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PASTEURELLOSIS OF GILTHEAD SEABREAM (*SPARUS AURATA*) IN GRAN CANARIA ISLAND, SPAIN.

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Abstract

In this report we describe the first epizootic pasteurellosis in gilthead seabream cultured in a fish farm located in South Gran Canaria (Spain), beginning in January 1996. Isolates from moribund fish conformed to *Pasteurella piscicida* biochemically and serologically. The measures which were applied to control the disease and the epidemiological hypothesis of the source of this outbreak are also discussed.

Introduction

Fish pasteurellosis has long been reported as an important disease affecting both natural and cultured populations of fish all over the world. *Pasteurella pisicida* has recently reassigned as *Photobacterium damsela* subsp. *pisicida* (Gauthier *et al.*, 1995). The bacterium has been isolated from populations of different fish species, such as white perch *(Morone Americanus)* (Snieszko *et al.*, 1964) striped bass (*Morone saxatilis*) (Paperna and Zwerner, 1976) and striped mullet (*Mugil cephalus*) (Lewis *et al.*, 1970). Some of the early reports from farmed fish were from Japan and mainly affected yellowtail (*Seriola quinqueradiata)*(Kubota *et al.*, 1970; Kusuda and Yamaoka, 1972) then spreading to other fish species, such as black sea bream (Muroga *et al.*, 1977; Ohnishi *et al.*, 1982) and red sea bream (Yasunaga *et al.*, 1983). Subsequently the disease was been reported from different countries as causing significant losses in several fish species. Some reports have been from European fish farm, such as Great Britain (Ajmal and Hobbs, 1967), Norway (Håstein and Bullock, 1976) and Hungary. Toranzo *et al.*, (1991) described the first outbreak of pasteurellosis in Spain in gilthead seabream (*Sparus aurata)* cultured in the north-west. Later, Balebona *et al.*, (1992) described the first epizootic of pasteurellosis in sea bass *(Dicentrarchus labrax)* cultured in several farms located in south-western Spain.

In this report we describe the first epizootic pasteurellosis in gilthead seabream cultured in a fish farm located in South Gran Canaria (Spain), beginning in January 1996. The microbiological and histopathological studies are presented. The measures which were applied to control the disease and the epidemiological hypothesis of the source of this outbreak are also discussed.

Material and Methods

Culture conditions

Juvenile gilthead seabream (*Sparus aurata)* cultured in a fish farm in South Gran Canaria (Spain), were stocked in tanks with an open flow circuit. They had been transported at the larval stage from a hatchery located in the South of Spain. The water temperature during the epizootic, fluctuated between 16°C and 19°C, salinity ranged between 32‰ and 36‰.The dissolved oxygen was maintained at saturation levels (7-8 ppm).

Pathology

Affected animals were observed and their clinical signs and gross lesions after necropsy were recorded.

Samples from all organs were fixed in neutral buffered 10% formalin, embedded in paraffin-wax, sectioned at 4µm and stained with haematoxylin and eosin (HE). Selected samples were also stained with Gram and PAS techniques.

Table I. Characteristic shown by *P. piscicida* strain isolated in Gran Canaria and reference strains.

Test	Strain isolated in G.C.	DI-21	ATCC 17911	ATCC 29690
Gram staining	-	-	-	-
Bipolar staining	+	+	+	+
Oxidase	+	+	+	+
Catalase	+	+	+	+
Motility	-	-	-	-
Nitrate reduction	-	-	-	-
H_2S production	-	-	-	-
Indole production	-	-	-	-
Citrate utilisation	-	-	-	-
O/F glucose	F	F	F	F
Gas glucose production	-	-	-	-
Growth at: 5°C	-	-	-	-
15°C	-	-	-	-
25°C	+	+	+	+
37°C	-	-	-	-
Growth in: 0% NaCl	-	-	-	-
1%NaCl	+	+	+	+
3% NaCl	+	+	+	+
5% NaCl	-	-	-	-
ß-Galactosidase (ONPG)	-	-	-	-
Arginine dehydrolase	+	+	+	+
Lysine descarboxylase	-	-	-	-
Ornithine descarboxylase	-	-	-	-
Voges Proskauer	(+)	(+)	(+)	(+)
Growth in Mac Conkey	-	-	-	-
Gelatinase	-	-	-	-
Caseinase	-	-	-	-
Urease	-	-	-	-
Sensitivity to:	-	-	-	-
Vibriostatic agent 0/129	+	+	+	+
Novobiocin	+	+	+	+
Acid from:		-	-	-
Glucose	+	+	+	+
Mannose	+	+	+	+
Gaaltose	+	+	+	+
Fructose	+	+	+	+
Maltose	-	-	-	-
Rhamnose	-	-	-	-
Arabinose	-	-	-	-
Amygdalin	-	-	-	-
Inositol	-	-	-	-
Melibiose	-	-	-	-
Mannitol	-	-	-	-
Sorbitol	-	-	-	-
Sacarose	-	-	-	-

Microbiology

Samples taken from spleen, liver and kidney of moribund or sick gilthead seabream were cultured in different culture media: Tryptic soy-agar (Difco), brain-heart infusion (Difco) and blood agar (Difco) with 1% and 2 % NaCl added.

Cultures were incubated at 22°C for 24 - 48 hours. Isolated colonies were subjected to biochemical tests (Toranzo *et al.*, 1991). Commercial miniaturised API-20E strips(Biomerieux, Spain) were also used to determine the characters of the isolates.

Two reference strains of *P. piscicida* ATCC 17911 and ATCC 29690, and a clinical strain DI-21 from the North of Spain (Toranzo *et al.*, 1991) were also included as controls. In order to confirm the diagnosis of pasteurellosis a slide agglutination test was applied (Toranzo *et al.*, 1991). We used a polyclonal antiserum against *Pasteurella piscicida* (ATCC 17911) raised in two rabbits. Each animal was injected twice with 2 ml by subcutaneous route and the inoculum was delivered by multiple (10 points) doses in separate sites. The inoculum consisted of 1 ml of sterile saline solution containing *P. piscicida* (ATCC 17911) (10^8 cells/ml) and 1 ml of incomplete Freund's adjuvant. A second injection was given six weeks after the first, and both animals were bled eight weeks after first boost (Harlow and Lane, 1988).

Antimicrobial resistance of the isolates was determined by the disk diffusion method on Mueller Hinton agar (Cultimed). The following antimicrobial agents (µg/disk) were tested: Kanamycin (30), sulphamethoxazole (125), nitrofurantoin (300), oxytetracycline (30), sulphonamides (300), doxycycline (30), novobiocin (5), the vibriostatic compound 0/129 (10 and 150) and rifampicin (30).

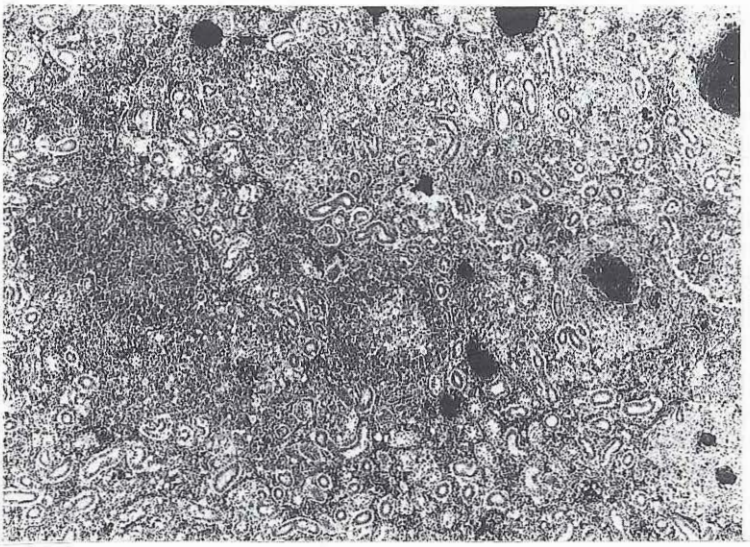

Figure 1: Gilthead seabream. Kidney. Severe multifocal granulomatous nephritis. H-E x 10.

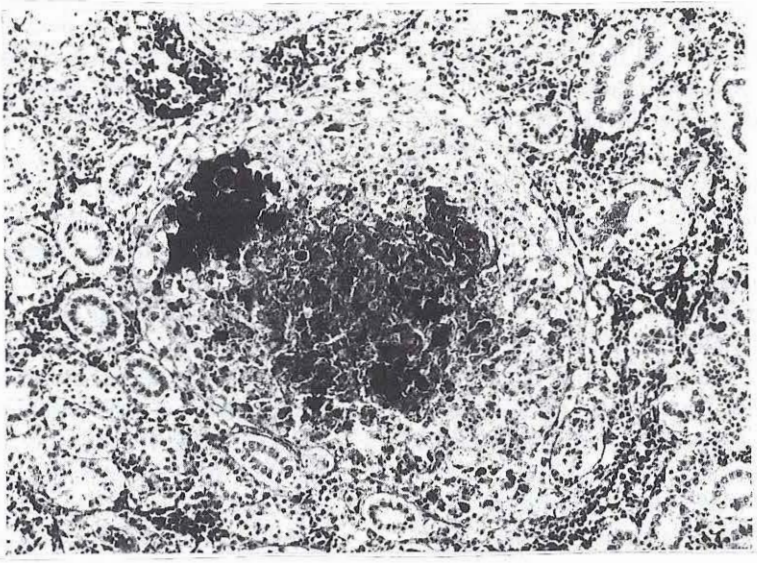

Figure 2: Gilthead seabream. Kidney. Well developed granuloma, numerous bacteria. H-E x 10

Results

In spite of their size, affecting fishes showed weakness and their growth was delayed. Moribund fish had usually no obvious external lesions, no parasites were seen on the fishes sampled, but preventive formalin baths against *Cryptocaryon irritans* infestation were usually applied. Affected fish usually died with no previous clinical signs.

Although, many fish did not show characteristic gross lesions of internal organs, several did show enlarged spleen and liver.

Histologically the most important changes were observed in the kidney, liver, spleen and myocardium.

Severe multifocal granulomatous nephritis associated with large numbers of small bacterial colonies was observed in the kidney (Figs. 1 and 2). The liver showed a severe multifocal necrotising hepatitis, with nuclear debris and infiltration of heterophils in and around the necrotic areas. Large numbers of gram-negative bacilli were found, closely associated with these hepatic lesions. No granulomata were observed in the liver. The spleen showed a severe multifocal necrotizing splenitis associated with large numbers of gram-negative bacilli. No granulomata were observed in the spleen. A multifocal necrotizing myocarditis with infiltration of numerous heterophils associated with small foci of bacterial colonisation was observed in the myocardium.

In addition, numerous basophilic bacterial colonies were observed in the blood vessels of the gills. Non-motile rod-shaped bacteria were observed in wet mount of spleen and liver tissue smears. Gram-staining showed gram-negative bacilli. Translucent and slightly colonies were seen after 48 hours in blood agar with 1% and 2% NaCl added. No growth was observed on TSA and BHI with 1% NaCl added.

Smears and staining showed the isolated organism as a non-motile, gram-negative rod that was oxidase and catalase positive, sensitive to the vibriostatic compound 0/129, failing to produce gas from glucose and having a salt requirement. The results of the biochemical tests are shown in Table 1, allowing us to identify the bacterium from gilthead seabream as *Pasteurella piscicida,* displaying the same characters as the reference strains (ATCC 17911, ATCC 29690 and DI-21) showed. Serologically our clinical strain showed a positive agglutination with raised against *P. piscicida* ATCC 17911. P. *piscicida* isolated from gilthead seabream showed sensitivity to doxycycline, oxytetracycline, nitrofurantoin, trimethoprim / sulphamethoxazole, novobiocin and the vibriostatic compound 0/129 and resistance to kanamycin, sulphonamides and rifampicin.

Discussion

Pasteurella piscicida has been isolated in different areas of Spain (Toranzo *et al.*, 1991, Balebona *et al.*, 1992), but this is the first reported outbreak of pasteurellosis in Gran Canaria. Although no virus isolation was attempted at this time, the characteristics of the isolated pathogen do not allow us any doubt of the diagnosis.

The characteristics of the isolated bacterium were similar to the reference strains of *P. piscicida* ATCC 17911, ATCC 29690 and the clinical strain DI-21, showing a positive reaction to the acid production from glucose, mannose, galactose and fructose. The Voges Proskauer test and the acid production from glycerol gave a variable reaction, but the acid production from carbohydrates depends on the basal medium used (Koike *et al.*, 1975).

API-20E strips are a rapid and accurate identification system for this infection as previously described (Santos *et al.*, 1993, Balebona *et al.*, 1992 and Candan *et al.*, 1996) always giving the same pattern : 2005004.

Histopathologically, only in the kidney was seen a granulomatous form typical of chronic pasteurellosis. This is only seen when the viability of the bacteria is decreased by the treatment (Kubota *et al.*, 1982). Liver, spleen and myocardium did not show this granulomatous form.

Probably, fish with no lesions found in the beginning of the epizootic died as result of a hyperacute endotoxic shock and septicaemia.

In general, histopathological changes observed agree with those previously described (Hawke *et al.*, 1987 Toranzo *et al.*, 1991, Balebona *et al.*, 1992).

Seemingly, because the temperature and physico-chemicals characteristics of the water were normal, these were not related with the appearance of the outbreak of pasteurellosis, although the density of fish was high. We believe the infection arrived in the Canaries with juvenile gilthead seabream which are usually imported from Iberian peninsula hatcheries.

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