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Original Article

Identification of pathogens in fish polyculture systems in southern Minas Gerais, Brazil

Identificação de patógenos em pisciculturas de policultivo no sul de Minas Gerais, Brasil

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Fish diseases represent a significant limiting factor in aquaculture systems. Among the many pathogens, bacteria are probably the most significant group of etiological agents. Thus, the objective of this work was to isolate and to report the incidence of streptococcosis and other possible pathogens in fish polyculture systems in the region of Lavras, state of Minas Gerais, Brazil. Fish samples were randomly collected from seven fish polyculture systems in the region, including the following municipalities: Lavras, Itutinga, Itumirim, Nepomuceno, Carrancas, São Sebastião da Vitória, and Ingaí. The specimens analyzed were: Tilapia (*Oreochromis niloticus*), Dourado (*Salminus brasiliensis*), Cará (*Geophagus brasiliensis*), Curimba (*Prochilodus lineatus*), Surubim (*Pseudoplatystoma corruscans*), and Piracanjuba (*Brycon orbignyanus*). Samples of kidney, brain, liver, spleen, and intestinal tract, and skin scraping was collected and sown in a culture medium. After the incubation period, the microorganisms were identified according to the culture, morphology, dyeing, and biochemical characteristics of the bacteria. Colonies of *Streptococci*, *Aeromonas*, and *Edwardsiella* were identified in Tilapia, Cara, Curimba, and Surubim fish species. The results showed the identification of the bacteria in fish polyculture systems in the region of Lavras, southern Minas Gerais. Therefore, greater sanitary control and the development of other studies to treat diseases related to these pathogens are necessary.

Palavras-chave:
Agente etiológico
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R E S U M O

As doenças em peixes representam um fator limitante nos sistemas de aquicultura, e as bactérias, dentre vários patógenos, provavelmente constituam o grupo de agente etiológicos economicamente mais significante. Desta forma, o objetivou-se com este trabalho, isolar e relatar a ocorrência da estreptococose e outros prováveis patógenos em pisciculturas de policultivo da região de Lavras – MG. Foram coletadas amostras aleatórias de peixes em sete criatórios de policultivo de peixes da região, que englobam os seguintes munícipios: Lavras, Itutinga, Itumirim, Nepomuceno, Carrancas, São Sebastião da Vitória e Ingaí. Exemplares de tilápia, dourado, cará, curimba, surubim e piracanjuba foram analisados. Amostras de órgãos dos peixes foram coletadas e semeadas em meio de cultura. Após o período de incubação, a identificação dos microrganismos foi realizada de acordo com as características de cultura da bactéria, morfológicas, tintoriais e bioquímicas. Foram identificadas colônias de estreptococos, aeromonas e edwardsiella, nas espécies de tilápia, cará, curimba e surubim. Os resultados demonstraram que foram identificadas as bactérias nas pisciculturas de policultivo da região de Lavras, sul de Minas Gerais. Portanto, há uma necessidade de maior controle sanitário e o desenvolvimento de outros estudos para tratamentos de doenças relacionadas a esses patógenos.

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INTRODUCTION

Aquaculture is the fastest growing sector of animal protein production in the world, with a total production increase from nearly 1 million tons in 1950 to approximately 52.5 million tons in 2008 (FAO, 2014). In this context, Brazil occupies the 12th position worldwide, with a production of approximately 707 thousand tons in 2012 (FAO, 2014); the southeast and center-west regions account for 30% of the national production (BRASIL, 2012; KUBITZA et al., 2012).

With the increase in productivity, there is also the increase in management difficulties related to water quality increased, stocking densities, and poor fish handling, which result in disease outbreaks that have caused severe economic losses for the producers (LI et al., 2018; BENHAMED et al., 2014; LEIRA et al., 2016). The literature presents a few cases of disease occurrence in Brazilian fish farms, which also cause high mortality rates (AZEVEDO; MOREY; MALTA, 2017; EIRAS; TAKEMOTO; PAVANELLI, 2010; KUBITZA et al., 2012). Among the main diseases, the ones caused by bacteria, especially of the Streptococcus (ZHANG; XU; KLESIUS, 2013), Aeromonas (HAWKE et al., 2013), and Edwardsiella (PARK; AOKI; JUNG, 2012) genera, are of great concern.

The occurrence of the infectious processes caused by *S. agalactiae* is still little studied in fish. The bacteria samples reported in fish presented a genetic pattern distinct from those isolated from humans and cattle, despite belonging to the same bacterial species (JOHRI; PAOLETTI; GLASER, 2006; PEREIRA; MIAN; OLIVEIRA, 2010).

The transmission of Streptococcosis occurs horizontally by the direct contact between the infected and healthy fish, as well as indirect contact by the bacteria present in the aquatic environment. This transmission causes the disease to gradually spread in the same property or through sources such as any inanimate object or substance capable of absorbing, retaining, and carrying infectious pathogens from one location to another, such as buckets and dip nets (BOTREL et al., 2010; EVANS; KLESIUS; GILBERT, 2000; SALVADOR, 2008). The virulence factors of the bacteria are related to the invasion, replication, and evasion of the host immune system, causing lesions during the disease pathogenesis (VILCHES et al., 2004). These rod cells, which are adapted to growth at temperatures ranging from 5 °C to 37 °C, are considered Gram negative, oxidative positive, facultative anaerobic, and are present on the skin and gills of fish (KOZINSKA, 2007).

The objective of this work was to isolate and to report the incidence of streptococcosis and other possible pathogens in fish polyculture systems in the region of Lavras, state of Minas Gerais, Brazil.

MATERIAL AND METHODS

Fish samples from seven fish polyculture systems in the region, including the municipalities of Lavras, Itutinga, Itumirim, Nepomuceno, Carrancas, São Sebastião da Vitória, and Ingaí, in southern Minas Gerais, Brazil, were collected. The study was accepted by the Bioethics Committee n°017/13 on April 18, 2013.

The fish polyculture systems were identified as A (16 fish), B (9 fish), C (19 fish), D (12 fish), E (8 fish), F (20 fish), and G (12 fish), in which the numbers in parenthesis represent the quantity of fish collected in each system. The sampled species were: Tilapia (*Oreochromis niloticus*), Dourado (*Salminus brasiliensis*), Cará (*Geophagus brasiliensis*), Curimba (*Prochilodus lineatus*), Surubim (*Pseudoplatystoma corruscans*), and Piracanjuba (*Brycon orbignyanus*). The collected fish presented no clinical signs of diseases and had an average weight of 700 grams.

Fish samples were desensitized according to Zahl; Samuelsen; Kiessling (2012), followed by external physical examination and necropsy for macroscopic changes recording. Samples of kidney, brain, liver, spleen, and digestive tract were collected in each fish. Skin scraping was also carried out.

The organ samples were initially seeded on BHI (Brain Heart Infusion – Difco) agar plates, supplemented with 5% defibrinated sheep blood, and incubated at 27 °C under aerophilic atmosphere for seven days. After the incubation period, the microorganisms were identified according to the bacteria culture, morphology, dyeing, and biochemical characteristics (KRIEG; HOLT, 1984).

The morphological analyses conducted in the plates containing BHI agar supplemented with blood suggested that *Streptococcus agalactiae* presented gray, translucent, circular, slightly convex, and punctate colonies instead of non-hemolytic colonies.

Subsequently, the cultures were submitted to the latex-agglutination test, in which the presence of the carbohydrate antigen indicates that the colonies belong to the B Lancefield group. The bacterial colonies were characterized by catalase, oxidase, KOH, and growth proof in methylene blue, 6.5% NaCl, with confirmation through PCR.

The bacterial genomic DNA were extracted from 20-50 mg of the organs using the Qiagen Kit for tissue extraction according to the manufacturer's protocol. The samples were diluted in 50 µL of autoclaved ultrapure water. A sequence of the *S. agalactiae* 16S rRNA target gene was amplified using a real-time PCR assay for quantification of equivalent cells/bacterial DNA. All real-time PCR reactions were performed in triplicates.

RESULTS AND DISCUSSION

Streptococcal colonies were found on the BHI agar. The colonies presented gray, translucent, circular, slightly convex, punctate, and nonhemolytic appearance. All strains presented negative results for the catalase tests, growth in 6.5% NaCl, 40% bile, esculin, and methylene blue. However, colonies with characteristics that are distinct from the streptococci characterized by PCR, such as *Aeromonas* and *Edwardsiella*, occurred (Table 1). Eight cases of *S. agalactiae* streptococcus were confirmed in two farms.

Table 1 – Identification and microbiological analyses of the bacteria found in the fish species reared in Southern Minas Gerais, Brazil (fish systems from A to G).

Fish System	N	N infected	KOH	Catalase	Oxidase	Morphology	PCR
A							
Tilapias	10	03	-	-	-	Coccus+	*S. Agalactiae*
Surubim	01		+	-	-	Coccobacillus-	Negative
Curimba	01	01	-	-	-	Coccus+	*S. Agalactiae*
Piracanjuba	02		-	-	-	Coccus-	Negative
Cará	02	01	+	+	+	Coccobacillus-	*Aeromonas*
B							
Tilapias	06		+	-	+	Bacillus+	Negative
Surubim	02		-	+	-	Bacillus+	Negative
Curimba	01		+	+	-	Coccus+	Negative
C							
Tilapias	13	02	-	-	-	Coccus+	*S. Agalactiae*
Dourados	01		-	+	+	Coccus +	Negative
Surubim	02		+	+	+	Coccus +	Negative
Curimba	01		-	+	+	Coccus -	Negative
Piracanjuba	01		+	+	+	Coccus +	Negative
Cará	01	01	-	-	-	Coccus +	*S. Agalactiae*
D							
Tilapias	09		-	-	-	Coccus -	Negative
Dourados	01		-	+	+	Coccus -	Negative
Surubim	01		-	+	-	Coccus -	Negative
Curimba	01		+	+	-	Coccus -	Negative
E							
Tilapias	06		+	+	+	Coccobacillus -	Negative
Surubim	01		+	+	-	Coccobacillus +	Negative
Curimba	01		-	-	-	Coccus -	Negative
F							
Tilapias	11		+	+	+	Coccobacillus +	Negative
Surubim	06	03	+	+	-	Coccobacillus -	*Edwardsiella*
Piracanjuba	01		+	-	+	Coccus -	Negative
Cará	02		+	+	-	Coccus -	Negative
G							
Tilapias	09		+	-	+	Coccus +	Negative
Dourados	02		+	+	+	Coccus +	Negative
Piracanjuba	01		+	+	+	Coccus -	Negative

Only three systems had the presence of pathogenic bacteria in fish: three in Tilapia and one in Curimba, which were contaminated with *Streptococcus* in the fish system A. Two Tilapia and one Cara were contaminated with *Streptococcus* in fish system C, while in the fish system F, three Surubim fish were contaminated by *Edwardsiella*. Bacteria were also found in Tilapia, Cara, and Curimba. A possible cause may be the high amount of fish placed in the tanks, excess organic matter, and the different species in the same tank. *Streptococci* are bacteria widely found in the aquatic environment, and their emergence is connected to stressful conditions (FIGUEIREDO, 2012).

Bacteria seem to be more prevalent in hot waters with large amounts of organic material associated with stressing factors. These factors include high stock densities, high temperatures, abrupt temperature

changes, trauma from handling, fish transfer and low oxygen levels, nutritional deficiency, external skin lesions, fungal or parasitic infections that contribute to physiological changes, and the increase of susceptibility to contamination (AOKI, 1999; HAWKE et al., 2013; PARK; AOKI; JUNG, 2012). Thus, when conditions are favorable, the occurrence of streptococcosis in fish may occur (BOTREL et al., 2010; PEREIRA; MIAN; OLIVEIRA, 2010).

Aeromonas and *Edwardsiella* are naturally present in the environment and typically inhabit the intestine of fish, which explain their occurrence in the studied fish. The diseases caused by these bacteria are common in fish farming throughout the world (HOLLIMAN et al., 1993).

The impact of these bacteria must be considered since several researchers affirm that the exposure to the aquatic ecosystem and its habitats can be precursors of diseases caused by *Aeromonas* and *Edwardsiella* (KIM et al., 2018; WIEDENMAYER; EVANS; KLESIUS; GILBERT, 2006). These are facultative pathogens that target hosts weakened or affected by other etiological agents, identified as secondary invaders, and establish themselves in the same way as other bacterial, viral, or parasitic infections, as well as those caused by nutritional or stress-related factors (BOTREL et al., 2010; PAVANELLI; MACHADO, TAKEMOTO, 1997).

The adequate characterization and identification of the diseases and their causal agents are essential to better understand their behavior, as well as to define the epidemiology and implement control methods. With such knowledge, it is possible to develop preventive measures, avoiding low fish production and high economic losses, expanding and consolidating the aquaculture activity, in addition to allowing the identification and selection of fish more resistant to pathogens (HAWKE et al., 2013).

Immunoprophylaxis, used as a preventive measure by vaccination, is the most appropriate form to control some bacterial diseases such as streptococcosis. It consists of integrated sanitary management, which combines the correct maintenance of the nurseries and biosafety in production. In order to achieve this goal, it is necessary to adjust the structure and production system, to train employees, and to organize the entire team. The management of larvae and water sources should also be monitored (PÁDUA; MENEZES FILHO; CRUZ, 2012).

CONCLUSION

This study showed the presence of *Streptococcus*, *Aeromonas,* and *Edwardsiella* in fish systems in the region of Lavras, state of Minas Gerais, Brazil, indicating the need for greater sanitary control and the development of epidemiological research at the national level.

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