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Prevalence for nematodes of hygiene-sanitary importance in fish from Colares Island and Vigia, Pará, Brasil*

Prevalência de nematoides de importância higiênico-sanitária em peixes da ilha de Colares e Vigia, Pará, Brasil

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Abstract

Zoonotic parasites can infect fish and be a serious threat to human health. The objective of this research was to estimate the prevalence for the main fish-borne zoonotic parasitic diseases of freshwater fish marketed in Colares Island and Vigia, Pará, Brazil. In February, 2012, 85 (40 of Colares and 45 of Vigia) fish were randomly sampled by means of net fishing. Eighty and 76% of fish sampled were parasitized, being silver croacker (*Plagioscion squamosissimus*), kumakuma (*Brachyplatystoma filamentosum*), and gilded catfish (*Brachyplatystoma rousseauxii*) the species most parasitized. The most prevalent parasites were *Anisakis* (50% in Colares and 49% in Vigia) and *Contracaecum* (60% in Colares and 40% in Vigia), followed by *Pseudoterranova* (2% in Colares and 11% in Vigia), *Eustrongylides* (10% in Colares and 0% in Vigia) and *Hysterothylacium* (2% in Colares and 7% in Vigia) in the species: silver croacker, kumakuma and gilded catfish, in both cities. Mesentery (55%) was the organ with highest level of intense infestation. A greater proportion of massive infestation was observed in females (57%) than in males (12%). Results of this study indicate that fish caught in Colares and Vigia could be of high risk for consumer.

Keywords: Anisakidae, *Eustrongylides,* zoonosis, Public Health, Amazonia.

Resumo

Parasitos zoonóticos podem infectar peixes e causar sérios agravos à saúde humana. O objetivo do presente estudo foi estimar a prevalência e identificar os fatores de risco para doenças parasitárias veiculadas por peixes de água doce comercializados na ilha de Colares e Vigia, Pará, Brazil. Em fevereiro de 2012, 85 peixes (40 de Colares e 45 de Vigia) foram coletados de forma aleatória com redes de pesca. Oitenta por cento e 76% dos peixes amostrados estavam parasitados, sendo a corvina (*Plagioscion squamosissimus*), piramutaba (*Brachyplatystoma filamentosum*) e dourada (*Brachyplatystoma rousseauxii*), as espécies mais parasitadas. Os parasitos mais prevalentes foram: *Anisakis* (50% em Colares e 49% em Vigia) e *Contracaecum* (60% em Colares e 40% em Vigia), seguido pelo *Pseudoterranova* (2% em Colares e 11% em Vigia), *Eustrongylides* (10% em Colares e 0% em Vigia) e *Hysterothylacium* (2% em Colares e 7% em Vigia) nas espécies: corvina, piramutaba e dourada, em ambos os municípios. O mesentério (55%) foi o órgão que apresentou infestação maciça em maior quantidade. A infestação maciça também foi observada com maior proporção em fêmeas (57%) do que em machos (12%). Os resultados deste estudo indicam que o peixe capturado em Colares e Vigia pode ser de alto risco para a o consumidor.

Palavras-chave: Anisakidae, *Eustrongylides,* zoonoses, Saúde Pública, Amazônia.

Introduction

The number of reports of fish-borne zoonotic diseases has increased as a result of several factors such as new diagnostic methods, increase of raw seafood consumption, and growth in the international market of fish (New et al., 1995; McCarthy and Moore, 2000; Chai et al., 2005; Nawa et al., 2005; Keiser and Utzinger, 2009; Robinson and Dalton, 2009).

Fish-borne zoonotic parasitic diseases include cestodes, trematodes and nematodes that infect humans by means of ingestion of contaminated raw fish. Anisakidae (nematodes) can cause allergic reactions to consumers (Ferre, 2001) and gastrointestinal perforation or obstruction (López-Serrano et al., 2000; Taniguchi et al., 2011). Anisakiasis in human is normally associated with seafood intake. The main genera involved are: *Anisakis*, *Pseudoterranova, Hysterothylacium,* and *Contracaecum* (Hochberg and Hamer, 2010).

The most involved species in human infection are *Pseudoterranova decipiens* and *Anisakis simplex* (EFSA, 2010). To evaluate zoonotic potential of *Contracaecum*, mammals were experimentally infected, resulting in damage to organism, which highlighted the zoonotic importance of this genus that belongs to Anisakidae family (Vidal-Martinez et al., 1994; Barros et al., 2004). Human infection by *Eustrongylides* sp. larvae has been described (Eberhard et al., 1989, Schantz, 1989; Wittner et al., 1989; Narr et al., 1996), and was characterized by abdominal pain and recovery of infectious larva from the abdomen.

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Data from Fisheries and the Ministry of Aquaculture reported that Brazil is ranked 23rd in fish production worldwide. The northern region is the most economically important in terms of continental fishery. Pará is the second most producing state (42,082.5 tons in 2009 and 50949 tons in 2010) (BRASIL, 2010) and fish is one the main part of the diet of many communities in the state. In view of the medical and economic importance of these data, and the scarceness of studies about fish zoonotic diseases in the region, prevalence studies of helminths of zoonotic potential would be fundamental to protect public health. The objective of this research was to estimate the prevalence for the main fish-borne zoonotic parasitic diseases of freshwater fish marketed in Colares and Vigia, Pará, Brazil.

Material and methods

Colares (00º55'38'' S, 48º17'04'' O) and Vigia (00º51'47'' S, 48º7'52'' O) are located in the northeastern region of Pará and salty micro-region, with amazonic equatorial climate, having an average temperature of 26°C (IBGE, 2010).

In February, 2012, 40 fish of the following species: silver croacker (*Plagioscion squamosissimus*), ripsaw catfish (*Oxydoras niger*), kumakuma (*Brachyplatystoma filamentosum*), and gilded catfish (*Brachyplatystoma rousseauxii*) were randomly sampled by means of net fishing from a river in Colares. The identification of fish species were based on data published in Fish Base site (FROESE and PAULY, 2015). During the same month, 45 fish of the same species were sampled in Vigia (except for silver croacker, which was replaced by white mullet, *Mugil curema*, due to its absence). The distribution of fish species by city is presented in Table 1. Fish were stored in Styrofoam boxes with innocuous ice for seafood conservation and were immediately transported to the laboratory of the Universidade Federal Rural da Amazônia, in Colares Island, to perform necropsy and parasite identification.

Necropsy was performed according to the method described by Moeller Jr. (2011). The plastron was removed to visualize parasites in the abdominal cavity. Subsequently, fish were filleted to search for parasites or parasitic cysts. When parasites were found, they were removed with an anatomical clamp and stored in bottles containing ethanol, formaline, and acetic acid (AFA) at room temperature. Alive parasites were inactivated using AFA at 60°C (Amato et al., 1991) for further identification.

Parasites were fixed in AFA for 24 hours, preserved in alcohol 70° GL, and clarified in lactophenol for identification according to Panesar and Beaver (1979), and Felizardo et al. (2009).

The prevalence of zoonotic parasites and fish species contaminated by *Anisakis* sp., *Contracaecum* sp., *Pseudoterranova* sp., *Hysterothylacium* sp., and *Eustrongylides* sp., was estimated according to Bush et al. (1997). Fish was defined as parasitized (binary variable) when at least one larva was present. Parasitic intensity (binary variable) was defined as massive when >10 larvae were recovered from a fish. Descriptive statistics were produced and the Chi-square or Fisher Exact test was used to test the association between prevalence of parasites and fish species, sex, affected organ or sampling location. The coexistence of parasites in the same fish was also assessed. Analyses were conducted using the R software version 2.14.2 (R Development Core Team, 2011). Statistical significance was defined as $P < 0.05$.

Results

A total of 85 fish were sampled, from which 47 and 53% were caught in Colares and Vigia, respectively. The number and gender of fish sampled are presented in Table 1 and Figure 1. Prevalence of nematodes parasitism was not different between cities (80 and 76% in Colares and Vigia, respectively, $P = 0.82$).

Table 1: Number of fish sampled by city studied during February 2012

Specie	Colares	Vigia	Total
Ripsaw catfish	10	10	20
Gilded catfish	10	10	20
Silver croacker	10	0	10
Kumakuma	10	12	22
White mullet	0	13	13
Total	40	45	85

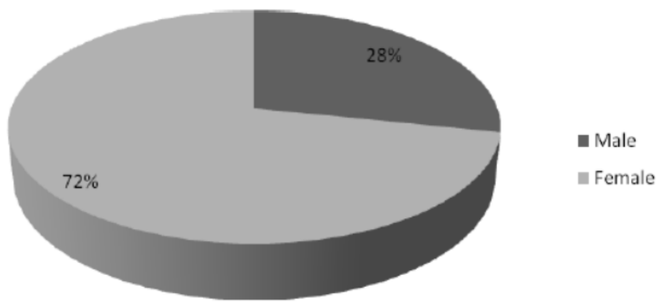

Figure 1: Prevalence of male and female sampled in Colares Island and Vigia, Pará, Brazil.

Among the species studied, the most parasitized species were silver croacker (*Plagioscion squamosissimus*), kumakuma (*Brachyplatystoma filamentosum*), and gilded catfish (*Brachyplatystoma rousseauxii*) (Table 2). The prevalence of each fish parasite studied can be observed in table 3. Except for *Eustrongylides,* the prevalence of parasitism was not different between cities.

Of the 85 fish sampled, 45% (38/85) presented massive infestation. The most contaminated organs were the mesentery (55%), stomach (15%), liver (7%), and ovary (7%) (Table 4). A greater proportion of massive infestation was observed in females (57%) than in males (12%), and in gilded catfish, silver croacker and kumakuma, as compared to the other species (Table 5).

The coexistence between parasites was observed for two Anisakidae genera. Of 42 fish parasitized by *Anisakis*, 32 (76%) were also parasitized by *Contracaecum* ($P < 0.01$). The coexistence between others pairs of parasites was not significant.

Table 2: Prevalence (%) of each parasite found in fish species analyzed in this study

Species	*Anisakis* sp.	*Contracaecum* sp.	*Pseudoterranova* sp.	*Hysterothylacium* sp.	*Eustrongylides* sp.	Total
Silver croacker	10(1/10)	70(7/10)	0 (0/10)	0 (0/10)	10 (1/10)	10
Kumakuma	9.09(2/22)	68.18(15/22)	18.18(4/22)	9.09(2/22)	9.09(2/22)	22
Gilded catfish	0 (0/20)	90(18/20)	10(2/20)	5(1/20)	0 (0/20)	20
Ripsaw catfish	5(1/20)	10(2/20)	0 (0/20)	5(1/20)	5(1/20)	20
White mullet	0 (0/13)	0 (0/13)	0 (0/13)	0 (0/13)	0 (0/13)	13
Total	4	42	6	4	4	85

Table 3: Prevalence of parasites detected in fish sampled in Colares and Vigia, Pará

Parasite	Colares	Vigia	*P-Value*
Anisakis sp.	50% (20/40)	49% (22/45)	0.91
Contracaecum sp.	60% (24/40)	40% (18/45)	0.06
Pseudoterranova sp.	2% (1/40)	11% (5/45)	0.12
Eustrongylides sp.	10% (4/40)	0% (0/45)	0.04
Hysterothylacium sp.	2% (1/40)	7% (3/45)	0.36

Table 4: Prevalence of intense infestation by organ evaluated in fish sampled during February 2012

Organ	Percentage of intense infestation [1]
Mesentery	55% (47/85)
Stomach	15% (13/85)
Liver	7% (6/85)
Ovary	7% (6/85)
Abdominal cavity	5% (4/85)
Gut	5% (4/85)
Gall bladder	4% (3/85)
Kidney	4% (3/85)

[1] Intense infestation was defined when > 10 larvae were found in the organ.

Table 5: Association between intense infestation and local caught, sex, and fish species sampled in this study

Category	Percentage of intense infestation[1]	*P-Value*
City		0.35
Colares*	50% (20/40)	
Vigia*	40% (18/45)	
Sex		< 0.01
Female	57% (35/61)	
Male	12% (3/24)	
Specie		< 0.01
Ripsaw catfish	5% (1/20)	
Gilded catfish	85% (17/20)	
Silver croacker	70% (7/10)	
Kumakuma	59% (13/22)	
White mullet	0% (0/13)	

[1] Intense infestation was defined when > 10 larvae were found in the organ.

Discussion

A high prevalence of infection by zoonotic parasites was observed in both locations studied, highlighting the importance of identifying factors that could be used to prevent the population from being exposed to such hazard. A high prevalence of zoonotic parasites (Anisakidae and *Eustrongylides*) was observed in silver croacker (*P. squamosissimus*), kumakuma (*B. filamentosum*), and gilded catfish (*B. rousseauxii*), which was not reported in the literature reviewed. Previous studies have been conducted to estimate the presence of zoonotic parasites in fish marketed in the Amazon region, including the following species: aimara (*Hoplerytrinus unitaeniatus*), trahira (*Hoplias malabaricus*), red piranha (*Pyogocentrus nattereri*), smooth weakfish (*Cynoscion leiarchus*), piraputanga (*Brycon micropleps*), piranha (*Serrasalmus marginatus*), tiger catfish (*Pseudoplatystoma fasciatum*), flatwhiskered catfish (*Pirinampus pirinampu*), gilded catfish (*Paulicea luetkeni*), spotted sorubim (*Pseudoplatystoma corruscans*), south American silver croacker (*Plagioscion squamosissimus*), and *Acestrorhynchus lacustris.* The prevalence of *Contracaecum* sp., and *Eustrongylides* sp., ranged from 6.6% to 100%, and 0.99% to 68.88%, respectively (Barros et al., 2006; Campos, 2006; Martins et al., 2009; Silva-Junior et al., 2011; Benigno et al., 2012).

Salgado (2011) showed prevalence of 15% (6/40) of *Anisakis* sp. in gilded catfish (*Brachyplatystoma rousseauxii*), 17.5% (7/11) of *Contracaecum* sp. in peackock cichlid (*Cichla* spp.), and 37.5% (15/40) of *Anisakis* sp. in smooth weakfish (*Cynoscion leiarchus*) marketed in southeast of Pará. However, in the present study, a lower prevalence of *Anisakis* in gilded catfish (0%) and silver croacker (10%) was found. In contrast, the prevalence of *Contracaecum* sp. was 90% and 70%, respectively (Table 2). Besides the presence of these parasites, *Eustrongylides* was also found which has zoonotic potential to the local population.

Dias et al. (2011) studied non-zoonotic species and reported a prevalence of 71.11% (32/45) of *Poecilancistrium caryphyllum* (order Trypanohryncha), in silver croacker caught in the Amazonic coast. The same authors reported that it would be necessary to perform epidemiological studies of parasitic diseases in Amazon fish, due to high levels of parasites found. This agrees with the present study because a high prevalence of massive infection was found. Massive infections can increase the risk of parasite migration to the muscles or to the abdominal cavity, which facilitates human exposure to larvae. In addition, such infections can cause significant economic losses due to condemnation of fish.

The prevalence of parasitism was highest in gilded catfish (*Brachyplatystoma rousseauxii*) and kumakuma (*Brachyplatystoma filamentosum*), and higher than that reported by Ibiwoye et al. (2004), who found 2.6 to 4.1 larvae per fish analyzed. Regardless of the parasitic species, we found a higher prevalence of parasitism in females than in males, which disagrees with results reported by Knoff et al. (2001). These authors reported that the proportion of infected males and females depended on the parasite studied. It can be hypothesized that females were more susceptible to infection due to a possible immunosuppression caused by their reproductive status (the study was conducted during spawning). Further studies could be performed to assess the importance of sex as a risk factor for infection because control measures to protect public health could be easily implemented (e.g., selective slaughter of fish).

As to place of parasitism, the most infected organ was the mesentery, which agrees with data reported by Barros et al. (2006) and Benigno (2012). However, the presence of *Eustrongylides* sp. in the oral cavity and mesentery, which was observed in this study, differs from results reported by Martins et al. (2009) and Benigno et al. (2012), which found *Eustrongylides* in muscle. The presence of these parasites in the oral cavity could be caused the migration from the esophagus or stomach after the death of the host, since the description by Ibiwoye et al. (2004) and Urdes et al. (2008) reported the presence of this nematode only in the body cavity, gut, muscle, and external surface of internal organs such as the liver.

Regarding to parasites migration to the muscle, Karl (2008) describes that many factors can influence and the probability and time (in life and *post-mortem*) of Anisakidae migration from gut to the muscle. This migration can be influenced by the parasites' physiology, ecological factors, fish immunology, and biochemical alterations of *post-mortem* in autolysate fish. Recently, the European Food Safety Authority reported that, based on scientific evidence, migration of Anisakidae is still poorly understood (EFSA, 2010).

For parasite inactivation, freezing of seafood by seven days under -20ºC or -35ºC for 15 hours is recommended (Howgate, 2007). Huss (2004) reported that inactivation occurs below -20ºC for 24 hours and also emphasizes that inactivation of infective stages of parasites occurs at 55ºC for one minute. Dias et al. (2010) and Silva-Junior et al. (2011) reported that gutting immediately after caught minimizes the probability of larvae migration to the muscle, as a function of temperature and time after fishery. However, fishermen of Colares and Vigia do not gut fish after catch, increasing the risk of migration to the muscle.

Conclusion

Results of this study indicate that fish caught in Colares and Vigia could be of high risk for human parasitism. These are important findings for supervisory agencies to guarantee the safety of fish consumption. Sanitary education campaigns and training of health agents is necessary to educate consumers and minimize the risk of infection.

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