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Isolation and characterization of *Streptococcus* spp. group B in Nile tilapias (*Oreochromis niloticus*) reared in hapas nets and earth nurseries in the northern region of Parana State, Brazil

Isolamento e caracterização de *Streptococcus* spp. do grupo B em tilápias do Nilo (*Oreochromis niloticus*) criadas em tanques rede e em viveiros de terra na região norte do Estado do Paraná, Brasil

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ABSTRACT

*The objective of this study was to isolate and characterize **Streptococcus spp.** in Nile tilapias (**Oreochromis niloticus**) reared in net-pens and earth nurseries. Eight intensive tilapia-rearing farms were investigated in north Paraná, Brazil from April 1st 2001 to April 30th 2002. The fish were reared in a system of hapas nets on four farms and in earth nurseries on other four farms. A total of 370 samples were analyzed of material collected from 120 fish (brain, liver, kidney, skin scrapes, ascites liquid and eye) that were sown on BHI agar (Brain Heart Infusion) supplemented with 1% yeast extract and sheep blood. **Streptococcus spp.** was isolated in 36 of the samples (18 brain, eight liver, eight kidney and two ascites liquid) from 25 fish. Streptococci were isolated in both systems, almost in the same proportion. First the streptococci were characterized by the catalase and esculin test, growth in methylene blue and sodium chloride at 6.5%. They were classified in groups by the Slidex Strepto-Kit (BioMerieux, France). The phenotypic characteristics were determined by the Api 20 Strep microtest system (BioMerieux, France). The 36 **Streptococcus spp.** samples did not present hemolysis and were classified as Lancefield group B. Further 16 samples were identified as **Streptococcus agalactiae** and 20 were not identified by the Api 20 Strep, but presented the same biochemical profile described for the reference strain of **Streptococcus difficile** (ND-2-22).*

Key words: *pisciculture, streptococci, infection, bacteriology.*

RESUMO

*O objetivo deste trabalho foi isolar e caracterizar o **Streptococcus spp.** em tilápias do Nilo (**Oreochromis niloticus**) cultivadas em sistema de tanque rede e em viveiros de terra. Oito propriedades de criação intensiva foram estudadas de 1 de abril de 2001 a 30 de abril de 2002. Em quatro propriedades, os peixes eram cultivados em sistema de tanque rede e em quatro em sistema de tanque terra. Ao todo foram analisadas 370 amostras de material colhido de 120 peixes (encéfalo, fígado, rim, raspado de pele, líquido ascítico e olho) que foram semeadas em ágar BHI (Brain Heart Infusion) contendo sangue ovino e extrato de levedura. **Streptococcus spp.** foi isolado de 36 amostras (18 amostras de encéfalo, oito de fígado, oito de rim e duas de líquido ascítico), provenientes de 25 peixes. Estreptococos foram isolados, quase na mesma proporção, nos dois sistemas de cultivo. Inicialmente os estreptococos foram caracterizados pela prova da catalase e esculina, crescimento em azul de metileno e NaCl a 6.5%. A classificação em grupos foi efetuada pelo Slidex Strepto-Kit (BioMerieux, França). As características fenotípicas foram determinadas pelo sistema de microtestes API 20 Strep (BioMerieux, França). As 36 amostras de estreptococos não apresentaram hemólise e foram classificadas no grupo B de Lancefield. Dessas, 16 amostras*

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*foram identificadas como **Streptococcus agalactiae** e 20 não foram caracterizadas pelo API 20 Strep, mas apresentaram o mesmo perfil bioquímico descrito para a cepa de referência de **Streptococcus difficile** (ND-2-22). A ausência de hemólise, classificação no grupo B e o perfil bioquímico sugerem que estes estreptococos podem pertencer à espécie **Styreptococcus difficile**.*

Palavras-chave: *piscicultura, estreptococococos, infecção, bacteriologia.*

INTRODUCTION

Fish farming is one of the fastest growing segments of animal production in the world (PEREZ, 1999). The world production of tilapia (***Oreochromis* spp.**) has grown expressively since 1984 and occupies an outstanding position among the species reared in fresh water (ALCESTE & JORY, 2000).

In Brazil, fish farming is in the phases of consolidation and expansion (CHAMMAS, 1997). Until 1979, the annual production of reared tilapias was not more than five thousand tons but currently it is 30 to 40 thousand tons annually (STICKNEY, 2001). Paraná state is the largest tilapia producer in the country, followed by São Paulo and Santa Catarina states.

The growth of fish farming in Brazil has been disorganized and characterized by high mortality rate, mainly because of bacterial diseases and low hygienic and sanitary quality (PEREZ, 1999). Among the bacterial diseases, septicemia by ***Streptococcus* spp.** is the largest problem in intensive tilapia rearing systems throughout the world (SURESH, 1998).

Several species of ***Streptococcus* spp.** can be involved in fish infections. WU (1970) reported the first isolation of ***Streptococcus* spp.** in tilapias and since then this pathogen has been blamed for high losses, especially in Japan (KITAO et al., 1981), Israel (HUBERT, 1989), Saudi Arabia (AL-HARB, 1994) and recently in the United States and Central America (PLUMB, 1997).

Streptococcus iniae, described for the first time by PIER & MADIN (1976), has been considered as the important pathogen in tilapias (PERERA et al., 1997; DODSON et al., 1999; SHOEMACKER et al., 2001). Other species are described as disease causers: ***Streptococcus faecium*** (KUSUDA et al., 1976; MINAMI, 1979), ***Streptococcus agalactiae*** (KUSUDA & KOMATSU, 1978), ***Streptococcus equi***, ***Streptococcus equisimilis***, ***Streptococcus pyogenes***, ***Streptococcus zooepidemicus*** (UGAJIN, 1981) and ***Streptococcus difficile*** and ***Streptococcus shilloi*** (ELDAR et al., 1994). BUNCH & BEJERANO (1997) studied the infection of fish by ***S. difficile*** and observed greater infection occurrence in fish cultivated in waters with high temperatures, showing the importance of ***S. difficile*** in locations with higher temperatures. The objective of this study was the isolation and characterization of ***Streptococcus* spp.** isolates of fish that presented clinical signs of bacterial infections in their fattening stage.

MATERIAL AND METHODS

Eight intensive tilapia rearing farms were studied in the Northern region of Paraná state, from April 1st 2001 to April 30th 2002. On four farms, the fish were reared in a system of hapas nets and on the other four, in earth nurseries. Fish presenting signs such as darkened skin, lethargy, erratic and circular swimming were captured and placed in plastic bags containing water from the location itself and oxygen under pressure, transported to the Microbiology and Infectious Diseases Laboratory at the Department of Preventive Veterinary Medicine at Londrina State University (UEL), and examined immediately. One hundred and twenty fish were collected, totaling 370 samples of biological materials, namely 120 brain, 120 liver, 120 kidney samples, six skin scrapings, two ascite liquids and two eyes.

In the laboratory, the fish were submitted to external physical examination and necropsy. Kidney, brain and liver samples were collected aseptically from all the animals along with other biological materials (skin scrapings, ascites liquid and eye) that presented macroscopic alteration. They were sown on plates containing BHI agar, supplemented with 1% yeast extract (BUNCH & BEJERANO, 1997) and sheep blood, and incubated at 30°C in aerophile atmosphere for five days.

The microorganisms were identified according to their cultural, morphological, tintorial and biochemical characteristics (HOLT et al., 1994). The streptococci that had been characterized previously by the catalase and esculin test, growth test in methylene blue and sodium chloride at 6.5%, were later classified in groups by Slidex Strepto-Kit (BioMerieux, France). The species were identified in 36 samples by their phenotypic characteristics, determined by the Api 20 Strep Microtest (BioMerieux, France) after incubation at 30°C and readings at four and 24 hours.

RESULTS AND DISCUSSION

The main clinical signs detected in the fish were lethargy, loss of appetite, spine displacement, uni or bilateral exophthalmia, abdominal extension and

erratic and circular swimming. Hemorrhagic lesions were observed on the skin, ascites, hepatomegalia and splenomegalia and pale liver were observed at the necrospy. Of the 120 fish researched, 25 (20.83%) presented ***Streptococcus* spp.** Growth. Streptococci were isolated in both systems, almost in the same proportion. Of the 77 biological materials from the 25 fish, ***Streptococcus* spp.** were isolated in 36 (46.75%) (18 samples of brain, eight liver, eight kidney and two ascitic liquid samples). The predominance of the isolation from the brain indicates the need for the routine bacteriological examination in this organ, because in fish with clinical suspicion of infection by ***Streptococcus* spp.** the clinical neurological signs resulting from meningoencephalitis can be observed. The relatively low isolation indexes may indicate little sensitivity in the bacterial culture, the participation of other etiological agents or further, low specificity of the clinical signs in the ***Streptococcus* spp**. infection in tilapias. Low correlation between the clinical signs and the etiological agents was described by ELDAR et al. (1995) and PLUMB (1999).

In the BHI agar the ***Streptococcus* spp.** colonies were gray colored, translucent, circular, slightly convex, pin head and not hemolytic. All strains presented negative results in the catalase and esculin tests, growth in sodium chloride at 6.5% and methylene blue. The 36 ***Streptococcus* spp.** samples submitted to the Slidex Strepto-Kit test presented the antigen carbohydrate belonging to the Lancefield group B and in the Api 20 Strep two phenotypical homogeneous groups were identified. Sixteen samples were identified as ***S. agalactiae***. The twenty remaining samples presented the same biochemical profile, but were not identified by the system used. This profile corresponded to that described by ELDAR et al. (1994) and VANDAMME et al. (1997) for the reference strain of ***Streptococcus difficile*** (ND-2-22) (Table 1).

According to VANDAMME et al. (1997) ***S. difficile*** belongs to group B type Ib and presents total cell protein indistinguishable from ***S. agalactiae***, but the biochemical profile of ***S. difficile*** is different from ***S. agalactiae***, but similar to that observed in other group B, Ib type ***Streptococcus* spp.** isolated in fish. BERRIDGE et al. (2001) also detected a significant homology (97.7%) in the sequence of nucleic acids between the 16S-23S rDNA, the ***S. difficile*** and ***S. agalactiae***.

S. difficile was described for the first time in Israel as the cause of septicemia and meningoencephalitis in reared tilapias and trout (***Oncorrhyncus mykiss***) (ELDAR et al., 1994). ***S. difficile*** is the most prevalent species in intensive tilapia rearing in Israel and is responsible for approximately 30% of the mortality on the farms studied (ELDAR et al., 1995).

Streptococcus* spp.** with beta hemolise and ***S. iniae biochemical profile were not identified. This species has been described as one of the most frequent in ***Streptococcus* spp.** infections in tilapias (PERERA & JOHNSON, 1994; ELDAR et al., 1995; BOWSER et al., 1998; EVANS et al., 2000). BUNCH & BEJERANO (1997) in a study carried out in Israel observed that ***S. iniae*** predominated in tilapias reared at low temperatures (15°C and 16°C) ***S. difficile*** predominated whereas in rearing at higher temperatures (26°C to 28°C). This fact might explain the non-isolation of ***S. iniae*** in this study, as the mean water temperature on the farms studied oscillated between 24° C and 26°C and at the same time reinforces

Table 1 - Comparison among sugar use characteristics and the enzymatic profile of ***S. difficile*** obtained by ELDAR et al. (1994) and VANDAMME et al. (1997) and 20 group B ***Streptococcus* spp.** isolated and submitted to Api 20 Strept (BioMerieux, France).

Sugar and substrates	***S. difficile***	Group B streptococci	Sugar and substrates	***S. difficile***	Group B streptococci
Pyruvate	+	+	Ribose	+	+
Hippurate	-	-	L-Arabinose	-	-
Esculin	-	-	Mannitol	-	-
PYRA[a]	-	-	Sorbitol	-	-
a- Gal[b]	-	-	Lactose	-	-
b-Gur[c]	-	-	Trehalose	-	-
b-Gal[d]	-	-	Inuline	-	-
PAL[e]	+	+	D-Raffinose	-	-
LAP[f]	+	+	Starch	-	-
ADH[g]	+	+	Glycogene	-	-

[a] Pyrrolidonylarylamidase, [b] α-galactosidase, [c] β-glucuronidase, [d] β- galactosidase, [e] Alkaline phosphatase, [f] Leucine arylamidase, [g] Arginine dihydrolase.

the possible involvement of ***S. difficile*** in the tilapia infections on the farms studied.

Although there are no reports involving ***S. difficile*** in diseases in man, its taxonomic relationship with ***S. agalactiae*** and ***S. iniae*** should alert the health authorities to this possibility (BERRIDGE et al., 2001) because other non hemolytic ***Streptococcus*** **spp.** from group B have been isolated in humans (ELLIOT et al., 1990).

CONCLUSION

The isolation of non hemolytic ***Streptococcus*** **spp.** from group B is strong evidence of the participation of these agents in the etiology of the septicemias and meningoencephalites in tilapias reared in the north of Paraná state, Brazil.

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