

ENDOPARASITES OF *Gymnotus* sp. (GYMNOTIFORMES: GYMNOTIDAE) FROM COMMERCIAL BAITFISH FARMING IN PANTANAL BASIN, CENTRAL BRAZIL

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ABSTRACT

This study evaluated the endohelminthic parasites of native baitfish *Gymnotus* spp. collected from different baitfish farming in the Pantanal basin, State of Mato Grosso do Sul, Central Brazil. From 99 fish collected for parasitological analysis, all of them were found to be parasitized by at least 1 parasite per host. A total of 4504 parasites were collected comprising the following taxa: Digenea, Nematoda, Pentastomida and Acanthocephala. *Rondonia rondoni* and *Goezia* sp. as well as *Alofia* sp. Were registered for the first time in farmed *Gymnotus* spp. The parasite species diversity in the host is discussed in this study.

Key words: biodiversity; fish parasites; gymnotiformes; neotropics.

ENDOPARASITOS DE *Gymnotus* sp. (GYMNOTIFORMES: GYMNOTIDAE) DE ISQUEIROS COMERCIAIS NA BACIA DO PANTANAL, BRASIL CENTRAL

RESUMO

Este estudo avaliou os parasitos endohelmintos do peixe nativo *Gymnotus* spp. coletado de diferentes isqueiros comerciais na bacia do Pantanal, Mato Grosso do Sul, Brasil Central. De 99 peixes coletados para análise parasitológica, todos estavam parasitados por pelo menos um parasito por hospedeiro. Um total de 4504 parasitos foram coletados compreendendo os seguintes táxons: Digenea, Nematoda, Pentastomida e Acanthocephala. *Rondonia rondoni* e *Goezia* sp. bem como *Alofia* sp. foram registrados pela primeira vez em *Gymnotus* spp. A diversidade de espécies de parasitos no hospedeiro é discutida neste estudo.

Palavras-chave: biodiversidade; parasitos peixes; gymnotiformes; neotropical.

INTRODUCTION

Gymnotus spp. commonly known as “swordfish”, “banded knifefish”, “tuvira”, “sarapó” or “itui”, depending on the geographical location, is widely distributed in South America and stands out as an important baitfish commercialized in the Pantanal Sul matogrossense, a region in which they are part of the feeding habit of piscivorous fish like “dourado” (*Salminus brasiliensis*) and “surubim” (*Pseudoplatystoma* spp.), and are appreciated by sports fishermen (PEREIRA and RESENDE, 2006) as bait.

Small fish like *Gymnotus* spp., plays an important ecological role, because the life cycle of the parasites they can act as intermediate and/or paratenic hosts, are food for other fish species and several groups of animals, which are considered definitive hosts (ISAAC *et al.*, 2004). Previous studies have shown high wealth endoparasites (16 species) in *Gymnotus* spp. collected in natural environments (ISAAC *et al.*, 2004; TAKEMOTO *et al.*, 2009; LUQUE *et al.*, 2011). In considering the rapid development of freshwater aquaculture, studies on endoparasite fauna of farmed fish have not gained special attention nor accompanied the burgeoning of continental fish farming (LIZAMA *et al.*, 2007).

No records were found on endoparasites living in baitfish under farming conditions. Parasitism at a high level can cause fish mortality and consequently, important economic losses to the local population have affected income (ISHIKAWA *et al.*, 2014). A deeper analysis of the maintenance of local or even adult specimens should be performed in order to assess the health of fish being marketed, due to the importance of live bait as a vector of pathogens to piscivorous fish aimed for human consumption. This study evaluated the endohelminthic fauna of *Gymnotus* spp. collected from different baitfish farmed in the Pantanal Sul matogrossense and presents new data on their diversity and host occurrence under farming conditions.

METHODS

A total of 99 fish (67.24 ± 33.50 g in weight and 27.72 ± 4.49 cm total length) were randomly collected in four different fish farms that keep the fish under similar conditions located in the Pantanal basin, State of Mato Grosso do Sul, Brazil. In all fish farms, the animals were kept in approximately 2000 L cement tanks and fed with crushed sardines, termites, dead gymnotids or ground beef. Samples were collected according to the following locations and number of fish sampled (n): fish farm A ($19^{\circ}00'25.78''$ S, $57^{\circ}39'04.63''$ W, n = 21), fish farm B ($21^{\circ}55'44.35''$ S $54^{\circ}34'54.98''$ W, n = 43), fish farm C ($21^{\circ}00'30.92''$ S $57^{\circ}16'51.51''$ W, n = 20), and fish farm D ($22^{\circ}08'06.04''$ S $53^{\circ}37'20:58''$ W, n = 15).

Fish were transported to the *Embrapa Agropecuária Oeste – Aquaculture Laboratory* in plastic bags with oxygen. After that, they were euthanized by deep anaesthesia in clove oil solution (150 mg L^{-1}) in accordance with procedures approved by the Ethics Committee of Animal Use n.º005/2011-CEUA/UFMG. For parasitological examination, the visceral cavity was opened for macroscopic observation to detect possible parasites. Internal organs as heart, liver, spleen, swim bladder, gonads, stomach, intestines, visceral cavity and muscle was removed and examined in order to collect endoparasites. The parasite specimens were collected, fixed, quantified and stained (EIRAS *et al.*, 2006), for identification according MORAVEC (1998) and THATCHER (2006). Prevalence, mean intensity and mean abundance of infection were calculated (BUSH *et al.*, 1997).

RESULTS AND DISCUSSION

All examined fish were parasitized by 4504 specimens of parasites and by at least one parasite per host. A total of 18 parasite species were observed belonging to the taxa Digenea, Nematoda, Acanthocephala and Pentastomida. Larvae of *Tylodelphys* sp. and *Diplostomum* sp. Henle, 1833, were observed in the celomatic cavity and *Clinostomum* sp. Leidy, 1856 (Clinostomidae) encysted in the skeletal muscle (Table 1). The most diversified were the nematodes comprised of type I, II and III larvae of *Contracaecum* Railliet and Henry, 1912 (Anisakidae), *Brevimulticaecum* sp. Mozgovoy, in Skrjabin, Shikhobalova, and Mozgovoy, 1951 (Heterocheilidae), *Eustrongylides* sp. Jägerskiöld, 1909 (Dioctophymatidae), *Goezia* sp. Zeder, 1800 (Raphidascarididae), *Rondonia rondoni* Travassos,

1920 (Atractidae) and two unidentified anisakid parasites. This study reports the first occurrence of *Goezia* sp. and *R. rondoni* in *Gymnotus* sp. in Brazil.

The highest prevalence was reported for *Contracaecum* sp. larvae type 1 and *Brevimulticaecum* sp. parasitising the celomic cavity and liver (Table 1), followed by *Eustrongylides* sp. in muscle. The acanthocephalan *Quadrigyrus* sp. and *Pomphorhynchus* sp. were also found in the intestine. Pentastomids, *Sebekia* sp. and *Alofia* sp. were observed in both spleen and mesentery. Of all the parasites observed, an increased prevalence (P) and mean abundance (MA) was found for *Tylodelphys* sp. (P = 56.57% MA = 36.48), followed by larvae of *Contracaecum* sp. type 1 (P = 36.36% MA = 10.42) and *Brevimulticaecum* sp. (P = 32.32% MA = 6.75). The highest average intensity of infection was (20.64) *Tylodelphys* sp. (Table 1). Based on the results it can be seen that the nematodes were the most prevalent (86.9%), followed by digeneans (81.8%), pentastomids (6.1%) and acanthocephalans (3%).

The parasite community of *Gymnotus* spp. in this study showed great parasite diversity in which nine species of nematodes, three digeneans, three acanthocephalans and two pentastomids were reported. The species diversity can be assigned to the fish that were collected in nature, which exhibit higher diversity (ISAAC *et al.*, 2004). Therefore, when they were maintained in conditions of captivity, an increase in the parasitic infection contributed to the transmission of parasites. This may also explain the fact that most of the parasites were found in larval stage, indicating an early infection. In addition, the wealth of species could be strongly associated with eating habits (ADRIANO *et al.*, 2012).

Digeneans are endohelminthic parasites with complex life cycle generally involving two hosts. They can be found parasitising several internal organs in definitive hosts (THATCHER, 2006; TAKEMOTO *et al.*, 2009) or as encysted metacercariae (BACHMANN *et al.*, 2007; SANTOS *et al.*, 2012). In the present study, metacercariae of *Tylodelphys* sp. and *Diplostomum* sp. were observed in the celomatic cavity and *Clinostomum* sp. in the muscle. In contrast to what was found here, *Clinostomum* sp. have related in the gonads and mesentery (ISAAC *et al.*, 2004). This parasite presents wide geographic distribution, a wide amplitude of hosts (CAFFARA *et al.*, 2011) and low host specificity (SERENO-URIBE *et al.*, 2013).

Moreover, human infections were also reported showing its zoonotic potential (CHUNG *et al.*, 1995; SHIRAI *et al.*, 1998; PARK *et al.*, 2009). In a study, analysing the composition of infracommunity structure in *Gymnotus* spp. from the river, a relationship was observed between the prevalence of *Tylodelphys* sp. and reproductive period of *Gymnotus* spp. (ISAAC *et al.*, 2004). It could be inferred that the host susceptibility is strongly related with hormonal and behavioural alterations as a result of stressful conditions in that period.

Larvae of *Diplostomum* sp. are opportunistic parasites registered in several fish species and amphibians (MACHADO *et al.*, 2005; LOCKE *et al.*, 2010; RELLSTAB *et al.*, 2011). Fish are an important vector for the parasite transmission to the final host. The cercariae emerge from the intermediate host (LOCKE *et al.*, 2010), and the feed habit of *Gymnotus* spp. including benthic organisms, may have favoured the parasitism by *Diplostomum* sp.

Table 1. Parasitological indices and site of infection of endohelminthic parasites in *Gymnotus* spp. examined from baitfish farming in the Pantanal basin of Mato Grosso do Sul.

Parasites	EF/PF	P	MI	MA	Site of infection
DIGenea					
<i>Diplostomum</i> sp. ^L	99/06	6.06	0.38 ± 31.99(1-215)	6.33	Celomatic cavity
<i>Clinostomum</i> sp. ^L	99/17	17.17	2.39 ± 9.01(1-71)	13.94	External muscle
<i>Tylodelphylus</i> sp. ^L	99/56	56.57	20.64 ± 42.86(1-325)	36.48	Celomatic cavity
Unidentified	99/01	101	0.01 ± 0.10(1-1)	1.00	Stomach
NEMATODA					
<i>Brevimulticaecum</i> sp. ^L	99/32	32.32	2.18 ± 4.50(1-25)	6.75	Liver/Celomatic cavity
<i>Contracaecum</i> type I ^L	99/36	36.36	3.79 ± 7.44(1-5)	10.42	Liver/Celomatic cavity
<i>Contracaecum</i> type II ^L	99/06	6.06	0.17 ± 0.74(1-5)	2.83	Liver/Celomatic cavity
<i>Contracaecum</i> type III ^L	99/05	5.05	0.33 ± 1.84(1-6)	6.60	Liver/Celomatic cavity
<i>Eustrongylides</i> sp. ^L	99/11	11.11	0.95 ± 3.77(1-22)	8.55	Muscle
<i>Goezia</i> sp.	99/03	3.03	0.11 ± 0.73(1-6)	3.67	Intestine/stomach
Anisakidae ^L	99/11	11.11	0.44 ± 1.54(1-9)	4.00	Liver/Celomatic cavity
<i>Rondonia rondoni</i>	99/01	1.01	0.29 ± 2.91(1-29)	29.00	Intestine
Unidentified	99/06	6.06	0.38 ± 1.72(1-11)	6.33	Intestine/stomach
ACANTHOCEPHALA					
<i>Pomphorhynchus</i> sp. ^L	99/01	1.01	0.01 ± 0.10(1-1)	1.00	Intestine/stomach
<i>Quadrigyrus</i> sp. ^L	99/02	2.02	0.02 ± 0.14(1-1)	1.00	Intestine/stomach
Unidentified	99/02	2.02	0.02 ± 0.14(1-1)	1.00	Intestine/stomach
PENTASTOMIDA					
<i>Alofia</i> sp. ^L	99/04	4.04	0.15 ± 0.97(1-9)	3.75	Spleen/Celomatic cavity
<i>Sebekia</i> sp. ^L	99/02	2.02	0.07 ± 0.50(1-4)	3.50	Spleen/Celomatic cavity

EF: examined fish; PF: parasitized fish; P: prevalence (%); MI: mean intensity of infection ± standard deviation and range between parentheses (min-max); MA: mean abundance; ^L: larva.

in the host. Although the highest prevalence of infection and damage caused to the host by these metacercariae are associated with their installation in the eyes (MACHADO *et al.*, 2005), there are reports of the presence of the metacercariae in the gills and adjacent pharyngeal plates (SATO and PAVANELLI, 2004). In this study, the metacercariae were found to be parasitising the celomatic cavity.

The presence of *Contracaecum* sp. and *Brevimulticaecum* sp. larvae in the liver and celomatic cavity corroborates some reports (CAMPOS *et al.*, 2009; VENTURA *et al.*, 2016). In the present study, all specimens were *Contracaecum* larvae of type I, II and III located in the mesentery and liver the gymnotid fish. Similarly, larvae of *Contracaecum* sp. were also reported in *Gymnotus* spp. (AZEVEDO *et al.*, 2007; TAKEMOTO *et al.*, 2009). On the other hand, larvae of *Brevimulticaecum* sp. in fish are scarce. To date, *Brevimulticaecum* larvae have been reported in the celomatic cavity, hepatic parenchyma, mesentery, skeletal muscle and stomach wall of *Gymnotus inaequilabiatus* (VIEIRA *et al.*, 2010) and GALINDO *et al.* (2017) suggest that *G. inaequilabiatus* acts as a paratenic host to nematodes in the larval stage.

Gymnotus sp. of natural environment the nematodes Capillariinae gen. sp., *Contracaecum* sp. (types I and II), *Eustrongylides* sp., *Hysterothylacium* sp., *Procamallanus* (P.) *peracuratus*, *Spiroxyx*

sp. *Brevimulticaecum* sp., and Ascaridoidea fam. gen. sp. were reported (ISAAC *et al.*, 2004). In a natural environment, *R. rondoni* has been reported in different river basins, Paraguay, Paraná, Amazonas and São Francisco (COSTA, 1963), parasitizing fish of different families Characidae, Cynodontidae, Doradidae, Heptapteridae, and Pimelodidae (KOHN *et al.*, 2011), *R. rondoni* is reported for the first time parasitizing *Gymnotus* sp. In Brazil four species of *Goezia* sp were described in different species of fish (VIEIRA-MENEZES *et al.*, 2017), but in the genus *Gymnotus* sp there are no reports of parasitism by this species. Though in the present study the prevalence of *Goezia* sp infection was low (3.03%), in cases of massive infection, causing gross lesions and high mortality among the fish (VIEIRA-MENEZES *et al.*, 2017).

Apart from the low host specificity of *Contracaecum* sp. larvae, they also present special importance for public health due to their elevated zoonotic potential (TAKEMOTO *et al.*, 2009). Nevertheless, these results indicate the low possibility of these helminths causing zoonosis as they were found in the liver and visceral cavity. However, *Eustrongylides* larvae were observed encysted in the muscle and could present a possible cause of human infection. Until the present, nematodes have been related only in *Gymnotus* spp. from the rivers (ISAAC *et al.*, 2004) and no reports have been found in farmed baitfish *Gymnotus*.

In this study, acanthocephalans were found in larval form encysted in the intestines and stomach. The main factor regulating the prevalence and intensity of infection by acanthocephalans is the predation of the crustaceans as intermediate hosts (AZEVEDO *et al.*, 2007). Similarly, larvae and adult of *Quadrigyrus* sp. were reported in *Gymnotus carapo* (ISAAC *et al.*, 2004). On the other hand, *Pomphorhynchus* sp. was found to parasitize gymnotid fish in Argentina (ARREDONDO and GIL DE PERTIERRA, 2010). Gymnotids were pointed out as an intermediate host for larvae of *Quadrigyrus machadoi* Fabio, 1983. Obligatory paratenic hosts in the life cycle of acanthocephalans feed on the intermediate host that does not constitute the common item in the diet of definitive host completing the life cycle (EIRAS *et al.*, 2010). The low infection found in this study could be strongly associated with the capture period of the specimens in the environment as the intermediate hosts were found in low amounts in this period, as also observed previously (CAMPOS *et al.*, 2009). Possibly, the helminthic fauna was introduced into fish farming with infected fish captured from the environment.

Pentastomids on their adult stage are normally found to parasitize the lungs of vertebrates mainly reptiles (BRITO *et al.*, 2012; CHRISTOFFERSEN and ASSIS, 2013) and during their larval stage they can be found in freshwater fishes (VARGAS, 1975). Larvae of *Sebekia* sp. and *Leiperia oxycephalum* were related in *Gymnotus* spp. (LUQUE *et al.*, 2013; CHRISTOFFERSEN and ASSIS, 2013) and are generalist parasites in terms of intermediate hosts (REGO and EIRAS, 1989).

CONCLUSION

Gymnotid fish are commonly used as live baitfish for sport fishing of carnivorous and food-appreciated fish but the endoparasitic diversity and definitive hosts in the aquatic ecosystem highlight the importance of these fish as an intermediate host. This study showed that the endoparasitic helminths in bait gymnotid fish are able to survive in fish farming conditions that in this case endured several weeks after capture until marketing. Additionally, this study registers *Gymnotus* spp. host of *Goezia* sp. and *R. rondoni* for the first time.

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