Anisakis spp. IN TRAHIRAS (*Hoplias lacerdae*) FROM TELES PIRES RIVER AFFLUENT, MATO GROSSO STATE, BRAZIL

Anisakis spp. EM TRAÍRÕES (Hoplias lacerdae) DE AFLUENTE DO RIO TELES PIRES, ESTADO DO MATO GROSSO, BRASIL

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SUMMARY

Anisakiasis is a parasitic disease caused by the consumption of raw or undercooked fish containing the third larval stage of anisakid nematodes. Safety in fish consumption from extractive fisheries is a challenge for public health authorities. This study presents the hematological analysis, necroscopic and histopathological examination of three *Hoplias lacerdae* parasitized by *Anisakis* spp. captured in an affluent of the Teles Pires River, in the municipality of Sinop, State of Mato Grosso, Brazil. At necropsy, intense parasitism was observed, characterized by free and adherent anisakid larvae in organs and mesentery. In the histopathological study, there were nephrotic alterations and hepatopathies, as well as the first report of the presence of Anisakis cyst adhered in the myocardium of *Hoplias lacerdae*. There was a correlation between the increase in the number of parasites and the circulating leukocytes, influenced by the number of neutrophils and eosinophils. However, other studies should be designed to elucidate the pathophysiological and epidemiological changes in *Hoplias lacerdae* by the parasitism of *Anisakis* spp., due to its socioeconomic importance, as well as the potential risk to public health.

KEY-WORDS: Amazon Basin. Anisakiasis. Public health Teleost fish. Zoonosis.

RESUMO

A anisaquíase é uma parasitose causada pelo consumo de peixes crus ou malcozidos contendo o terceiro estágio larval de nematoides anisaquídeos. A segurança no consumo de pescado proveniente da pesca extrativista representa um desafio para as autoridades em saúde pública. Este estudo apresenta a análise hematológica, exame necroscópico e histopatológico de três *Hoplias lacerdae* parasitadas por anisaquídeos capturadas em um afluente do rio Teles Pires, no município de Sinop, Estado do Mato Grosso, Brasil. Com a abertura da cavidade celomática dos animais observou-se intenso parasitismo, caracterizado por larvas de anisaquídeos, livres e aderidas, em órgãos e mesentério. Fragmentos de órgãos foram avaliados microscopicamente, na qual foi constatado nefropatia, hepatopatia e cardiopatia. Salienta-se ainda o primeiro registro da presença de cisto parasitário de *Anisakis* spp. aderido em miocárdio de *Hoplias lacerdae*. Observou-se correlação entre o aumento do número de parasitos ao aumento do número de leucócitos com marcado aumento de neutrófilos e eosinófilos. Diante do exposto, este trabalho vê a necessidade de futuro estudo minucioso para a real caracterização fisiopatológica e epidemiológica da anisaquiose em trairões do rio Teles Pires, visto sua importância socioeconômica na região, como também grande risco para saúde pública.

PALAVRAS-CHAVE: Bacia Amazônica. Anisaquíase. Saúde pública. Peixe teleósteo. Zoonose.

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INTRODUCTION

The catch of fish in the Amazon basin for human consumption is very common for the riverside communities, due to the socioeconomic-cultural characteristics of this population in this area (OLIVEIRA et al., 2010). Affluent belonging to the Amazon Basin, the Teles Pires River is an important source of income, food and recreation for the population of the municipality of Sinop, Mato Grosso (GALLARDO et al., 2017; SMERMAN, 2007).

Among the species that are part of the Brazilian ichthyofauna, which is appreciated by the population, *Hoplias* spp., popularly known as trahira, belong to the order Characiformes (Erythrinidae family), present piscivorous habit, fact which favor the infection by endoparasites, such as nematodes anisakids (RODRIGUES et al, 2017).

Anisakiasis is a parasitic disease caused by the consumption of raw or undercooked fish containing the third larval stage of anisakid nematodes, more commonly belonging to the genera Anisakis and Pseudoterranova, or more rarely by Contracaecum (KASSAI, 1988). They present cosmopolitan distribution and about two thousand people are affected by the disease each year (WOOTTEN, 2012).

The purchase of safe fish for consumption is crucial. According to Lopes et al. (2016), most fish consumers do not care about their origin (whether from extractive or aquaculture), and about 35% do not know about the practice of raising these animals for human consumption. Based on the public health importance and the need to understand the pathophysiological changes of anisakiasis to fish, this study evaluated the pathological changes observed in three trahiras with *Anisakis* spp. captured in a Teles Pires River affluent in the municipality of Sinop, Mato Grosso.

CASE REPORT

This study comes from the monitoring of fishermen living in the municipality of Sinop, State of Mato Grosso, Brazil. The individual fishing with spinneret and artificial bait was conducted on an affluent of the Teles Pires River, located geographically at latitude 11 ° 56'47.2 "S and longitude 55 ° 38'43.0" W. And three specimens of trahira, *Hoplias lacerdae*, were caught (Figure 1).

Figure 1 - Specimen of *Hoplias lacerdae* captured in aaffluent of the Teles Pires River, Sinop, Mato Grosso, Brazil.

Immediately after capture, to attenuate handling stress, the fish were anesthetized by immersion in 1: 10,000 (v: v) aqueous benzocaine solution, pre-diluted in 98ø alcohol (0.1 g / mL) and completing the volume for one liter of water (WEDEMEYER, 1970). Samples of blood were collected by caudal vessel puncture using pre-heparinized disposable syringes and needles (HEMOFOL®). An aliquot of whole blood was used to measure the glycemic values using the Accu-Chek® monitor Performa model (MORAES et al., 2018). After blood collection, the fish were euthanized by anesthetizing by benzocaine at a ratio of 1: 500 (v:v) and submitted to bleeding.

The biological samples (blood and fish) were stored at refrigeration temperature until the analyzes were carried out at the UFMT / Sinop Animal Pathology Laboratory. Hematological values were obtained by counting erythrocytes, leukocytes and thrombocytes in a Neubauer chamber, using the solution of Natt and Herrick (1952) and following the methodology of Prado et al. (2018). For differentiation and determination of the proportion of thrombocytes and leukocytes in the blood, blood stains stained with May-Grunwald-Giemsa-Wrigth (BELO et al., 2014) were performed. After establishing the percentage of each cell type of interest in 300 cells counted.

Considering that the fish presented considerable parasitism, it was decided to count parasite numbers and to study the morphometric changes in the heart, liver, spleen, intestine, kidney and renal cranial portion, as well as to determinate the somatic index by relating the proportion between organ weight (OW) and body weight (BW) expressed as: Organ Somatic index (I) = (OW X 100) / BW, according to Weibel et al. (1969).

For histopathological evaluation, a fragment of each organ was removed from the lesion site and fixed in 10% buffered formaldehyde solution, after 24 hours, the organs were transferred to 70% alcohol and then, dehydrated, diaphanized, paraffin embedded 5μm thick slices to be stained with hematoxylin and eosin (HE) and periodic acid-Schiff (PAS).

The parasites referred to the Laboratory of Parasitology of the UFMT / Sinop were compatible with Anisakis nematodes, according to morphological analysis, described using the dichotomous key proposed by Tavares and Luque (2006).

The hematological and anatomopathological information of each animal are detailed in Table 1. Although the results came from a small sample (three fish), they cannot be neglected, since the specimens were randomly captured in a natural environment.

Table 1 - Hematological, anatomopathological and glycemic values of *Hoplias lacerdae* captured in the affluent of the Teles Pires river, in the municipality of Sinop, Mato Grosso, Brazil.

Trahira		**Female 03**	**Male 04**	**Male 05**
Parasite	**N°**	14	12	24
Weight[1]	**g**	714	274	536
Heart		0,0811	0,0657	0,0935
Liver		1,3050	1,5489	1,2782
Spleen		0,0419	0,0518	0,0427
Gut	**%**[5]	5,6730	3,2285	2,8759
Kidney		0,2660	0,2077	0,3168
Cranial kidney[2]		0,1824	0,2606	0,0558
HT[3]		27,7	25,7	27,9
Blood glucose	**mg/dL**	20	15	33
Erythrocytes		1445000	1045000	1285000
Thrombocytes		20727	13394	20011
Neutrophils		1244	824	3638
Eosinophils	**N°/µL**	553	412	1040
Lymphocytes		51265	19164	19232
Monocytes		1658	206	2079
GSC[4]		553	0	0

[1]Body weight of trahiras; [2]Renal cranial portion; [3] Hematocrit; [4] Granulocytic special cells; [5] Percentage by organ weight / animal weight * 100 (somatic index).

In this sense, an exploratory analysis of the data was performed to identify and investigate patterns among the variables, through the analysis of main components (JOLIFFE & MORGAN, 1992; HAIR et al., 2009). According to this mathematical model it was possible to summarize interrelationships of the variables, associating in a particular way in each of the three copies of the group *H. lacerdae* (Figure 2).

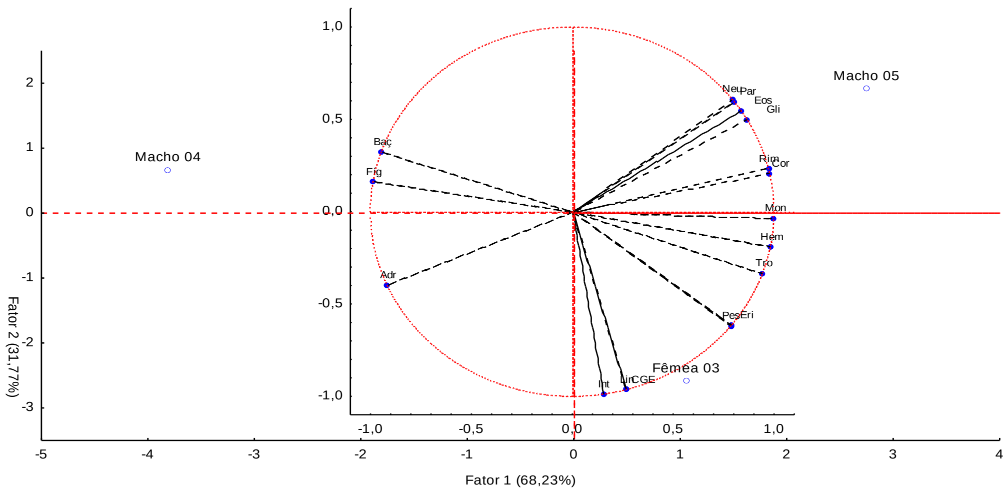


Figure 2 - Patterns of variables (Weight = Pes, Erythrocytes = Eri, Hematocrit = Hem, Blood Glucose = Gly, Heart = Color, Liver = Spleen, Spleen, Intestine = Int, Kidney, Adrenal = Adr, Parasite = Pair, Thrombocyte = Tro, Neutophil = Neu, Eosinophil = Eos, Lymphocyte = Lin, Monocyte = Mon, Granulocytic Special Cell = GSC) in relation to the sample units (Trahira: female 03, male 04, male 05) according to principal component analysis (component principal 1 = factor 1 and principal component 2 = factor 2).

In order to better clarify the degree of interrelation between variables, a linear correlation analysis was performed according to Pearson's linear correlation coefficient test (Table 2). To assure such a correlation test, the assumption of the analysis that constituted a P value> 0.05 according to the Shapiro-Wilk test was evaluated.

Table 2 - Anisakiosis in trahiras (*Hoplias lacerdae*) variables that obtained the significant Pearson linear correlation.

CORRELATION **P-value** matrix	**Neutrophil**	**Eosinophil**	**Glucose**	**kidney**	**Hematocrit**	**Thrombocyte**	**Erythrocyte**
Parasite	$0,011^{0,99984}$	$0,038^{0,99826}$	$0,074^{0,99325}$	$0,259^{0,91833}$	$0,531^{0,67235}$	$0,624^{0,55729}$	$0,827^{0,26783}$
Neutrophil		$0,049^{0,99707}$	$0,085^{0,99105}$	$0,270^{0,91121}$	$0,542^{0,65921}$	$0,635^{0,54258}$	$0,839^{0,25081}$
Eosinophil			$0,036^{0,99836}$	$0,222^{0,94007}$	$0,493^{0,71483}$	$0,586^{0,60528}$	$0,790^{0,32418}$
Heart				$0,015^{0,99973}$	$0,257^{0,91978}$	$0,350^{0,85276}$	$0,554^{0,64519}$
Liver					$0,021^{-0,99947}$	$0,114^{-0,98405}$	$0,318^{-0,87814}$
Spleen						$0,008^{-0,99993}$	$0,211^{-0,94547}$
Weight							$0,003^{0,99999}$

At necroscopy, the opening of the coelomic cavity of all specimens revealed the presence of free parasites in the cavity and adhered to intestinal serosa, abdominal fat, musculature and liver (Figure 3). Two animals presented rounded structures with yellowish hepatic parenchyma compatible with cysts, noting the presence of larvae in their interior. On the myocardium tissue one of the specimens presented encapsulate structure, translucent with the presence of nematode inside. In the musculature, it was also possible to observe the presence of encapsulated parasites. In the female abdominal, it was evident the increase of the ovaries that showed a high number of oocytes.

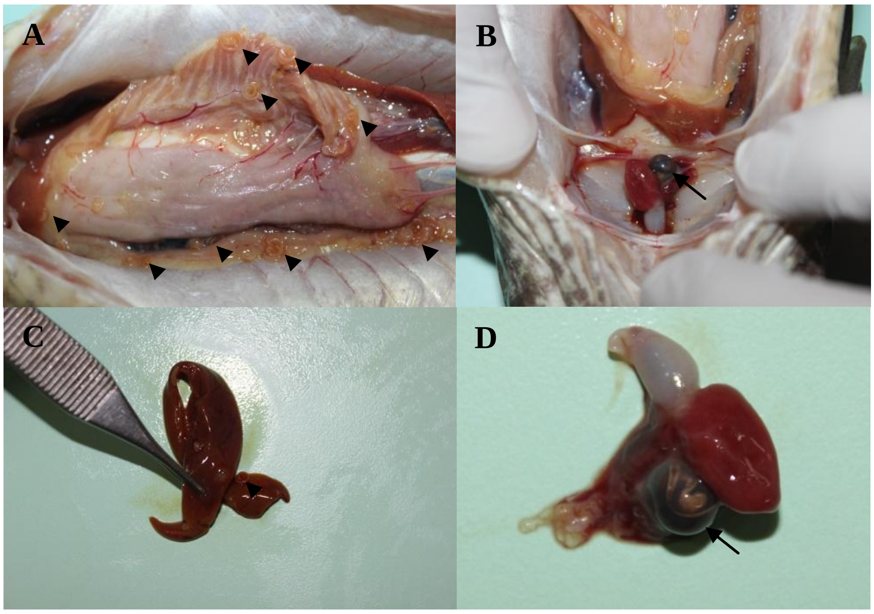
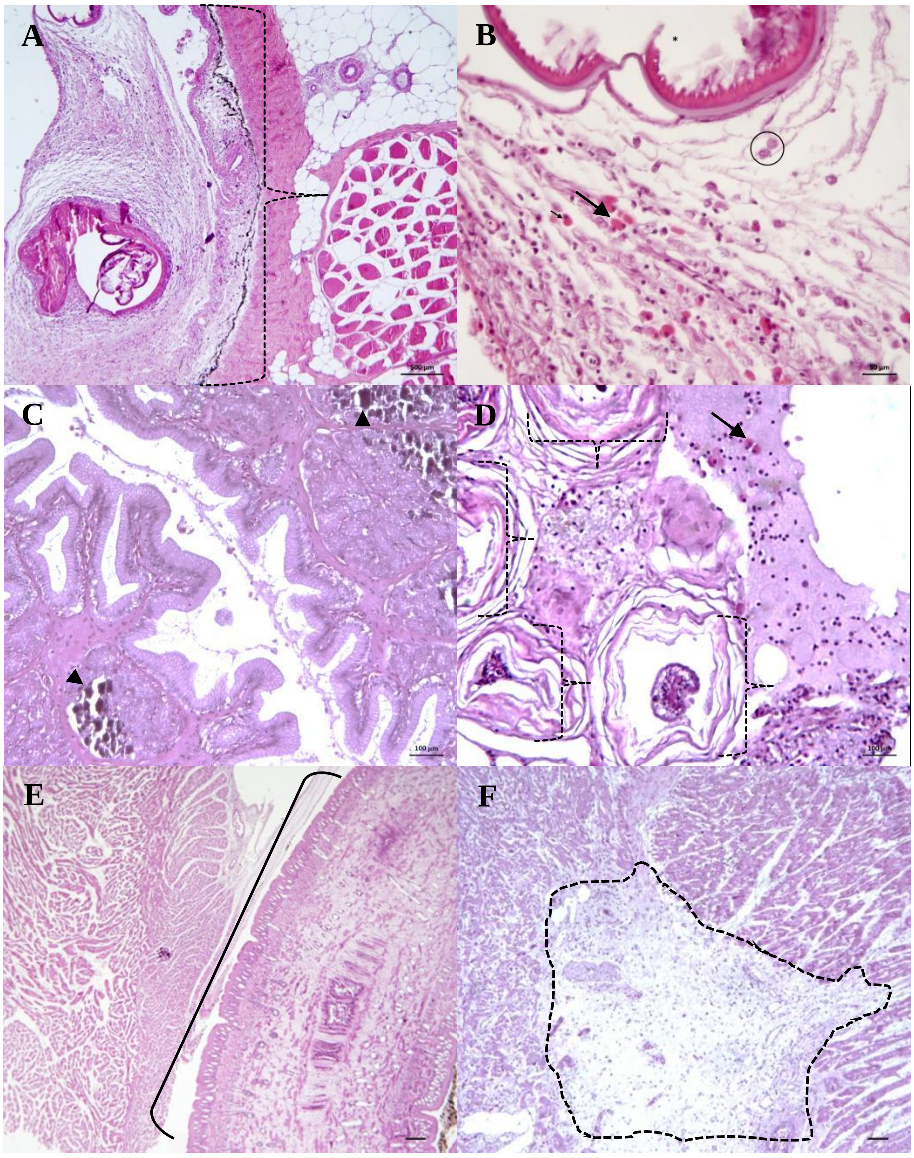


Figure 3 - Endoparasite larvae (arrowhead) in a cellomatic cavity (A and B), with cyst (arrow) in liver (C) and heart (D) of *Hoplias lacerdae* captured in the affluent of the Teles Pires, Sinop, Mato Grosso, Brazil.

According to the two mathematical models presented in Figure 2 and Table 2, positive correlations were observed in the male 05 between the high number of parasites at the number of neutrophils and eosinophils, the latter also positive correlation of glycemic levels. In addition, the same animal had a positive correlation between the cardiac and renal index, where both organs presented increased. In the female, there were also acceptable positive correlations between high numbers of erythrocytes and higher body weight. However, it is associated with acceptable negative correlations, in which there is a higher percentage of hematocrit and a high number of thrombocytes, respectively correlated with a lower hepatic and splenic index.

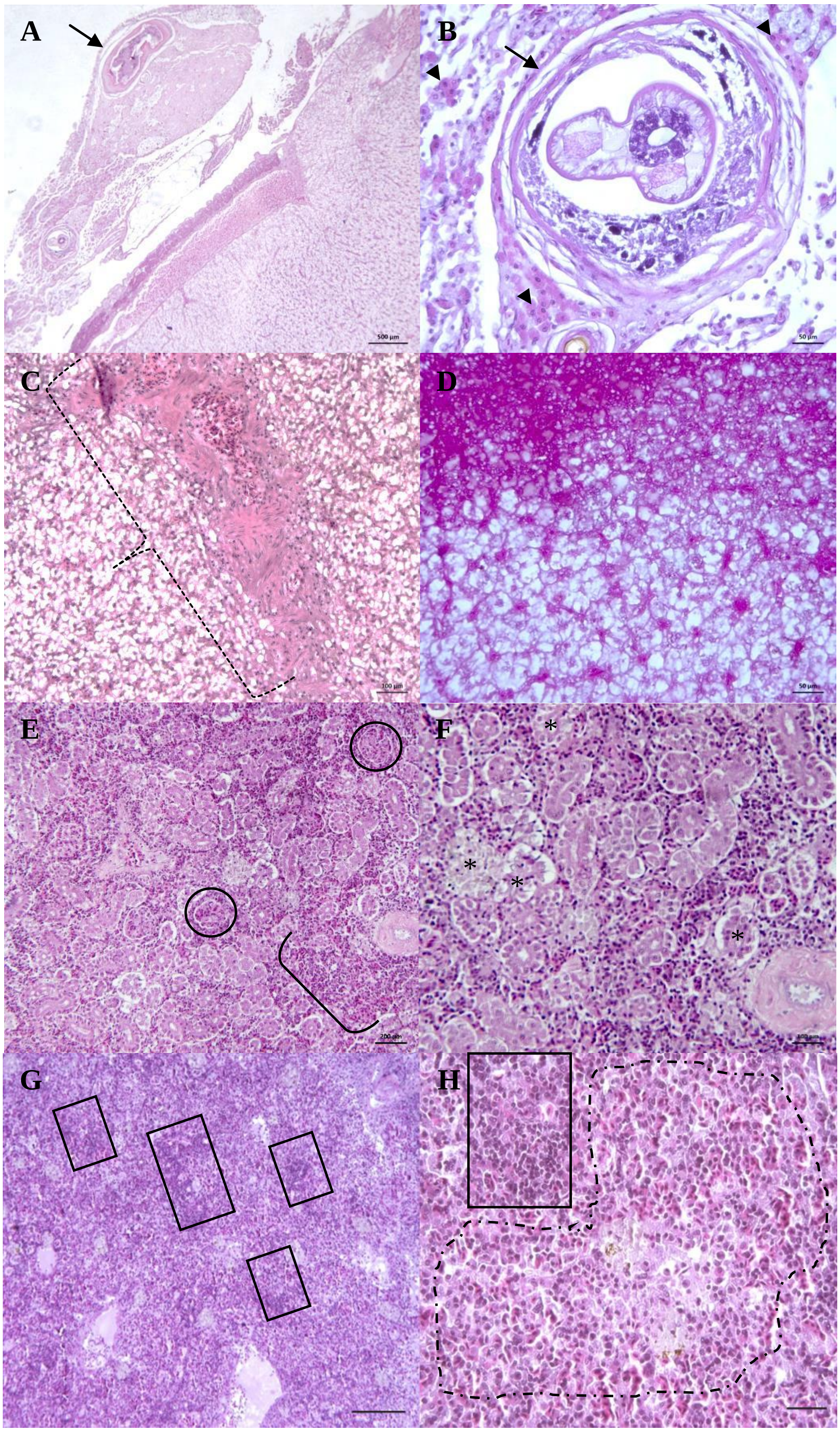
In the skeletal muscle tissue (Figure 4), we observed an intense inflammatory infiltrate, with emphasis on eosinophilic granulocytic cells around the cystic capsule and the parasite was in the center. In the small intestine, calcification was observed in crypts, as a well as parasitic cystic form was observed in the surface of the serous with moderate and suppurative inflammatory infiltrate.



Figures 4 - Histopathological photomicrographs of anisakiasis in trahiras. Muscle (A and B), parasitic cyst (key) surrounded by inflammatory infiltrate, macrophages (circle) and eosinophil (arrow). Small intestine (C), calcification (arrowhead) in crypt and parasitic cysts with inflammatory infiltrate in serosa (D). Heart (E and F), parasite (bracket) and extensive fibrotic region (dashed area). Hematoxylin and eosin (HE) staining.

The heart presented a parasitic cyst below the epicardium in the atrioventricular region, formed by a thin capsule of connective tissue that circumscribed the endoparasite. A discrete inflammatory infiltrate and an extensive fibrotic region were also observed.

In hepatic tissue (Figure 5), diffuse vacuolation was observed in hepatocytes and two animals presented parasitic cysts, surrounded by capsule of dense connective tissue, with intense inflammatory infiltrate with predominance of foamy macrophages around the capsule and discrete multifocal congestion. More severe lesions were observed in the female, where there was also intense vacuolization in hepatocytes associated with moderate fibrosis and congestion. In the special staining by periodic acid-Schiff, a positive reaction was observed for glycogen with evident accumulation of cytoplasmic lipids, characterizing alterations of fibrotic steatohepatitis.



Figures 5 - Histopathological photomicrographs of anisakiasis in trahiras. Liver (A, B, C and D), parasitic cyst (arrow) surrounded by intense marginal inflammatory infiltrate (arrowhead); Female with hepatic fibrosis (key) and positive glycogen reaction (area with violet color) with evident accumulation of cytoplasmic lipids (area with vacuole); Kidney (E and F), female with renal interstitial multifocal lymphocytic inflammatory infiltrate (arrow) and expansion of glomerular mesangial matrix with decreased Bowman space (circle) associated with tubular hydropic degeneration (asterisk). Spleen (G and H), female with evident splenic germinal centers (rectangle) and few red blood cells in the parenchyma (dashed area). Hematoxylin and eosin staining (A, B, C, E, F, G and H) and PAS- periodic acid-Schiff staining (D).

Changes in renal tissue was also observed in the female, characterized by collapsing proliferative glomerulonephritis, since mesangial matrix expansion was observed, resulting in a decrease in Bowman's space, associated with tubular hydropic degeneration and interstitial multifocal lymphocytic inflammatory infiltrate.

In the female splenic tissue, germinal centers with few erythrocytes in the parenchyma were observed in relation to the white pulp, corroborating the morphometric study and confirming the splenic contraction.

DISCUSSION

The trahira has been drawing attention in the scientific community in the area of public health, since it is a potential host in harboring zoonotic parasites (REIS et al., 2017). Among the worrisome emerging zoonotic diseases, fish-borne outbreaks are highlighted the *Anisakis* spp. (BUCHMANN & MEHRDANA, 2016), their larval form are easily found in the mesentery of parasitized fish and usually migrate to the muscles after the host death (CIPRIANI, 2016).

It was possible to observe the occurrence of free and encapsulated larvae in the cavity and adhered to organs of all studied fish, also reported by Rodrigues et al. (2017) and is justified by the migration that occurs after host death (WOOTTEN, 2012). According to Eiras and Rego (1989), the organism usually reacts by encapsulating the parasite, and there is also cellular hyperplasia, forming nodules which are easily visualized, corroborating with the macroscopic findings in this report.

The involvement of the hepatic parenchyma by cysts with presence of larvae in their interior reported in this study, corroborate with macroscopic findings of Barros et al. (2007), where they verified the presence of Anisakidae family nematodes in the abdominal cavity and even infecting the hepatic tissue.

This is the first report of the presence of *Anisakis* spp. larvae in the heart of trahira. This fact is related to high parasitic infection and consequent installation in an organ responsible for blood circulation. In addition, the same animal was associated with increased heart and kidney somatic index. Cardiorenal interaction in congestive heart failure has been increasingly recognized in medicine (SARNAK, 2014; DAMMAN & TESTANI, 2015).

It was established that cardiac performance and renal function are closely interconnected and dysfunction of one organ often leads to impairment of the function of the other, called cardiorenal syndrome (RONCO et al., 2008). In this context, it is possible to attribute the simple parasite accommodation near the atrioventricular cardiac region, associated with an inflammatory lesion on the cardiac wall, observed microscopically, caused congestive heart failure, which consequently influenced the degenerative changes, both in the tubules and in the renal glomeruli in our study. In that it made possible the development of the chronic cardiorenal syndrome of type II described by Ronco et al., 2008.

Trahiras with higher number of parasites load have a high neutrophil and eosinophil counts. This leukocytosis may be associated with lesions caused by the parasite presented in the histopathological evaluation. These lesions were also observed by Noguera et al. (2009), where the authors report an intense inflammatory infiltrate with predominance of eosinophils and melanomacrophages, necrosis and hemorrhage in the rectum and anus associated with anisakiasis.

According to Wu et al. (2011) and Odegaard and Chawla, (2013), eosinophils can participate of important mechanism of glucose homeostasis by paracrine processe in conjunction with macrophages and adipose tissue. This fact can be the hypothesis to justify the elevated blood eosinophil counts with higher glycemic levels observed in trahiras from this study.

The high weight of the trahira female was associated with higher erythrocyte counts. It is very probable that this association is related to the reproductive phase, since at the moment of the anatomopathological evaluation there was a remarkable number of oocytes in both ovaries.

According to Egawa et al. (2003), granulosa cells in the preovulatory follicles are observed an increase of the expression of proteins denominated of EphB1. Results of Anselmo et al. (2016) and Suenobu et al. (2002) demonstrated that EphB1 and EphB4 receptors are respectively an important signaling pathway involved in erythropoiesis. In zebrafish, Zhang et al. (2016) reveal an important role for EphB4 and EfnB2 signaling in embryonic development. Thus, this pathway can be the hypothesis to justify the increased number in the female erythrocytes counts.

Due to the female reproductive period, particularly in the state of oogenesis, associated with a high energetic demand to interrupt and repair constant parasitic lesions, it may be one of the strong reasons for the development of fibrotic steatohepatitis, since vitellogenesis in fish, represents a major catabolic effort for the maternal organism, directly involves hepatic function by stimulating estradiol from follicular cells and stimulating the synthesis of vitellogenin, favoring absorption by the vitellogenic follicles (LUBZENS et al., 2010). In this sense, studies indicate that changes in hormone activity in the gestational period cause an increase in serum free fatty acids, making it possible to lipotoxicity in hepatocytes, culminating in the activation of inflammatory pathways and cellular necrosis to hepatic insufficiency (LIU et al. al., 2017).

The increased hematocrit levels observed, correlated with the lower hepatic index, may be due to the development of fibrotic steatohepatitis. Li et al. (2014) found that elevated hematocrit levels were strongly associated with fibrotic steatohepatitis, where they believed to be an important marker in the study of non-alcoholic fatty liver disease. A possible plausible explanation for the increase in the number of circulating erythrocytes, be it sinusoidal distortion caused by hepatocellular steatosis, reducing intra-sinusoidal volume, and microvascular blood flow, reflecting some degree of hypoxia (SEIFALIAN et al., 1999), stimulating physiologically erythropoiesis, which leads to increased levels of hematocrit (RICHARDSON et al., 2008).

The female had a higher thrombocyte count correlated with the lower splenic index. This fact can be explained by the migration of thrombocytes into the bloodstream in endoparasite infection in fish (BELO et al., 2013), whose assigned function is hemostasis and homeostasis, conferring on the splenic tissue the important function in producing cells of this lineage (TAVARES-DIAS & MORAES, 2004).

FINAL CONSIDERATION

It was possible to confirm the occurrence of anisakiasis in trahiras from the river Teles Pires affluent, in the municipality of Sinop, MT. The disease is characterized by parasitic cysts in organs, promoting inflammatory and degenerative lesions in liver, heart and kidney, which have repercussions with hematological alterations. In view of the above, there is a need for a detailed study for the pathophysiological and epidemiological characterization of trahira anisakiasis in this River, considering the socioeconomic importance of the extractive fishing for the region, as well as the great risk of anisakiasis for public health.

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