ポルトガルで養殖ターボットに発生したStreptococcus parauberis感染症

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Streptococcus parauberis Infection in Turbot Scophthalmus maximus in Northern Portugal

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ABSTRACT—High mortalities of turbot Scophthalmus maximus were observed at a fish farm in northern Portugal from May to August in 2004. Moribund fish exhibited typical symptoms of streptococcosis, such as uni- or bilateral exophthalmia and hemorrhages and edema in the dorsal trunk region and the base of fins. Gram-positive cocci were isolated from moribund fish and identified as Streptococcus parauberis based on physiological, biochemical and serological characteristics and 16S rDNA-targeted PCR. Histopathological examinations revealed occurrence of meningoencephalitis and presence of bacteria in the connective tissue of examined organs. This is the first report of S. parauberis infection in cultured turbot in Portugal.

Key words: Streptococcus parauberis, streptococcosis, Scophthalmus maximus, histopathology, turbot

Turbot Scophthalmus maximus is a very important marine fish species in southwestern Europe aquaculture, with an estimated production of 9,000 tons/year in the latest years by the main producer countries, including Spain, France and Portugal (FAO Fishery Statistics 2010). The expansion of intensive aquaculture has led to increased disease susceptibility of farmed fish and control of the various bacterial fish pathogens has become increasingly difficult1-39.

Streptococcosis is among the most important fish diseases. The disease is caused by Gram-positive bacteria from the genera Streptococcus, Lactococcus, and Vagococcus4,5, known to cause both acute and chronic infections with high mortality rates over a period of 3-7 days or low daily mortality rates over several weeks6, respectively. The first diagnosed outbreak of streptococcosis was recorded in 19577 in rainbow trout Oncorhynchus mykiss cultured in Japan and since then, it has been reported in many other fish species, such as yellowtail Seriola quinquemaculata, Japanese eel Anguilla japonica, Gulf menhaden Brevoortia patronus, striped mullet Mugil cephalus, striped bass Morone saxatilis, turbot, Atlantic salmon Salmo salar, golden shiner Notemigonus crysoleucas and olive flounder Paralichthys olivaceus1,5-10.

Streptococcosis seems to be endemic in some turbot farms, posing a putative danger of new outbreaks19. From May to August in 2004, severe mortalities possibly occurring from streptococcosis were observed in a Portuguese grow-out facility of turbot where fish were held in circular concrete tanks with an open flow circuit of seawater (30 ppm, 12-14°C). This study describes bacteriological and histopathological examinations on the disease.

Materials and Methods

Monthly, moribund specimens were collected and killed by spinal cord severance and subjected to necropsy in order to determine the etiology of the high and progressive mortalities. A total of 110 fish (90-1,020 g in body weight) were examined. Sterile swabs from liver, kidney, spleen, brain and eyes were streaked on Columbia blood agar (bioMérieux), Tryptic Soy Agar (Merck) supplemented with 0.5% (w/v) NaCl (TSA) and Thiosulfate Citrate Bile salts Sucrose agar (TCBS, Difco). The inoculated plates were incubated at 22°C for 24-48 h. Single colonies from plates with culture growth were re-streaked on TSA to obtain pure isolates. Stock cultures were maintained at -80°C in Tryptic Soy Broth (Merck) supplemented with 0.5% (w/v) NaCl and 15% (v/v) glycerol.

Pure cultures of the isolated colonies were subjected to morphological, physiological and biochemical analysis performed on standard plate and tube tests16. Biochemical identification was also done with the commercial identification system Api 20 Strep (bioMérieux). S. parauberis NCDO 2020 was used as a positive control, and two strains of S. uberis (174 and 230/1) isolated from cow mastitis outbreaks were used as negative controls. Slide agglutination test was performed with rabbit anti-S. parauberis serum. Susceptibility pattern of bacterial isolates to erythromycin, ampicillin, trimethoprim-sulfamethoxazole, penicillin, cefalotin, chloramphenicol, nitrofurantoin, furazolidone, tetracycline and streptomycin were tested by using the Kirby-Bauer method on Muller Hinton agar (Difco).
Bacterial DNA was extracted and purified with the Bacterial Genomic DNA Mini-Prep Kit (V-gene) according to manufacturer’s instructions. The primers developed by Hassan et al. were used to amplify the bacterial 16S rRNA gene, with an expected size of 884-bp for S. parauberis. The amplification was carried out with SuperHot 2 x PCR Master Mix (Bioron) according to the manufacturer’s instructions. Samples were run in agarose gel and visualized by UV trans-illuminator.

Some moribund fish (n = 10) were subjected to histopathological examinations. Samples of the liver, kidney, spleen, brain, eyes and skin were fixed in 10% phosphate-buffered formalin, routinely processed for histopathology, stained with haematoxylin and eosin (H & E), Gram, Gram-Twort and Periodic Acid Schiff’s (PAS) and examined under the optical microscope.

Results and Discussion

The disease occurred throughout the sampling period with severe outbreaks in May, July and August 2004 when mortality was higher. Clinical signs, gross pathology and histopathology observed in infected turbot closely resembled those observed in streptococcosis. Some fish also showed poor appetite and abnormal swimming behavior with sharp and sudden erratic movements. Moribund fish were emaciated, with uni- or bilateral exophthalmia and hemorrhages in the periorbital tissue (Fig. 1A, B). Edemas were observed in the dorsal region (Fig. 1A) and at the base of the fins, and hemorrhages in the dorsal and caudal fins, occasionally around the mouth and abdominal petechia. Internal signs included pale, friable liver, occasionally hepatomegaly, hemorrhagic and friable kidney and ascitic fluid in the peritoneal cavity.

Bacterial growth was observed on blood agar and TSA from all examined organs, with colonies 1–2 mm in diameter, raised, grayish-white with entire margin and α-haemolysis producers on blood agar and colonies 1–3 mm in diameter, raised, white with entire margin on TSA. No colonies were formed on TCBS agar. The phenotypic characteristics of the present isolates (n = 10) are observed, Gram-Twort, Bar = 20 μm. (D) Hyperplasia of the meninges (arrowheads). H & E, Bar = 200 μm. (E) Hyperplasia of the meninges – dura-mater (arrow) with large amounts of bacteria. H & E, Bar = 10 μm. (F) Meninges with mononuclear inflammatory cells with bacteria (arrows) and some necrosis. PAS, Bar = 20 μm. (G) Meninges with mononuclear inflammatory cells with bacteria (arrowhead) and some necrosis. Gram, Bar = 10 μm. (H) Mononuclear inflammatory cells (+) with Gram-positive bacteria in the eye choroid (arrows). Gram-Twort, Bar = 20 μm. (I) Mononuclear inflammatory cells with Gram-positive bacteria in the liver portal space (arrows), but not inside the blood vessels. Gram-Twort, Bar = 20 μm.
from turbot were in agreement to those published for *S. parauberis*\textsuperscript{9,12}: nonmotile, Gram-positive cocci grouped in pairs or in short chains; facultative anaerobic; oxidase and catalase negative; producing acid from glucose, mannitol, sorbitol and lactose; arginine dihydrolase, lysine and ornithine decarboxylase negative. Using the Api 20 Strep test system, it was not possible to obtain an accurate identification of the isolates. All isolates gave a positive and strong reaction in the slide agglutination test, and gave the expected 884-bp band typical of *S. parauberis* in 16S rDNA-targeted PCR amplification, whereas the *S. uberis* strains showed a weak reaction and no detectable PCR amplification.

In *in vitro* antibiotic tests, all isolates examined were sensitive to ampicillin, penicillin, erythromycin, trimethoprim-sulfamethoxazole and to a lesser extent to trimethoprim-sulfamethoxazole and to a lesser extent to amoxicillin and to cefalotin, chloramphenicol and nitrofurantoin, while *in vivo* only erythromycin showed some effectiveness in controlling the disease at the fish farm, but it did not entirely eliminate the streptococcal problems.

The histological analysis of the different organs of turbot showed that *S. parauberis* induced subacute to chronic inflammations. In the skin, small chains of Gram-positive bacteria could be observed in the dermal stratum spongiosum, with some areas showing necrotic lesions, extending to the muscle beneath the injured skin (Fig. 1C). In the brain, bacterial cells could be observed in the meninges (Fig. 1D). Hyperplasia, hydropic degeneration and large amounts of Gram-positive cocci were detected in the dura-mater (Figs. 1D, E) with an associated infiltration of leucocytes and a high number of bacteria presented inside mononuclear cells (Figs. 1F, G). In heavily infected meninges, necrotic tissue was also observed. Meningitis was reported for streptococcal infections in other fish species\textsuperscript{50–23} but the typical ischemia-like lesions\textsuperscript{20} were not observed in turbot brain. The erratic behavior and lethargy of the fish could be attributed to the meninges infection. In heavily infected fish eyes, bacteria (Fig. 1H) and inflammation associated with hypertrophy and necrosis of choroid connective tissue could be observed. In the liver and kidney, Gram-positive bacteria were observed in the portal space connective tissue (Fig. 1) or the supportive connective tissue (not shown), with both organs presenting some necrosis. Furthermore, the dominant inflammatory cells observed were macrophages. No neutrophils were found in the brain, eyes or visceral organs, in opposition to Roberts\textsuperscript{24} and Fänge\textsuperscript{25} that reported large numbers of neutrophils in the early stages of inflammation.

In conclusion, this is the first report of *S. parauberis* infection in cultured turbot in Portugal.

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**References**

海生甲殻類から分離した病原卵菌類のITS1 メソルの塩基配列による同定とアルテミア卵化幼生に対する病原性
村長保志・佐野文雄・畑高義雄

海生甲殻類から分離した病原卵菌類27株について形態分類を行うとともに、対照とした卵菌類6株と合わせて、ITS1 領域の塩基配列を比較した。その結果、塩基配列に基づく系統関係は形態的分類に基づく良好なグループが形成され、種の同定に有用であることが示唆された。また、9 種18株の病原卵菌についてアルテミアの卵実生卵に投与する浸透攻撃試験（1 × 10^6 zoospores/mL, 25℃）によって卵菌卵によって大きく異なる。


感染実験からみた病魚卵菌のサケ科魚類卵内感染機序
小原昌和・笠井久会・吉永 守

ニジマスおよびアマゴ卵を用いて Flavobacterium psychrophilum, Renibacterium salmoninarum および Aeromonas salmonicida の感染実験を行い、卵内感染機序を検討した。F. psychrophilum は卵の吸水時に卵内から侵入すると考えられた。F. psychrophilum 感染症は、汚染水吸水卵より卵表面汚染後に吸水させた卵で有意差を示し、条件は 10 CFU/mL 以上であった。また、高濃度の R. salmoninarum で表頭卵卵で卵内感この卵においても卵内感染がみられた。F. psychrophilum または A. salmonicida 感染卵卵を吸水させたところ、F. psychrophilum は卵内侵入後に増殖したが、A. salmonicida は卵内侵入後に減izした。


タイのテラピア養殖場から分離された Aeromonas hydrophila 多剤耐性株
N. Tipmongkolset · C. S. del Castillo · 引間順一
T-S. Jung · 近藤秀裕・原野育生・青木 宇

タイのテラピア養殖場の感染魚より 55株の A. hydrophila を分離し、11薬剤の最小発育阻止濃度を調べた。その結果、全ての分離株が 1～8 割の粗合せの耐性を示し、薬剤耐性菌が約半数を占めた。これらの薬剤耐性株中、1 割から ABPC, CP, SM, SMMX および TC の 5 割に対して耐性を示す伝達性 Rプラスミドを検出した。このプラスミドは、耐性遺伝子として blaOXA-35, cat2, aadA1, sul1 および tetA を含んでいた。

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河川アユにおける Edwardsiella ictaluri 不類性感染
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川村修・飯田悦也・満浅 賢・中村敏博

2008年から2010年にかけて、広島県下の1河川において E. ictaluri の保菌調査を実施した。アユからは本菌が高頻度で分離され、特に9月以降の保菌率は高く平均 45.4% であった。アユ以外の魚種では1尾のギキから分離されたとすぎた。また、指を指して探ろうとおった但放流アユ稚苗からは本菌はまったく検出されなかった。一方、E. ictaluri の指標としてのファージが河川水から周年にわたって検出されたことから、本菌は河川環境に常在化し、それが河川アユへの感染源になると考えられた。

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在来マス及びアユに対する Yersinia ruckeri の病原性
坂井貴光・中島千早・伊東高史・三輪 理
大迫政久・飯田貴幸

ニジマス、イワナ、アマゴ及びヤマメの腹腔内に Y. ruckeri を 7.1 × 10^8 CFU/魚体重 (g) 接種して攻撃した。各魚種の累積死亡率は、100%, 50%, 30%, 30% であり、すべての死亡魚がレッドマス病の症状を示した。1.5 × 10^8～1.5 × 10^9 CFU/魚体重 (g) で腹腔内接種したアユの累積死亡率は、0%～87%であった。また、浸漬攻撃でも高い累積死亡率が観察された。死亡したアユにサケ科魚類と同様のレッドマス病の症状は見られず、眼球の突出や出血、腹水の貯留が観察された。

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ポルトガルで養殖ターポットに発生した Streptococcus parauberis 感染症
M. F. Ramos · J. F. Marques · J. V. Neves · T. Barandela
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2004年5月から8月にかけてポルトガル北部の1養殖場でターポット（体重 90～1,020 g 水温 12～14℃）に、眼球突出、背部や腹部の出血と浮腫、また病理組織学的には肺浸潤を特徴とする大量死亡が発生した。病魚の内臓諸器官からグラム陽性の α溶血性球菌が分離され、それらは生化学的・血清学的性状および遺伝学的性状（16S rDNA を標的とした PCR）から S. parauberis に同定された。これはポルトガルにおける本菌感染症の初報告である。

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