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First report of *Streptococcus agalactiae* isolated from *Oreochromis niloticus* in Piura, Peru: Molecular identification and histopathological lesions

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ABSTRACT

The aim of this study was to identify the bacterium *Streptococcus agalactiae* isolated in farmed Nile tilapia (*Oreochromis niloticus*) from Piura, Peru and to characterize the histopathological lesions caused by this pathogen. Sixteen tilapias were sampled with clinic signs of the disease such as erratic swimming, exophthalmia and haemorrhages on the body and fins. Qualitative PCR in real time and histopathological analysis were performed. Nine fishes positives to *S. agalactiae* were found. The main histopathological findings were fibrinosuppurative epicarditis, periesplenitis, meningitis and panophtaltmitis with predominance of mononuclear infiltration in all tissues. The correlation between qPCR and histopathological findings demonstrated nine fish (prevalence of 56.25%) with Cq lower than 30, associated to high degree of tissue injuries. This study reports the first isolation of *S. agalactiae* by PCR in real time in tilapia farmed in Peru and characterizes the major histopathological changes caused by this bacterium.

1. Introduction

The tilapia industry has positive prospects for productive development in Peru (Sandoval et al., 2010). The country has about 154,000 ha available to develop tilapia farms in earthen ponds, concrete or geomembranes (Baltazar, 2009). The departments of San Martin and Piura have suitable areas for the production of this species (PRODUCE, 2011), because the waters offer exceptional conditions for breeding, such as warm temperatures (20–30 °C), low pollution, turbidity and good oxygenation (Baltazar and Palomino, 2004).

The culture of Nile tilapia is one of the fastest growing aquaculture activities worldwide (Conroy et al., 2008). However, the intensification of fish production has resulted in the occurrence of stress (Belo et al., 2005, 2012), a fact that increases the susceptibility to diseases outbreaks, such as bacteria (Amal and Zamri-Saad, 2011), parasites (Manrique et al., 2012, 2015a, 2016; Padua et al., 2015), nutritional deficiencies (Sakabe et al., 2013; Belo et al., 2014; Castro et al., 2014a) and fungi (Jimenéz, 2010; Iregui et al., 2014). Among them, bacterial are the main obstacle to the sustainable development of the culture of tilapia (Yuasa et al., 2008).

The genus *Streptococcus* is one of the most important Gram-positive bacteria that affect the culture of tilapia (Conroy, 2009; Jimenéz, 2010). Within this, the species *S. agalactiae* is the most prevalent worldwide (Sheehan et al., 2009). *S. agalactiae* is a coccus, organized in pairs or short, catalase and oxidase negative and positive CAMP chains; it may or may not be haemolytic (Abuseliana et al., 2010; Buller, 2014). The lesions are characterized by loss of appetite, exophthalmia, eye haemorrhage, corneal opacity, distended abdomen, curvature of the spinal cord, erratic swimming, stiffness and bleeding on the base of the fins (Yanong and Francis-Floyd, 2002; Pulido and Iregui, 2010). Castro et al. (2014b) studied

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the Kinect of cell accumulation in swim bladder of tilapias during acute aerocystitis by *S. agalactiae* and verified high number of granulocytes in site of inflammation, while Pulido and Iregui (2010) described infiltration of mononuclear cells with subsequent granuloma formation and meningoencephalitis in chronic inflammatory response.

The diagnosis of *S. agalactiae* is based on clinical signs, histopathological lesions and microbiological isolation (Wongsathein, 2012). However, the culture methods and biochemical tests can be confused for the variable reactions of the different strains of *S. agalactiae* (Wongsathein, 2012). For this, several authors suggest the specific identification of this bacterium through molecular techniques such as PCR (Jimenez et al., 2011).

Tilapias reared in Piura, the main production area in Peru, have presented clinical signs and lesions described in outbreaks of *S. agalactiae* (Sedano, 2013). However, the presence of this bacterium has not been confirmed so far. The objective of this study was to identify by PCR in real time the presence of *S. agalactiae* and characterize histopathological lesions in diseased tilapias from nursery and grow-out phases.

2. Materials and methods

2.1. Study design

The study design was cross-sectional. Eight tilapias in each of the phases nursery (33.5 ± 3.6 g) and grow-out (670.5 ± 22.6 g) were selected between February and June 2014. The criterion of selection of individuals was the presence of clinical signs such as erratic swimming, exophthalmia, lethargy, bleeding around the body and superficial location in the water column. The fish were euthanized with a lethal dose of sodium benzocaine 1:500 (v/v) previously diluted in pure alcohol (0.1 g/mL) (Manrique et al., 2015b), then proceeded to the necropsy according to the protocol described by Ferguson (2006).

The sampling was performed aseptically for the microbiology analysis of the following organs: spleen, brain, liver and kidney. For histopathology, besides were taken samples of gills, heart, eye and skeletal muscle of approximately 1.0 cm^2 and fixed in 10% buffered formalin.

2.2. Location

The fish farm was located in the department of Piura (4° 38′27″S, 80° 32′55″W), Northern Peru. The water surface area was 19.26 ha, supplied by a water reserve of the valley. The tanks were concreted with stoking density of 15–19 kg of biomass by m^3 of water. The study was conducted between February and June 2014, ambient temperatures ranging from 24 to 35 °C. The average values of the water quality parameters reported by the fish farm during the period can be seen in Table 1.

2.3. Microbiology

Samples of spleen, brain, liver and kidney were plated on blood agar (enriched with 5% sheep blood) and incubated at 30 °C for 24–48 h. Then, there were identified the morphology of the colonies, Gram positive cocci, catalase and oxidase negative, as isolated presumptive of *Streptococcus* spp.

2.4. DNA extraction

The DNA of the isolated presumptive to *Streptococcus* spp. was extracted with the PureLink Genomic DNA Kit (Invitrogen, USA), following the manufacturer's instructions for Gram positive bacteria.

For purification of DNA, it was used the same commercial kit PureLink Genomic DNA Kits (Invitrogen, USA). The material was centrifuged at 13,000*g* for 1 min at room temperature (24 °C) followed by two washes with 40 mL of elution buffer in each wash. The concentration and quality of DNA extracted was measured with Nanodrop spectrophotometer 2000 UV–vis (Thermo Scientific, USA). Finally, the genetic material was stored at −20 °C until use.

The positive control of the reaction was strain of *Streptococcus agalactiae*, donated by the Laboratory Genomic Medicine, University Surcolombiana – Colombia. The DNA extraction was a performed with commercial PureLink Genomic DNA Kits (Invitrogen, USA), following the manufacturer's recommendations. As negative control DEPC water was used (Invitrogen, USA).

2.5. Sensitivity and specificity of real-time PCR

Previously, tests for PCR were performed in real time with different dilutions to optimize the reaction, from a concentration of 1/1 (15 ng/μL) until the concentration of 1/100,000. Fig. 1 shows the DNA extracted from three species of *Streptococcus*: *S. pyogenes*, *S. pneumoniae* and *S. iniae*, which shows the high specificity of the test to identify the species *S. agalactiae*.

2.6. Real-time PCR

PCR was performed in qualitative real time. Primers were designed using the intergenic fraction of the 16S-23S rRNA as amplification region, which were synthesized from the strain *S. agalactiae* SA 66-07, access number AF064441, reported in Genbank, using the Primer Express 3.0 Applied Biosystems (Life Technologies, USA) (Betancur et al., 2012) software. The primers used for the detection of *S. agalactiae* were Forward (5′ CATTTGCGTCTTGTTAGTTTTGAG 3′) Reverse (5′ GGAGCCTAGCGGATCGA 3′) and probe (5′ VIC- AGAGCGCCTGCTTTGCACGCA-TAMRA 3′) (Bioneer Corporation, Korea).

The PCR reaction was performed in triplicate, briefly: 12.5 μL Taqman Universal PCR Master Mix (2x) (Invitrogen, USA), 0.5 μL probe (10 μM), 1.25 μL each primers (10 μM), 1 μL of DNA (20 μg/μL) and 8.5 μL of DEPC water (Invitrogen) to a total volume of 25 μL. Thermal conditions were: denaturation at 95 °C for 10 min followed by 40 cycles of 15 s at 95 °C, 2 min annealing at 50 °C and 1 min of extension at 60 °C, using the Applied Biosystem equipment 7300 (Life Technologies, USA).

2.7. Histopathology

Samples of spleen, gills, brain, heart, eye and liver were fixed in 10% buffered formalin and processed according to the standard protocol for histological studies of fixed tissues. Sections of thickness 3 μm were cut and mounted on slides and were stained with hematoxylin eosin (H&E).

3. Results

3.1. Necropsy

The main necropsy findings were observed: diffuse haemorrhagic areas around the mouth and fins (3) 18.75%, exophthalmos and eye opacity (4) 25%, hyphema (2) 12.5%, ascites (4) 25% congested brain (7) 43.75%, liver and spleen congestion (3) 18.75%, cloudy spots in liver and heart (12) 75%, hepatomegaly, splenomegaly and ascites (5) 31.25%, dilated gut (4) 25%, melanosis (2) 12.5%.

Table 1
Parameters of water quality (mean ± SD) obtained in the tanks during the period of study (February to June, 2014).

	Temperature (°C)	Oxygen (mg/L)	CO_2 (mg/L)	pH	Nitrites (mg/L)	Ammonium (mg/L)	Hardness (mg/L)
Nursery	25.4 ± 1.1	4.2 ± 0.8	3.1 ± 0.5	7.3 ± 0,4	0.0	0.14 ± 0.03	92.5 ± 3.2
Grow-out	25.2 ± 0.9	4.0 ± 0.6	3.0 ± 0.4	5.8 ± 0.8	0.0	0.34 ± 0.02	93.2 ± 2.8

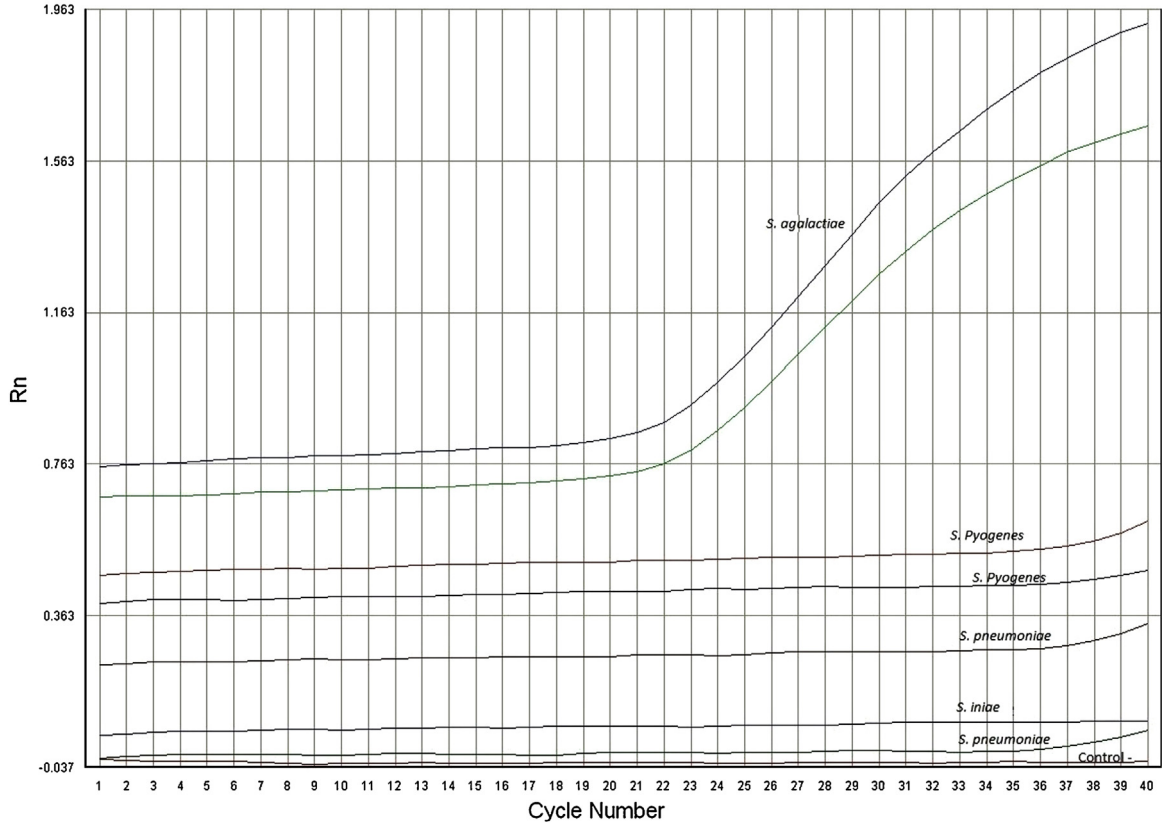

Fig. 1. Real time PCR with different species of *Streptococcus* to determinate the specificity of the assay.

3.2. Microbiology

Were identified 30 isolated presumed for *Streptococcus* spp. The distribution of the isolated by organs was the following: Liver (1) 3.33%, brain (8) 26.67%, kidney (10) 33.33% and spleen (11) 36.67%. These isolated belong to 16 tilapias positives presumed to *Streptococcus* spp.

3.3. Histopathology

The most important histopathology findings were: acute or chronic suppurative fibrin type epicarditis (11) 68.75%; suppurative myocarditis (5) 31.25%; acute suppurative meningitis (10) 62.5% and acute suppurative panophthalmitis. Presence of dispersed pigment in epithelial cells (12) 75%, coagulation necrosis in muscle (14) 87.5%, necrosis and mononuclear infiltration in the liver (9) 56.25%, hyperplasia and fusion of secondary lamella (12) 75%, congestion (5) 31.25%, increased of melanomacrophages center (MMC) in spleen (5) 31.25%, acute suppurative periesplenitis (1) 6.25%, lymphocytolisis (6) 37.50%, fat and hydropic degeneration (8) 50% (Fig. 2–5).

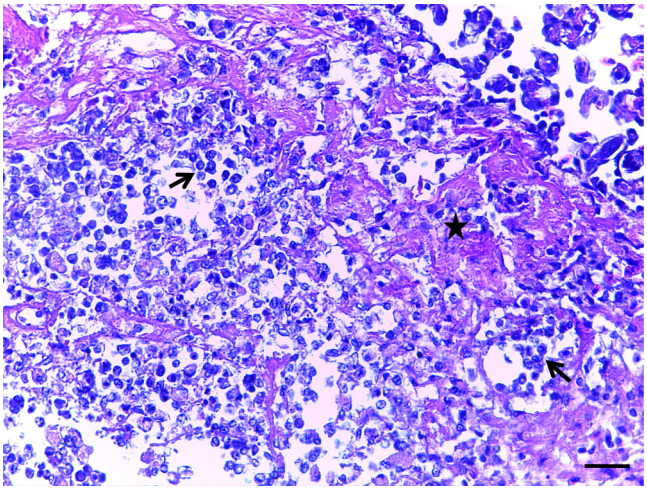

Fig. 2. Histological section of heart. Fibrinosuppurative epicarditis (star) and mononuclear infiltrate (arrow). Magnification: 400×. H&E.

3.4. Relationship between qPCR and histopathological findings

Fifteen fishes were positive to *S. agalactiae* and one sample was inconclusive by qPCR (Cq 39.9). This result is near to the technical limit of detection. The correlation between qPCR and histopathological findings (Table 2) showed fifteen fish positives (prevalence of 93.75%) with qPCR lower than 40 cycles (Cq <40). These fish presented lower Cq associated to high degree of tissue injuries.

Table 2
Values of the Cq (<40) and its correlation with the histopathological findings caused by *S. agalactiae* in *Oreochromis niloticus*.

Fish	qPCR	Histopathological Findings							
	(Cq)	Brain	Eye	Gills	Heart	Muscle	Gut	Liver	Spleen
1	25.5		Panophthalmitis	Fused lamella and hyperplasic		Coagulative necrosis and mononuclear infiltrate	Necrosis and mononuclear infiltrate	Necrosis of hepatocytes Fatty and hydropic degeneration	
2	36.8	Meningitis	Ophthtalmitis	Fused lamella	Epicarditis	Coagulative necrosis		Necrosis of hepatocytes and necrotic foci	Lymphocytolisis
3	37.5		Edema and ophthalmitis	Lamellar hyperplasia and fusion				Mononuclear infiltrate Fatty and hydropic degeneration	Congestion
4	36.9			Fused lamella		Coagulative necrosis and mononuclear infiltrate	Necrosis and hyperplasia	Mononuclear infiltrate	
5	38.6	Meningitis		Congestion and fused lamella	Epicarditis Myocarditis	Coagulative necrosis	Necrosis and hyperplasia	Necrosis of hepatocytes Fatty and hydropic degeneration Mononuclear infiltrate	Depletion of lymphocyte and necrosis
6	26.8	Suppurative meningitis Congestion	Panophthalmitis and dispersed pigmentary epithelium cells	Fused lamella	Chronic fibrino suppurative epicarditis Myocarditis			Necrosis of hepatocytes Mononuclear infiltrate	Congestion
7	32.5		Panophthalmitis and congestion	Hyperplasia and congestion	Epicarditis	Coagulative necrosis		Necrosis of hepatocytes	Depletion and necrosis
8	22.0	Meningitis and congestion	Panophthalmitis	Fused lamella, congestion and hyperplasia	Chronic fibrino suppurative epicarditis Myocarditis	Coagulative necrosis	Necrosis	Necrosis of hepatocytes Fatty and hydropic degeneration.	
9	25.5	Meningitis	Ophthalmitis		Fibrino suppurative necrosis	Coagulative necrosis		Necrosis of hepatocytes	Necrosis and depletion
10	20.7	Suppurative meningitis	Ophthalmitis dispersed pigmentary epithelium cells	Lamellar hyperplasia and congestion		Coagulative necrosis		Necrosis of hepatocytes Mononuclear infiltrate	Necrosis and lymphocytolisis
11	22.5	Meningitis	Panophthalmitis	Lamellar hyperplasia and fusion	Chronic fibrino suppurative epicarditis and paniculitis Myocardial necrosis	Coagulative necrosis		Necrosis of hepatocytes Fatty and hydropic degeneration Perihepatitis fibrino suppurative.	Perisplenitis, necrosis
12	19.6		Panophthalmitis Congestion Dispersed pigmentary cells	Congestion and fused lamella	Fibrino suppurative epicarditis	Coagulative necrosis		Perihepatitis Necrotic focus Mononuclear infiltrate	Congestion Necrosis MMC increased
13	38.1	Congestion and meningitis	Ophthtalmiltis		Epicarditis Myocarditis	Coagulative necrosis		Fatty and hydropic degeneration Congestion Necrosis of hepatocytes	Necrosis and congestion
14	21.4		Severe panophthalmitis with dispersed pigmentary epithelium cells		Chronic fibrino suppurative epicarditis Miocarditis	Coagulative necrosis and mononuclear infiltrate		Necrosis of hepatocytes Mononuclear infiltrate	Necrosis and depletion
15	39.9					Coagulative necrosis			
16	18.3	Suppurative meningiits		Fused lamella and congestion	Fibrino suppurative epicarditis Myocardial necrosis	Coagulative necrosis and mononuclear infiltrate		Necrosis of hepatocytes Fatty and hydropic degeneration Mononuclear infiltrate	Necrosis and lymphocytolisis

4. Discussion

The specie *S. agalactiae* is the most widely distributed in Latin American countries (Sheehan et al., 2009). However, its geographical distribution may be undervalued due to the absence of formal reporting and inadequate identification (Pulido and Iregui, 2010). In 16 sampled fishes, the molecular analysis showed fifteen positive animals. Several authors have used the PCR for the identification of *S. agalactiae*, such us multiplex PCR by detection of *Aeromonas* spp., and *Streptococcus* spp. (Lukkana et al., 2014). Also, Rodkhum et al. (2012) developed a duplex PCR for the detection of *S. agalactiae* and *S. iniae*. However, the real-time PCR assay is a faster method for

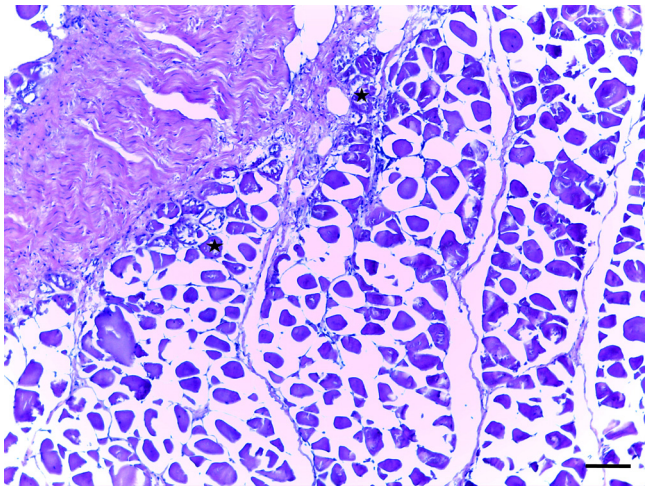

Fig. 3. Histological section of skeletal muscle. Necrosis (star). Magnification: 400×. H&E.

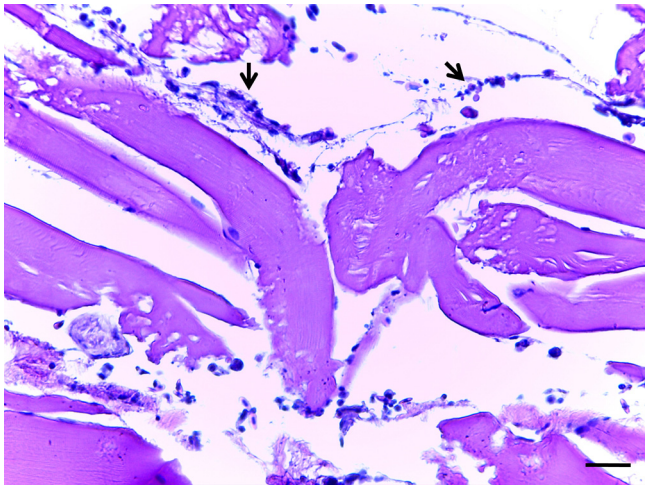

Fig. 4. Histological section of skeletal muscle. Infiltratation mononuclear (arrow). Magnification: 400×. H&E.

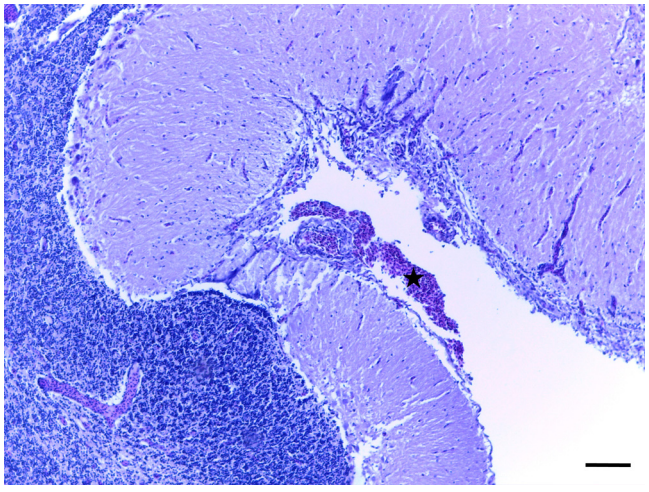

Fig. 5. Histological section of brain. Suppurative meningitis (star). Magnification: 400×. H&E.

identification of *S. agalactiae*, which was used in the present study. It has become the most commonly used molecular tool because it requires less labor, provides greater cost-effectiveness and is easily adapted to process large-scale samples compared with other methods (Sebastião et al., 2015). This study described the real-time PCR assay using Taqman probes that can use DNA extracted directly of a pure isolated.

Macroscopic findings found at the necropsy, such as unilateral or bilateral exophthalmia, hyphema, corneal opacity, ascites, melanosis, bleeding in the anal pore, haemorrhagic and necrotic lesions on the skin and muscle tissue, fluid in the abdominal cavity, hepatomegaly, splenomegaly are suggestive of *Streptococcus agalactiae* infection, in accordance with the described by Amal and Zamri-Saad (2011), and also reported by Salvador et al. (2005) and Figueiredo et al. (2006) in tilapias infected naturally.

The histopathological description of the fish sampled showed correlation with qPCR results, because animals with low values of Cq (<40) had more histopathological lesions. According with Iregui et al. (2014), to identify DNA in tissues do not mean that the fish has the disease, and only the correlation between the results from molecular techniques, anamnesis and microscopic findings can offer more reliable diagnoses (Sebastião et al., 2015). In this study, the tissues most affected by the positive fish were the heart, liver, eye, spleen and brain. Such results suggest that they would be target organs for this bacterium, which has also been reported by Alsaid et al. (2013). These findings indicate septic process and spread this agent as reported by Yuasa et al. (2008). Also, the findings of epicarditis and meningitis found in this study are consistent with those described by Zamri-Saad et al. (2010) in *O. niloticus* infected naturally with *S. agalactiae*. Alsaid et al. (2013) and Pulido et al. (2004) mentioned that inflammation in the epicardium may vary in different degrees, while other organs could be less affected, corroborating the findings observed in this study with types of acute or chronic epicarditis. In the spleen was found congestion, necrotic foci and increased of MMC. Similar histological lesions in the splenic tissue of red tilapia were described by Alsaid et al. (2013). Other authors observed increased number of MMC in the spleen of fish during bacterial infection (Manrique et al., 2014).

The lesions observed in the liver, as necrosis and mononuclear infiltration, described in this study are consistent with those reported by Mohamed et al. (2014) in tilapias infected experimentally with *Streptococcus* spp. It is noteworthy that the infiltration of mononuclear cells, mainly macrophages, observed in this study in all organs were also described by Filho et al. (2009) and Iregui et al. (2014).

In Asia and Latin American, were isolated near of 500 cases of *Streptococcus* sp. coming from tilapia, which 82% of prevalence were isolates of *S. agalactiae* and 18% of *S. iniae* in the *Oreochromis* sp. Some authors mention that *S. agalactiae* is among the most prevalent species in Latin American countries. In Brazil and Colombia, this bacterial infection has caused high mortality in tilapia farms (Iregui et al., 2014; Marcusso et al., 2015). This study was the first to identify by qPCR *S. agalactiae* in naturally infected tilapia from nursery and grow-out phases in Piura, Peru, and to characterize histopathological lesions caused by this bacterium. The correlation between the histopathological findings with the qPCR can provide greater support in relation to the diagnostic and specific treatment for this disease.

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References

Abuseliana, A., Daud, H., Aziz, S., Bejo, S., Alsaid, M., 2010. *Streptococcus agalactiae* the etiological agent of mass mortality in farmed red tilapia. (*Oreochromis* sp.). J. An. Vet. Adv. 9, 2640–2646.

Alsaid, M., Mohd, H., Mohamed, N., Khairani, S., Mohamed, Y., Farag, A., Hayati, R., 2013. Pathological findings of experimental *Streptococcus agalactiae* infection in red hybrid tilapia (*Oreochromis* sp.). In: International Conference on Chemical, Agricultural and Medical Sciences (CAMS), Dec. 29–30. Kuala Lumpur (Malasia), pp. 70–73, http://dx.doi.org/10.15242/IICBE.C1213075.

Amal, M.N.A., Zamri-Saad, M., 2011. Streptococcosis in tilapia (*Oreochromis niloticus*): a review. Pertanika J. Trop. Agric. Sci. 34 (2), 195–206.

Baltazar, P.M., Palomino, A.R., 2004. Manual de cultivo de tilapia. Acuerdo de Colaboración Institucional AECI/PADESPA – FONDEPES. Sub proyecto: Programa de Transferencia de Tecnología en Acuicultura para Pescadores Artesanales y Comunidades Campesinas, pp. 115.

Baltazar, P.M., 2009. Infopesca Internacional. Número 40. Cultivo de tilapia en Perú, 21–26.

Belo, M.A.A., Schalch, S.H.C., Moraes, F.R., Soares, V.E., Otoboni, A.M., Moraes, J.E.R., 2005. Effect of dietary supplementation with vitamin E and stoking density on macrophage recruitment and giant cell formation in the teleost fish, *Piaractus mesopotamicus*. J. Comp. Pathol. 133, 146–154.

Belo, M.A.A., Moraes, J.R.E., Soares, V.E., Maritns, M.L., Brum, C.D., Moraes, F.R., 2012. Vitamin C and endogenous cortisol in foreign-body inflammatory response in pacus. Pesq. Agropec. Bras. 47, 1015–1021.

Belo, M.A.A., Moraes, F.R., Yoshida, L., Prado, E.J.P., Moraes, J.R.E., Soares, V.E., Silva, M.G., 2014. Deleterious effects of low level of vitamin E and high stocking density on the hematology response of pacus, during chronic inflammatory reaction. Aquaculture 422–423, 124–128.

Betancur, J., Ostos, H., Quintero, J.C., Barreiro, F., 2012. Sanidad Acuícola-Estudio De Infección Por Bacterias Del género *Streptococcus* spp. En Sistemas De Producción De Tilapia En El Departamento Del Huila. Universidad Surcolombiana, pp. 63.

Buller, N., 2014. Bacteria and Fungi from Fish and Other Aquatic Animals. A Practical Identification Manual, 2da edic. CABI International, pp. 919.

Castro, M.P., Claudiano, G.S., Bortoluzzi, N.L., Garrido, E., Fujimoto, R.Y., Belo, M.A.A., Shimada, M.T., Moraes, J.E.R., Moraes, F.R., 2014a. Chromium carbochelate dietary supplementation favored the glucocorticoid response during acute inflammation of *Piaractus mesopotamicus*. Aquaculture 432, 114–118.

Castro, M.P., Claudiano, G.S., Petrillo, T.R., Shimada, M.T., Belo, M.A.A., Machado, C.M.M., Moraes, J.R.E., Moraes, F.R., 2014b. Acute aerocystitis in Nile tilapia bred in net cages and supplemented with chromium carbochelate and *Saccharomyces cerevisiae*. Fish Shellfish Immunol. 36, 284–290.

Conroy, G., Conroy, D., Klesius, P., Shoemaker, C., Evans, J., 2008. Importantes enfermedades infecciosas y parasitarias en tilapias cultivadas. Intervet—Schering-Plough Animal Health, pp. 171.

Conroy, G., 2009. Estreptocococis en tilapia: prevalencia de las especies de *Streptococcus* en América Latina y sus manifestaciones patológicas. En: Memorias de *Streptococcus* en peces de aguas cálidas. 3, 15–20.

Ferguson, H., 2006. Systemic Pahtology of Fish: A Text and Atlas of Normal Tissues in Teleosts and Their Responses in Disease, second ed. Scotian Press, pp. 367.

Figueiredo, H., Carneiro, D., Faria, F., Costa, G., 2006. *Streptococcus agalactiae* associado à meningoencefalite e infecção sistêmica em tilápia-do-nilo (*Oreochromis niloticus*) no Brasil. Arq. Bras. Med. Vet. Zootec. 58, 678–680.

Filho, C.I., Müller, E.E., Pretto-Giordano, L.G., Bracarense, A.P.F.R.L., 2009. Histological findings of experimental *Streptococcus agalactiae* infecion in Nile tilapias (*Oreochromis niloticus*). Braz. J. Vet. Pathol. 2, 12–15.

Iregui, C., Barato, P., Rey, A., Vásquez, G., Verjan, N., 2014. Epidemiology of. https://www.researchgate.net/profile/Paola_Barato/publication/261876026_Epidemiology_of_Streptococcus_agalactiae_and_streptococcosis_in_tilapia_%28Oreochromis_sp%29/links/0a85e535c0b6079009000000.pdf.

Jimenéz, A.P., 2010. Disertación de maestría en microbiología. In: Detección De *Streptococcus Agalactiae* Por PCR En Tejidos De Tilapias Rojas (*Oreochromis* spp.) Menores De 20 g. Universidad Nacional de Colombia, Colombia, pp. 101.

Jimenez, A.P., Tibatá, V.M., Junca, H., Ariza, F., Verjan, N., Iregui, C.A., 2011. Evaluating a nested-PCR assay for de-tecting *Streptococcus agalactiae* in red tilapia (*Oreochromis* sp.) tissue. Aquaculture 321, 203–206.

Lukkana, M., Jantrakajorn, S., Piamsomboon, P., Wongtavatchai, J., 2014. Simultaneous detection of *Streptococcus* spp. and *Aeromona* spp. from diseases tilapia (*Oreochromis niloticus*) using Multiple Polymerase Chain Reaction. Isr. J. Aquacult.-Bamid. 66, 1023–1030.

Manrique, W.G., Claudiano, G.S., Pereira, M.A., Petrillo, T.R., Moraes, J.R.E., Moraes, F.R., 2012. Myxosporidiosis in intensively-reared *Piaractus mesopotamicus:* histopathological diagnosis by means of Ziehl-Neelsen staining. Pesqui. Vet. Bras. 32, 1133–1137.

Manrique, W.G., da Silva, C.G., Petrillo, T.R., Pardi de Castro, M., Figueiredo, M.A.P., Moraes, J.R.E., Moraes, F.R., 2014. Response of splenic melanomacrophage centers of *Oreochromis niloticus* (Linnaeus) to inflammatory stimuli by BCG and foreign bodies. J. Appl. Ichthyol. 30, 1001–1006.

Manrique, W.G., Silva, G.C., Castro, M.P., Petrillo, T.R., Figueiredo, M.A.P., Belo, M.A.A., Berdeal, M.I.Q., Moraes, J.R.E., Moraes, F.R., 2015a. Expresion of celular inflamatory response in *Piaractus mesopotamicus* model. PLoS One 10, e0121625.

Manrique, W.G., Figueiredo, M.A.P., Belo, M.A.A., Martins, M.L., Moraes, F.R., 2015b. First report of *Myxobolus* sp. infection in the skeletal muscle of Neotropical freshwater fish *Piaractus mesopotamicus*. Parasitol. Res. 114, 2041–2044.

Manrique, W.G., Figueiredo, M.A.P., Belo, M.A.A., Martins, M.L., Azevedo, C., 2016. Ultrastructural description of *Myxobolus cuneus* (Myxosporea) in the skeletal muscle and kidney of tropical farmed fish *Piaractus mesopotamicus* (Characiformes: characidae). Parasitol. Res. 115, 2505–2510.

Marcusso, P., Eto, S., Claudiano, G., Vieira, F., Salvador, R., Moraes, J., Moraes, F.R., 2015. Isolamento de *Streptococcus agalactiae* em diferentes órgaos de tilápias do Nilo (*Oreochromis niloticus*) criadas em tanques-rede. Biosci. J. 31, 549–554.

Mohamed, N., Mahmoud, K., El-Sayed, M., NaguibMohamed, M., Abd-Elrahman, S., 2014. Streptoccosis in tilapia: clinico-pathological picture of experimentally infected tilapia. Life Sci. J. 11, 1005–1012.

Padua, S.B., Menezes, R.N., Martins, M.L., Belo, M.A.A., Ishikawa, M.M., Nascimento, C.A., Saturnino, K.C., Carrijo, J.R., 2015. A survey of epitheliocystis disease in farmed Nile tilapia (Linnaeus, 1758) in Brazil. J. Appl. Ichthyol. 31, 927–930.

Pulido, E., Iregui, C., 2010. Hibridación *in situ* para la detección de *Streptococcus agalactiae* en tejidos de tilapia (*Oreochromis* sp.). Rev. Fac. Med. Vet. Zoot. 57, 11–22.

Pulido, E., Iregui, C., Figueroa, J., Klesius, P., 2004. Estreptococosis en tilapias (*Oreochromis* spp.) cultivadas en Colombia. Rev. AquaT 20, 97–106.

PRODUCE, 2011. Ministerio De La Producción. Estadística De Cosecha En Acuicultura. PRODUCE, Lima http://www2.produce.gob.pe/produce/boletines/estadistico/2011/05_03.pdf.

Rodkhum, C., Kayansamruaj, P., Pirarat, N., Wongtawatchai, J., 2012. Duplex PCR for simultaneous and unambiguous detection of *Streptococcus iniae* and *Streptococcus agalactiae* associated with streptococcosis of cultured tilapia in Thailand. Thai. J. Vet. Med. 42, 153–158.

Sakabe, R., Moraes, F.R., Belo, M.A.A., Moraes, J.E.R., Pilarski, F., 2013. Kinects of cronic inflammation in Nile tilapia supplemented with essential fatty acids n-3 and n-6. Pesq. Agropec. Bras. 48, 313–319.

Salvador, R., Eckehardt, E., de Freitas, J., Leonhadt, J., García, L., Alves, J., 2005. 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. Ciencia Rural 35, 1374–1378.

Sandoval, N., Lam, K., Manchego, A., 2010. Reporte De Enfermedades Que Se Han Presentado Y Existen En Las Áreas De Cultivo De Las Especies Acuícolas: Trucha, Langostino, Concha De Abanico Y Tilapia. Ministerio de la Producción62.

Sebastião, F de A., Lemos, E.G., Pilarski, F., 2015. Validation of abosultue quantitative real. time PCR for the diagnosis of *Streptococcus agalactiae* in fish. J. Microbiol. Methods 119, 168–175.

Sedano, N., 2013. Disertación de Médico veterinario. In: Lesiones Anatomo-histopatológicas Procedentes De Tilapia Roja (*Oreochromis* spp.) De Cultivo En El Departamento De Piura Provincia De Sullana, Distrito De Lancones, Caserío De Chilacos Pelados. Universidad Nacional Mayor de San Marcos, Perú, pp. 134.

Sheehan, B., Lee, Y.S., Wong, E.S., Chan, J., Labrie, L., Komar, C., Wendover N., Grisez, L., 2009. Aquavac Strep Sa: Una novedosa vacuna para el control de las infecciones causadas por *Streptococcus agalactiae* Biotipo 2 en tilapia de granja. En memorias: Manejo de Streptococcus en peces de aguas cálidas, pp. 21–26.

Wongsathein, D., 2012. Doctoral dissertation. In: Factors Affecting Experimental *Streptococcus Agalactiae* Infection in Tilapia, *Oreochromis Niloticus*. University of Stirling, UK, pp. 169.

Yanong, R., Francis-Floyd, R., 2002. Streptococcal infections of fish. Report from University of Florida. Series from the Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, pp. 5.

Yuasa, K., Kamaishi, T., Hatai, K., Bahnnan, M., Borisuthpeth, P., 2008. Two cases of streptococcal infections of cultured tilapia in Asia. In: Bondad-Reantaso, M.G., Mohan, C.V., Crumlish, M., Subasinghe, R.P. (Eds.), Diseases in Asian Aquaculture VI. Fish Health Section, Asian Fisheries Society, Manila, Philippines, pp. 259–268.

Zamri-Saad, M., Amal, M.N., Siti-Zahrah, A., 2010. Pathological changes in red tilapias (*Oreochromis* spp.) naturally infected by *Streptococcus agalactiae*. J. Comp. Pathol. 143, 227–229.