTION

Pasteurella piscicida was initially isolated from an epidemic of white perch (*Morone americanus*) and striped bass (*Morone saxatilis*) in 1963 from Chesapeake Bay of the USA (6) After this the first isolation of *P. piscicida*, the disease has been reported from yellow tail (*Seriola quinqueradiata*) (7), ayu (*Plecoglossus altivelis*) (8), black sea bream (*Mylio macrocephalus*) (9) and red sea bream (*Acanthopagrus schlegeli*) (13) in Japan. Pasteurellosis was not reported from Europe until June 1990. Although there are some reports indicating isolation of fish pathogenic Pasteurella like bacteria from different kind of fish in Europe but, the characteristics of the bacterium were found to be similar to atypical Aeromonas salmonicida rather than *P. piscicida* (12). The first isolation of *P. piscicida* from Europe was done in Italy in 1990 (5). It has been reported that the outbreak was observed in many euryhaline fish species as sea bass (*Dicentrarchus Labrax*), mullets (*Mugilidae*), gilthead bream (*Pagrus pagrus*) and Dover sole (*Solea solea*). Pasteurellosis was also observed in both wild and farmed fish species of Mediterranean cost of continental France and of Corsica. In mean time suspected case was seen in Greece (4) *P. piscicida* were also isolated from cultured sea bream (*Sparus aurata*) and turbot (*Scophtalmus maximus*) in Spain (12).

From February to May 1993 *Pasteurella piscicida* was recognized as a cause of mortality in farmed gilthead sea bream (*Sparus aurata*) in İzmir Bay. In this report the first epizootic of pasteurellosis in sea bream cultured in a marine farm were documented in Turkey. The isolation and identification of the causative organism and attempted treatments are presented.

MATERIAL and METHODS

During February and May 1993, an epizootic occurred approximately 100 gr of size sea bream (*Sparus aurata*) which cultured in a sea bream and sea bass farm in the external side of İzmir Bay which is not detoriated part of the Bay. Fish were stocked 15 kg/m^3 in net cages with an open circuit, and were fed with commercial dry pellets. The sea water temperature was 14°C. When the mortality was begun 40-50 fish were dying every day. The mortality reached the peak after 4 weeks of the beginning of the outbreak while the water temperature reached to 16 °C. The disease was observed in one cage than spread to other sea bream cages, in which the weight of the fish was 30 to 100 grams but not to any of the sea bass cages.

For bacterial isolation, samples were taken from spleen, liver, and kidney of darkened and sluggish sea bream. Samples were cultured on tryptic soy agar (TSA, Oxoid) brain hearth infusion agar (BHIA,Oxoid) cytophaga agar (CA) and thiosulfate citrate bile sucrose agar (TCBS, Hi-media, Atabay). The first three media were prepared with distilled water containing 25% sterilized natural sea water, which aged for at least 2 weeks and autoclaved for 60 minutes in order to destroy its natural antibiotic activity. TCBS (Hi media, Atabay) was prepared by the following the manufacturer instruction. The plates were incubated at 20 °C up to 2 weeks. Smears from different organs were examined after and before Gram and Methylene Blue stains. In order to make sure purity of isolated colonies were checked by streaking on the TSA and then their phenotypic traits were determined following the procedures of Austin (2, 3).

To determine the pathogenicity of the isolated *P. Piscicida* for sea bream 10 bealthy looking fish about 100 gr were maintained in a 100 liter aquarium of sea water at 18-20 °C with aeration. Other 10 fish containing one aquarium was spearated as control group. While the experimental ingection group was subjected to 3 times phosphate buffered saline (PBS) washed 3 x 10^5 bacteria by intraperitoneal injection, the control group subjected to sterile PBS. Both groups were maintained in same condition.

Chemotheropotic sensitivity test was performed by using disk diffusion techniques on Mueller Hinton agar (Oxoid) adding 2 % sodium chloride.

RESULT

Diseased fish had no obvious external lesions. Some of them had a few Furnestia sp. on the gill. Diseased fish were showing darkening, and loss of a few scales, darkening of skin, swimming top of the water and sluggishness.

Internally, hepato splenomegalie was visible. Enlarged spleen

1 : Research Assistant. Ege University, Fisheries Faculty, Department of Fish Disease, Urla, İZMİR.

Table 1. Phenotypic Characteristics of Isolated *Pasteurella piscicida*.

Fluorescent pigment	-	Beta galactosidase	-
Green pigment	-	Hemolysis of sheep eritrocytes	-
Gram stain	-	Gelatin degradation	-
Rod (R) Cocci (C)	Bipolar cocobacil	Urea degradation	-
Presence of aerial hyphae	-	Methyl red	+
Presence of microcyst	-	VP	+
Presence of endospores	-	Growth at 37 °C	-
Acid-Fast staining reaction	-	Growth on 0% NaCl	-
Motility by flagella	-	Growth on 7% NaCl	-
Gliding motility	-	Swarming colony	-
Requiremet for L-Cystine HCL	-	Sensivity to O/129	+
Aerobic growth	+	Growth on TBCS	-
O/F metabolism	F	Acid production from sugars glucose	+(Weak)
Catalase	+	Arabinose	-
Oxidase	+(Late)	Inositol	-
Indol production	-	Lactose	-
H2S	-	Mannitol	-
ADH	-	Salicin	-
LDC	-	Sucrose	-

was containing numerous, white, up to 2-3 mm diammeter tubercles. Large numbers of non-motile rod-shaped bacteria were observed in native examination of spleen and kidney tissue Stained smears were Gram negative bacilli and showing a distinct bipolar staining.

Isolated colonies were shiny, raised entire, translucent, and 1-2 mm diameter after 72 hour incubation in TSA. There was no growth on TCBS and 0% sodium chloride containing TSA. Bacteria werenon-motile, Gram negative bipolar staining rod, oxidase weak positive, catalase positive, fermentative and sensitive to O/129. The additional phenotypic tests listed on Table1, in which allowed us to identify the bacterium is *Pasteurella piscicida*.

Table 2. Chemothrepotic Sensivity tesTest Results of Isolated *P. piscicida*.

Chemotherapotics (µg/disk)	Sensivity to Different Chemotherapotics
Ampicilline (10)	+++
Cloramphenicol (30)	-
Erythromycine (15)	-
Flumequine (26)	+++
Kanamycine (30)	++
Nitrofurantoin (300)	-
Oxytetracycline (30)	+
Sulphamethaxasole-trimethroprim (25)	+++

According to the result of antibiotic sensitivity diseased fish were treated with appropriate chemotherapotics. The result of the antibiotic sensitivity tests were presented in Table 2. Diseased fish were tried to treat using Ampicilline 20 mg/kg in feed for 7 days, Chloramphenicole 75 mg/kg for 7 days, Flumequine 12.5 mg/kg for 7 days and Sulphadoxin-trimethoprim 50 mg/kg for 5 days in feed, but after stopping the treatment there was no obvious positive improve in condition of fish population. Nevertheless etiologic agent was isolated 3 days after the treatments ends. Total mortality was calculated as 22% against to all treatments. Even there was not any control group, it seems that pasteurellosis could not be controlled by Ampicillline, Chloramphenicole, Sulphadoxin-trimethoprim and Flumequine.

The result of the experimental infection showed that Koch's Postulates were confirmed.

DISCUSSION

Sparus aurata has been reared in Aegean sea coast of Turkey since 1980's. The outbreak of pasteurellosis reported here is the first case of the disease in Turkey. It has been belived that before 1990, There was not pasteurellosis outbreak in Europe. The disease was reported from Italy and France affecting mainly the sea bass and sea bream in 1990 (5 th International Conference E.A.F.P., Budapest, 25-29 August 1991).

P. piscicida isolated from yellowtail yielding strongly positive reactions for acid production from glucose, mannose, galactose, and fructose (7). Some variable results were also reported in biochemical characteristics of *P. piscicida* by different researchers, from different countries depending on different geographic locations (10). While Japanese isolates were showing Voges-Proscouer (VP) test weakly but oxydase strongly positive, this isolate was showing strongly positive VP, but weak positive in acid production from glucose and late as well as weak and oxydase positive (after 60 seconds). It may possible like that delay in oxydase test when working with marine bacteria (11). However typical morphology, and the other biochemical characterisitics of the isolated bacteria as well as confirmation by reference laboratory are showing that this isolete is *P. piscicida*.

While Ceschia et al., (5) was reporting mortality in sea bass (*Dicentrarchius labrax*) and sea bream (*Sparus aurata*), Baudin-Laurencin (4) did not observe mortality in farmed sea bream. In this research farmed sea bass (*D. labrax*) kept close to sea bream cages but did not appear susceptible to the disease.

In this research in vitro chemotherapotic sensitivity tests were positive for Ampicilline, Flumequine and Sulphadoxin-trimethohprim. However none of the treatment was effective in vivo. Possibly this is related with the drug can not reach into the tubercle.

Acknowledgements

The author would like to thank to Dr. D. Bruno from SOAFD Marine Laboratory, Scotland for confirmation of the isolate as *Pasteurella piscicida*.

REFERENCES

1. Austin B (1982) Toxonomy of Bacteria Isolated from a Coastal, Marine Fish-Raring Unit. Journal of Applied Bacteriology, 53, 253-268.
2. Austin B (1987) Bacterial Fish Pathogens: Disease in Farmed and Wild Fish. Ellis Horwood Limited, Chichester, pp. 364.
3. Austin B (1989) Methods in Aquatic Bacteriology, John Willey and Sons Ltd., Chichester, pp. 425.
4. Baudin-Laurencin F, Pepin JF, Raymond JC (1991) First observation of pasteurellosis in farmed and wild fish of the French Mediterranean coast. EAFP fifth International Conference Diseases of Fish and Shellfish. 25-29 August, p 17 Book of Abstract. Fish Culture Research Institute Szarvas, Hungary.
5. Ceschia G, Quaglio F, Giorgetti , Bertoja G, Bovo G (1991) Serious outbreak of Pasteurellosis (*Pasteurella piscicida*) in the Italian coast EAFP Fifth International Conference Diseases of Fish and Shellfish 25-29 August, p 26 Book of Abstract. Fish Culture Research Institute Szarvas, Hungary.
6. Conroy DA (1984) Agents: Bacteria In: O. Kinne (Ed.) Diseases of Marine animals Vol. 4, Part 1, Biologische Anstalt Helgolad, Hamburg, FRG., pp. 48-85.
7. Kubota S, Kimura M, Egusa S (1970) Studies on a bacterial tuberculoids of the yellowtail. 1. Symptomatology and histopathology. Fish Pathology, 4, 111-118.
8. Kusuda R, Miura W (1972) Characteristics of a Pasteurella sp. pathogenic for pond cultured ayu. Fish Pathology. 7, 51-57.
9. Muroga K, Sugiyama T, Ueki N (1977) Pasteurellosis in cultured black sea bream Mylio macrocephalus. J. Fac. Fish Anim. Husb., Hiroshima Univ., 16, 17-21.
10. Robohm RA (1983) *Pasteurella piscicida*. In: DP Anderson, M Dorson and P Dobourget (Ed.) Antigens of Fish Pathogens: Development and Production for Vaccines and Serodiagnostics. Collection Foundation Marcel Merieux, Lyon, pp. 161-175.
11. Sakazaki R, Balows A (1981) The Genera Vibrio, Pseudomonas, and Aeromonas. In: Starr et al. (Ed.) The Prokaryotes A Handbook on Habitats, Isolation, and identification of bacteria. Vol. 2 Berlin, Springer Verlag pp. 1272-1301.
12. Toranzo AE, Barreiro SB, Casal JF, Figueras A, Magarinos B, Barja JL (1991) Pasteurellosis in Cultured Gilthead Sea bream (*Sparus aurata*): First Report in Spain. Aquaculture, 1-15.
13. Yasunaga N, Hatai K, Tsukahara J (1983) *Pasteurella piscicida* from an epizootic of cultured red sea bream. Fish Pathology, 18, 107-110.