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Leptospira spp. serogroups circulating in two neotropical fish species in a quilombola area in Maranhão State, Brazil

Serogrupos de *Leptospira* spp. que circulan en dos especies de peces neotropicales en un área quilombola en el estado de Maranhão, Brasil

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ABSTRACT

Despite fish's relevance as a reservoir of several zoonotic bacteria, their role in maintaining and outspreading *Leptospira* remains poorly known. The objective of the study was to report the serovarieties of *Leptospira* spp. circulating in *Hoplerythrinus unitaeniatus* and *Cichlasoma bimaculatum* living in a quilombola area in Maranhão State, Brazil; 42 fish specimens (21 *H. unitaeniatus* and 21 C. *bimaculatum*) were captured and transported alive in isothermal boxes filled with water, from the location they were placed into a laboratory environment. Blood samples of these fish were collected through brachial vein conduit and its serum was subjected to anti-leptospiral agglutinins assessment against 25 serovarieties belonging to the *Leptospira* spp. complex, based on the serum-agglutination microscopy technique. Total anti-leptospiral agglutinins prevalence reached 80,95 % (n=34/42) – with titles that range from 1:100 to 1:400. The most frequent serovarieties belonging to the *Leptospira* complex were Pomona, Hebdomadis, Butembo, Copenhageni, Cynopteri, Panama, Sentot, Bratislava, Icteriohaemorragiae, Pomona variant Bubalinos, Pyrogenes, Whitcombi. Leptospirosis is a globally documented infectious disease observed in several animal species and in humans. It infected native fish caught in Ponta Bonito quilombola community at high prevalence rate. It is essential investigating fish's infection sources and the application of proper measures to prevent leptospirosis in this geographic area.

Keywords: traditional peoples, leptospirosis, native fish, serum-agglutination microscopy.

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RESUMEN

A pesar de la relevancia de los peces como reservorio de varias bacterias zoonóticas, su papel en el mantenimiento y propagación de *Leptospira* sigue siendo poco conocido. El objetivo del estudio fue reportar las serovariedades de *Leptospira* spp. circulante en peces pertenecientes a las especies *Hoplerythrinus unitaeniatus* y *Cichlasoma bimaculatum* que viven en un área quilombola en el estado de Maranhão, Brasil; 42 peces (21 *H. unitaeniatus* y 21 *C. bimaculatum*) fueron capturados y transportados vivos en cajas isotérmicas llenas de agua, desde el lugar donde fueron recolectados hasta el laboratorio. Se recolectaron muestras de sangre de estos peces a través del conducto de la vena braquial y el suero se sometió a evaluación de aglutininas anti-leptospira contra 25 serovariedades pertenecientes al complejo *Leptospira*, basado en la técnica de microscopía de aglutinación de suero. La prevalencia total de aglutininas antileptospirales alcanzó el 80,95 % (n=34/42) – con títulos que van de 1:100 a 1:400. Las serovariedades más frecuentes pertenecientes a *Leptospira* complejo fueron Pomona, Hebdomadis, Butembo, Copenhageni, Cynopteri, Panamá, Sentot, Bratislava, Icteriohaemorragiae, Pomona variante Bubalinos, Pyrogenes, Whitcombi. La leptospirosis es una enfermedad infecciosa documentada a nivel mundial que se observa en varias especies animales y en humanos. Infecta peces nativos pertenecientes a la comunidad quilombola de Ponta Bonito con una alta tasa de prevalencia. Es fundamental investigar las fuentes de infección de los peces y aplicar las medidas adecuadas para prevenir la leptospirosis en esta área geográfica.

Palabras clave: pueblos tradicionales, leptospirosis, peces nativos, microscopía de aglutinación de suero.

INTRODUCTION

Fish are among the most traded and consumed protein food in Brazil (Souza *et al.* 2013). Fishing and aquaculture in Maranhão State are important activities to provide animal-origin protein for human nutrition (Monteles *et al.* 2010). Among areas accounting for the greatest fish production in the country, one is the Western Plain of Maranhão State, since this region encompasses an integrated hydrographic system comprising rivers, lakes, and floodable river plains. Such a feature gives this region the status of the biggest lake basin in the Northern Region (Pereira *et al.* 2016).

The Western Plain of Maranhão State holds countless traditional quilombola communities who practice artisanal fishing, as an important means of survival and source of protein throughout the year (Bernardes *et al.* 2011). *Cichlasoma bimaculatum* (Linnaeus 1758) (black acara) and *Hoplerythrinus unitaeniatus* (Agassiz 1829) (jeju) are important fishing resources for human consumption in quilombola communities, in Maranhão State (Gurgel *et al.* 1994, Viana *et al.* 2014). Both species are distributed in shallow water environments close to submerged or riverside vegetation (Leal *et al.* 2010, Froese and Pauly 2016).

Despite the importance of artisanal fishing in Brazil, it has been strongly affected by anthropic and ecological changes that have led to the loss of several fish species, every year (Maldonado and Santos, 2006). Besides the countless environmental impacts statewide (deforestation and pollution resulting from domestic sewage discharge), it is also possible mentioning domestic animals' husbandry and animals' breeding (for livestock activity purposes) under precarious sanitary conditions, such as those provided to pigs, buffaloes and cattle, since these animals can host bacterial etiological agents like *Leptospira* sp.

Despite fish's relevance as a reservoir of several zoonotic bacteria, their role in maintaining and outspreading *Leptospira* remains poorly known. Research about leptospirosis in fish, carried out in Brazil, is scarce; there are only a

few records to point this class of vertebrates out as potential spirochete carriers (Esteves *et al.* 2005).

Leptospirosis is an emerging zoonosis of global distribution that affects both humans and animals that are directly or indirectly exposed to environments contaminated with urine, blood or tissue of infected animals (Thibeaux *et al.* 2018a, Vincent *et al.* 2019). The etiological agent is a bacterium that belongs to genus *Leptospira*, which encompasses intermediate and pathogenic saprophytic species (Perolat *et al.* 1998, Helmerhorst *et al.* 2012, Marteli *et al.* 2020). According to Thibeaux *et al.* (2018b) and Vicent *et al.* (2019), *Leptospira* is highly heterogeneous and genetically different from other bacteria; it is composed of 35 species and more than 300 serovars.

Despite the importance of aquaculture and fishery in Maranhão State, there are no studies addressing the occurrence of zoonotic bacteria in fish. Accordingly, the aim of the current study was to report the serovarieties of *Leptospira* sp. circulating in two neotropical fish species in a quilombola area in Maranhão State, Brazil.

MATERIALS AND METHODS

Research ethics

This research was approved by the Ethics Committee on Animal Experimentation (CEEA) of State University of Maranhão – UEMA – under protocol n. 08/2021, since it meets the Resolutions of the Federal Veterinary Medicine Council (CFMV) n. 879/2008, 1000/2012, and Federal law n. 11794/2008, which provides on Ethical procedures in Animal Experimentation.

Study site

The study was carried out in Ponta Bonita Quilombola community, which is located in the rural municipality of Anajatuba - Maranhão State, between geographic coordinates: latitude 03°15'50" S and longitude 44°37'12" W. This municipality belongs to the Western Plain of Maranhão State, a region known as the Maranhão Lowlands due to the flood-prone areas.

Collection of fish and recording water abiotic parameters

Abiotic parameters: temperature (°C), pH and salinity were measured *in situ* with the aid of a multi-parameter instrument, before fish capture in riverside lagoons (floodable environment), in the rainy season (feb 2021). In total, 42 specimens were collected, 21 individuals belong to *H. unitaeniatus* and 21 belong to *C. bimaculatum*. Mesh gill net 4 (20 mm), actively operated (drags / sieges), and cast net with mesh 4 (20 mm) were used to catch the fish.

Blood collection and transport

After fish were collected, the specimens were transported alive in 100-L polystyrene boxes added with water and oxygenation equipment. Fish were transported alive to the Aquatic Resources Reproduction Laboratory – LARAQUA – of UEMA, where they were placed in water tanks with constant oxygenation for, at most, twelve hours in order to let them rest. Subsequently, blood samples of the specimens were collected at the Infectious Disease Diagnostic Laboratory of the Agricultural Sciences Center with the aid of 1-mL disposable syringes and 8 x 0.30 mm sterile needles – the gill vein was channeled by introducing a needle at the angle of ± 45°. After the collection procedure, blood samples were placed into sterile tubes at sloped position, at room temperature, to delay coagulation and serum liberation process.

Serological diagnosis

Serum was separated from blood through centrifugation at 2000 rpm, for 10 min, in the Infectious Disease Diagnostic Laboratory of the Agricultural Sciences Center. The serum samples were placed in Eppendorf-type ampoules and stored at -20° C until the time for serological tests. The blood serums were subjected to anti-leptospiral agglutinins against 25 serovars belonging to the *Leptospira* complex, from the bank of University of São Paulo – they were kept in the aforementioned laboratory (Table 1).

The presence of anti-leptospiral agglutinins was observed through the Serum-agglutination microscopic (SAM) technique, according to Faine *et al.* (1999), Vasconcellos *et al.* (1997) and OIE (2021), based on 1/100 dilution (cut-off point). Agglutination of at least 50 % of leptospiras in the microscopic field at 100x magnification was the criterion to classify serums as reactive. Reactive serums were titled under geometric series of four ratio-two dilutions; the title was provided as reciprocal to the greatest dilution presenting agglutination. Descriptive analysis was used to calculate the frequency of anti-leptospiral agglutinins through absolute and relative distribution.

Table 1. Collection of antigens belonging to the *Leptospira* complex that were used in the Serum-agglutination Microscopy (SAM) technique to diagnose the *Leptospira* serovariety circulating in *Cichlasoma bimaculatum* and *Hoplerythrinus unitaeniatus*

Species	Serogroup	Serovarieties
L. interrogans	Australis	Australis
		Bratislava
	Autumalis	Autumnalis
L. kirschneri	Autumalis	Butembo
L. borgpetersenii	Ballum	Castellonis
L. interrogans	Batavia	Bataviae
	Canicola	Canicola
L. borgpetersenii	Calledoni	Whitcombi
L. kirschneri	Cynopteri	Cynopteri
	Gripotyphosa	Gripotyphosa
L. interrogans	Hebdomadis	Hebdomadis
	Icteriohaemorragie	Copenhageni
		Icteriohaemorragiae
L. borgpetersenii	Javanica	Javanica
L. noguchi	Panama	Panama
L. interrogans	Pomona	Pomona
		Pomona variante bubalinos
		Pomona RG
L. interrogans	Pyrogenes	Pyrogenes
L. interrogans	Serjroe	Hardjo Prajitno
L. borgpetersenii		Hardjo Bovis
L. santarosai		Guaricurus
L. santarosai	Shermani	Shermani
L. borgpetersenii	Tarassovi	Tarassovi
L. interrogans	Djasiman	Sentot

RESULTS AND DISCUSSION

The sampled environment showed favorable features for *Leptospira* survival, such as low water speed, pH of 7.31 and temperature of 28.3 °C. According to Correa and Correa (1992), Brasil (1995) and Simões *et al.* (2016), leptospires habitat includes backwater, moistened soil, decomposing organic matter, plants, animals and man. Survival is favored by humidity, slightly warm temperature (28 ºC to 30 ºC) and neutral backwater with slightly moderated pH (7.2 to 7.6).

This is the first study carried out in Maranhão State about serovars of *Leptospira* circulating in fish – results have shown 80,95 % prevalence (n=34/42) of anti-leptospiral agglutinins with titles ranging from 1:100 to 1:400. Esteves *et al.* (2005) carried out a serological study with animals and employees of Uberaba Municipal Zoo and found six samples of *Oreochromis niloticus* (Linnaeus, 1758) (tilapia) reagent to *Leptospira* sp., serovar Canicola. Overall, these studies have evidenced fish importance as *Leptospira* reservoirs and/or hosts, and also point out the need of carrying out further studies focused on diagnosing leptospiral infection in this class of vertebrates.

In total, 100 % (n= 21/21) and 61,90 % (n= 13/21) of the analyzed *H. unitaeniatus* and *C. bimaculatum* serum samples were reactive to *Leptospira*, respectively. The most frequent serovars belonging to the *Leptospira* complex in the analyzed samples were Pomona (14/42; 33, 33 %), Hebdomadis (12/42; 28,59 %), Butembo (10/42; 23, 80 %), Copenhageni (10/42; 23,80 %), Cynopteri (10/42; 23, 80 %), Panama (10/42; 23,80 %), Sentot (10/42; 23,80 %), Bratislava (8/42; 19,04 %), Icteriohaemorragiae (8/42; 19,04 %), Pomona variant Bubalinos (8/42; 19,04 %), Pyrogenes (8/42; 19,04 %), Whitcombi (8/42; 19,04 %), Grippotyphosa (6/42; 14,28 %), Hardjo Prajitno (6/42; 14,28 %), Hardjo Bovis (6/42; 14,28 %), Australis (6/42; 14,28 %), Autumnalis (6/42; 14,28 %), Castellonis (6/42; 14,28 %), Tarassovi (6/42; 14,28 %), Bataviae (4/42; 9, 52 %), Pomona variante Bubalinos (4/42; 9,52 %), Guaricurus (2/42; 4,77 %), Javanica (2/42; 4,77 %) and Shermani (2/42; 4,77 %).

The identification of serovars Pomona (the most frequent one) and Grippotyphosa highlight the importance of pigs (Oliveira and Lima 1996), bred extensively without sanitary control, as source of infection caused by *Leptospira* in fish collected in Ponta Bonita quilombola community. Oliveira & Lima (1996) point out that it is essential identifying this serovar in pigs, since it causes leptospirosis through two types of different infections are observed: (i) when pigs are the ultimate host, and the infection is caused by serovar Pomona; and (ii) when pigs are accidental hosts, and the infection is caused by serovar Grippotyphosa. Also, according to these researchers, serovar Grippotyphosa is often found in rodents who can urinate in and contaminate the environment; they can also be the very source of infection in different animals bred in the same area.

The herein diagnosed serovars: Australis, Bataviae, Bratislava, Copenhageni, Hebdomadis, Grippotyphosa, Guaricurus, Sentot and Shermani are indicative of fish's contact with environments contaminated with *Leptospira* assumingly deriving from synanthropic and wild species. Results in studies conducted in Brazil, and in Maranhão State have shown the high percentage of serovars that affect wild animals, but that are also found in domestic and livestock-interest animals (Silva *et al.* 2010, Silva *et al.* 2012, Coelho *et al.* 2014, Carvalho *et al.* 2015, Paixão *et al.* 2016, Guedelha *et al.* 2019). Opossums, deer, capybaras, turtles and other wild species can act as *Leptospira* reservoirs to other animals, when they find a proper habitat (Castro *et al.* 2008, Silva *et al.* 2010, Rocha *et al.* 2019, Gonçalves *et al.* 2020). Based on such epidemiological aspect, Castro *et al.* (2008) highlighted the importance of a broad antigenic battery composition, since serovars like Shermani, Hebdomadis and Autumnalis, oftentimes are not found in antigenic routine examinations.

The official recommendation for the SAM technique applied to leptospirosis lies on the application of antigens that have at least one representative per serum-group. Thus, it is essential including isolated autochthonous serovars in a country or region, since it would increase the discriminating capacity of the serological technique (Sarmento *et al.* 2012). Based on this official recommendation, the Pomona variant Bubaline was included in the antigenic battery used with 19,04 % (n=4/21) frequency of reagent fish was recorded.

Serovars Hardjo, species *L. interrogans* (Hardjo prajitno) and serovar Hardjo, especies *L. borgpetersenii* (Hardjo tipo bovis) (14,28 %) presented the same frequency in the current study. Identifying these serovars was a way to reinforce the theory introduced by Pellegrin *et al.* (1999),

who have associated the occurrence of these serovarieties, and of their variants, with environmental factors linked to animal husbandry management, such as inappropriate discharge of placental remains, bacterial multiplication in flooded areas, animal watering in contaminated areas and pollution – these situations are similar to those found in the floodable area in the assessed quilombola community. In addition, researchers have reported that bovines are the preferred hosts of serovariety Hardjo (Silva *et al.* 2010, Silva *et al.* 2012).

For serovar Icterohaemorrhagiae, a high frequency was recorded in the sampled fish (19,04 %). This serovar is frequently isolated from rodents and is of great relevance to public health (Brasil 2005). Many wild animals, such as rodents, are adapted to *Leptospiras*, therefore, they do not manifest any of its clinical signs and lesions. They play the role of main leptospirosis reservoirs, since they are healthy carriers of it and host spirochetes in their kidneys – they eliminate these spirochetes in the urine and contaminate the water, the soil and food (Brasil 1995).

The simultaneous breeding of different domestic animal species in the same area can favor leptospirosis occurrence. Another point to be discussed was the presence of co-agglutination in 16 fish, and it involved all the 25 tested serovars and different combinations to the highest frequency of Pomona/Pomona variant Bubalins (42,86 %) and Hebdomadis/Grippotyphosa (25,58 %); Copenhageni/Pyrogenes (19,05 %) reaction types. Base on Juliano *et al.* (2000), the herein identified co-agglutination can be explained by the concomitant infection of several *Leptospira* serovars, or by crossed reactions between serovars belonging to the same serum-group.

Based on the present results, fish belonging to species *H. unitaeniatus* and *C. bimaculatum* living in a quilombola area in Maranhão State are exposed to different *Leptospira* sp. serovarieties. It is also necessary investigating fish infection sources, as well as applying appropriate measures to prevent leptospirosis in this geographic area.

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PARTICIPATION OF THE AUTHORS

IAP, LRS, JSM, GSJ, VMSM, MVRS, DCB and NPCB collected samples in the field; IAP, LRS, VMSM, MVRS, DCB, HPS and NPCB carried out blood collections and serological analyzes for the diagnosis of Leptospira sp.; IAP, DCB, VCSC, HPS, HMP and NPCB contributed to the analysis, data interpretation and writing of the article.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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