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Anisakidae nematodes and Trypanorhyncha cestodes of hygienic importance infecting the king mackerel *Scomberomorus cavalla* (Osteichthyes: Scombridae) in Brazil

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ABSTRACT

From February to October 2007, thirty specimens of the king mackerel, *Scomberomorus cavalla* (Cuvier, 1829) were purchased from markets in the municipalities of Niterói and Rio de Janeiro. The fishes were measured, filleted and further had their organs investigated for helminths. Ten out of the thirty fish specimens were parasitized with anisakid nematodes represented by *Anisakis* sp. and *Contracaecum* sp. with prevalence of 1% and 16%, mean intensity of 2 and 3.31 and mean abundance of 0.02 and 0.53, respectively. The infection range with *Contracaecum* sp. was 1–9. The sites of infection were the stomach serosa and mesentery. Seventeen fish specimens (53%) out of the 30 investigated were parasitized with Trypanorhyncha metacestodes, identified as *Callitetrarhynchus gracilis*, *Pterobothrium crassicole*, *Callitetrarhynchus speciosus* and *Tentacularia coryphaenae* in the mesentery, with prevalence of 26, 20, 6, 3%, intensity and mean intensity of 3.25, 3.5, 1, 2 and mean abundance of 0.86, 0.7, 0.06 and 0.06, respectively. The infection range due to *C. gracilis* and *P. crassicole* were of 1–5 and 1–20, respectively. *Anisakis* sp., *C. speciosus* and *P. crassicole* are reported in *S. cavalla* for the first time. Considerations on the zoonotic potential of the parasites and their rules in sanitary inspection are presented.

1. Introduction

The king mackerel, *Scomberomorus cavalla* (Cuvier, 1829) occurs in the occidental Atlantic, from Massachusetts, in the USA, to the south of Brazil. Basically, specimens of this species fed on other fishes, mollusks and crustaceans, mainly squids and shrimps (Figueiredo and Menezes, 2000).

In Brazil, data on the fishery of this species indicate its high economic value related to the amount of fish obtained, taking into account the evaluated expressive internal acceptance of the product; moreover meat of the king mackerel has been exported to the USA, France, Spain, Argentina, Portugal and Japan (IBAMA, 2007). The species is still poorly studied considering hygienic-sanitary procedures regarding ichthyoparasitological approaches. Among the possible harbored parasites by specimens of the king mackerel, two groups are of concern: the Anisakidae nematodes, with zoonotic potential and the Trypanorhyncha cestodes, due to their importance during sanitary inspection, if one considers the harm that the repulsive aspect of infected meat certainly causes to consumers.

The presence of parasites in fish products indicates a harmful sanitary problem not to be underestimated. Even considering that most of the parasitic agents is not pathogenic to humans, some species can be associated to serious diseases due to the ingestion of contaminated

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fish as occurs, as widely known, in those caused by larval anisakid nematodes (González, 2003).

Human infections with those parasites can settle after the ingestion of either raw or poorly processed fish meat thus preserving the larval viability. Historically, those infections were most frequent in localities where typical alimentary habits are adopted. Nevertheless, presently, some of these infections are of surpassing medical concern in several countries, due to the increasing popularity of some oriental foods and to the encouraging appeals of naturalists that recommend the ingestion of either raw or poorly cooked fishes (Ferre, 2001).

To prevent the infection is necessary that people avoid ingesting fish meat either prepared under these conditions or inadequately processed during cooking, freezing, salting, smoking procedures or try to inactivate the parasites before the commercialization of infected fishes. In accordance with the guidelines of the North American agency "Food and Drug Administration" (FDA, 2003), fish meat bound to ingestion and prepared under temperatures below 60 °C has either to be frozen under −35 °C for, at least, 15 h or under −20 °C for seven days. The visual analysis of contaminated specimens to be further discharged is recommended and foreseen by the legislation of several countries including Brazil, Spain and France, among others (Brasil, 2007). In industries and in this type of analysis, the candling table can be utilized in the samplings of filleted meat.

According to Smith and Wootten (1978) fish evisceration immediately after capture is a control procedure that prevents larval migration from the viscera to the musculature; nevertheless, viscera are not to be discharged in water collections. Also some authors suggest that the hypoaxial musculature may be eliminated considering its high worm burdens, aiming at the reduction of potential risks related to anisakiasis (Herreras et al., 2000).

Since the detection of the parasitism and associated lesions in fishes infected with anisakid larvae and the appearance of the symptoms in humans, this parasitosis has been considered as emerging and a target of investigations aiming at the development of techniques to support the safe alimentary production as well as reliable methodologies for laboratorial diagnosis, besides the adoption of adequate preventive procedures. In several countries studies aim at the knowledge of hosts, infection dynamics, the relation between fish parasites and the resulting problems of public health importance. Records of anisakid larvae parasitizing marine fishes from the littoral of Rio de Janeiro have been previously reported (Knoff et al., 2007) and although to date there are no findings related to human anisakiasis in Brazil, this fish parasitosis can become an emergent zoonosis in our country (Germano and Germano, 1998) taking into account the increasing ingestion of food containing raw fish.

Scombrid fishes utilized in the preparation of raw or semi-raw food as the *cebiche*, in the Latin American continent has been investigated in order to associate the parasitism to the appearance of symptoms in humans (Torres et al., 2000; Laffon-Leal et al., 2000; Mercado et al., 2001; Timi et al., 2005).

In specimens of *S. cavalla* the only report of the parasitism of this host with anisakids is related to adults of *Hysterothylacium fortalezae* (Klein, 1973) in the stomach, small and large intestines (Klein, 1973). Other helminth groups have been referred in this host; Fernandes and Kohn (1984), reported to the digenean *Hirudinella ventricosa* (Pallas, 1774) in the stomach of specimens captured in the littoral of Rio de Janeiro; also, monogenenoids were reported from the gills of this fish species: *Gotocotyla acanthura* (Parona & Perugia, 1891), by Lamothe-Argumedo et al. (1996) in Mexico, *Scomberocotyle scomberomori* (Koratha, 1955) in Puerto Rico by Williams and Bunkley-Williams (1996) in Brazil and Colombia by Hayward and Rodhe (1999b), *Thoracocotyle crocea* Mac Callum 1913 in Brazil by Hayward and Rodhe (1999a), and *Mexicocotyla mexicana* (Meserve, 1938) in Brazil and Bahamas by Rodhe and Hayward (1999).

The Trypanorhyncha comprises a great diversity of species that parasitize fish and sea invertebrates. Adults infect the intestine of elasmobranchs, sharks and rays whereas larval forms are found in the celomatic cavity and musculature of teleosteans, crustaceans and cephalopods (Campbell and Beveridge, 1994).

The importance of this group of parasites is related to the disagreeable aspect they present to potential consumers of infected fishes that most of times are discharged either in processing facilities or during inspection procedures, thus causing economic losses. Reports of parasitism by Trypanorhyncha cestodes in marine fish from the coast of Rio de Janeiro have been made by several authors (São Clemente et al., 2007). The species *Tentacularia coryphaenae* Bosc, 1802 and *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931, although not reported from *S. cavalla* in Brazil, were referred in other scombrid fishes (Alves and Luque, 2006). Overseas, trypanorhynchs were recovered from specimens of *S. cavalla*, in Miami, USA, Ward (1954) in Battes (1990) reported to the presence of *T. coryphaenae*, and Palm and Overstreet (2000) referred to *C. gracilis* in the Gulf of Mexico.

In accordance with Dollfus (1942) cysts of Trypanorhyncha are not transmissible to homeothermic vertebrates and the re-encapsulation of the post-larvae does not occur in this group of hosts; nevertheless, according to Deardorff et al. (1984) the localization of larvae in the musculature of fishes can release toxins and, consequently, affect humans. Some reports have demonstrated that extracts from a species of Trypanorhyncha are responsible for immune responses in mice, indicating the possibility of allergic reactions in humans (Rodero and Cuéllar, 1999; Vázquez-López et al., 2001, 2002; Gòmez-Morales et al., 2008).

The present paper aimed the study of the parasitism of larval Anisakidae and Trypanorhyncha worms infecting the king mackerel *S. cavalla* (Cuvier, 1829), commercialized in the State of Rio de Janeiro, Brazil, with data on the helminth species, parasitological indexes related to prevalence, mean intensity, mean abundance, infection range, sites of infection and sanitary aspects due to their importance in fish hygiene and public health programs.

2. Materials and methods

From February to October 2007, 30 specimens of *S. cavalla* (Cuvier, 1829) with total lengths of 81.1–141 cm

Table 1
Prevalence (P), intensity and mean intensity (I/MI), mean abundance (MA), infection range (IR), infection site and CHIOC deposit number of larval Anisakidae nematodes and Trypanorhyncha metacestodes recovered from *Scomberomorus cavalla* (Cuvier, 1829), commercialized in the State of Rio de Janeiro, from February to October/2007.

Helminth species	P (%)	I*/MI	MA	IR	Infection site	CHIOC no.
Anisakis sp.	1	2*	0.02	–	Stomach serosa	35634
Contracaecum sp.	16	3.31	0.53	1–9	Mesentery	35635
Callitetrarhynchus gracilis	26	3.25	0.86	1–5	Mesentery	35630
Pterobothrium crassicole	20	3.5	0.7	1–20	Mesentery	35632
Callitetrarhynchus speciosus	6	1*	0.06	–	Mesentery	35631
Tentacularia coryphaenae	3	2*	0.06	–	Mesentery	35633

* Intensity is indicated when only a host was collected.

(110.5 cm) were purchased from fish markets in the municipalities of Niterói and Rio de Janeiro, State of Rio de Janeiro, Brazil. Specimens were identified in accordance with Figueiredo and Menezes (2000). Fishes were preserved in isothermal boxes with ice, to be further transferred to the Laboratory of Inspection and Fishery Technology of the Veterinary School, Fluminense Federal University, where specimens were identified, measured, submitted to necropsy and filleted. Helminths were recovered in Petri dishes with a 0.65% NaCl solution. Nematodes were fixed with hot (60 °C) AFA, preserved in 70° GL-5% ethanol–glycerin, clarified with Aman's lactophenol. Plerocercoids and plerocerci of Trypanorhyncha were transferred to distilled water, where cysts of plerocerci were broke open with the aid of sharp needles under the stereomicroscope to release the larvae that were put in the refrigerator for at least 24 h to permit the relaxing of scolices and tentacular extroversion. Further, larvae were fixed in cold AFA, stained with Langeron's carmine, differentiated in a 5% chloridric ethanol solution, dehydrated in a crescent alcoholic series, clarified in beechwood creosote and preserved either as whole mounts in Canada Balsam or in 70° GL ethanol. Taxonomic generic identification of larval nematodes was based on Rego et al. (1983) and Petter and Maillard (1988); Trypanorhyncha cestodes were identified in accordance with Carvajal and Rego (1985), Campbell and Beveridge (1994, 1996). Indexes of prevalence, intensity, mean intensity, mean abundance and infection range follow Bush et al. (1997). Representative parasite specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Rio de Janeiro, Brazil.

3. Results

Thirty-three percent of the 30 purchased specimens of *S. cavalla* were infected with third-stage Anisakidae larvae, representing the species *Anisakis* sp. and *Contracaecum* sp.; 53% with the Trypanorhyncha cestodes: *T. coryphaenae*, *Pterobothrium crassicole* Diesing 1850, *C. gracilis* and *Callitetrarhynchus speciosus* (Linton, 1897) (Carvajal & Rego, 1985).

Parasitological indexes related to prevalence, intensity and mean intensity, infection range, mean abundance, as well as the infection sites and CHIOC deposit numbers are depicted in Table 1.

Anisakis Dujardin, 1845. *Anisakis* sp. Main morphological characteristics based on two third-stage larvae: cuticle with thin transversal striation. Anterior extremity with a dorsal and two poorly developed ventro-lateral lips. Six cephalic papillae, one pair in the dorsal lip and a pair in each ventro-lateral lip. Boring tooth below the oral aperture, between the two ventro-lateral lips. Excretory pore opening beneath the boring tooth. Deirids inconspicuous. Ventriculus longer than large. Ventricular appendix and intestinal cecum absent. Two nearly spherical rectal glands. Tail conical, mucron present.

Contracaecum Railliet & Henry, 1912. *Contracaecum* sp. Main morphological characteristics based on ten third-stage larvae: cuticle with thin transversal striation, more evident in the posterior extremity of the body. Anterior extremity with a dorsal lip and two poorly developed ventro-lateral lips. Six cephalic papillae, one pair in the dorsal lip and a pair in each ventro-lateral lip. Boring tooth near the oral aperture, between the two ventro-lateral lips. Excretory pore opening beneath the boring tooth. Deirids inconspicuous. Ventriculus small and sub spherical. Ventricular appendix nearly twice longer than the intestinal cecum. Two almost spherical rectal glands. Tail conical, mucron absent.

Tentacularia Bosc, 1797. *Tentacularia coryphaenae* Bosc, 1802. Main morphological characteristics based on two plerocercoids: scolex craspedote with distinct velum. Four elongate and narrow sessile bothria. Bothrial margins entirely fused to scolex. Tentacles short. Metabasal armature homeacanthous homeomorphous. Characteristic basal armature present, basal hooks homeomorphous, of about 8–9 tridentate hooks, arranged in bilateral symmetry. Sheats sinuous or straight. Pars vaginalis short. Pars bulbosa in anterior region of pars bothrialis. Bulbs short, ellipsoidal. Retractor muscles originate in the base of bulb.

Pterobothrium Diesing, 1850. *P. crassicole* Diesing 1850. Main morphological characteristics based on ten plerocerci: plerocercus with blastocist. Scolex elongate, acraspedote. Bothria, pyriform, on mobile pedicels, in cruciform arrangement. Pedunculus scolecis subcylindrical, narrower than pars bothrialis. Principal rows of metabasal armature form alternating half-spiral of 5 large heteromorphous, hollow hooks; small interpolated hooks between principal rows on bothrial and antibothrial surfaces. Distinctive basal armature and swelling present on internal and external faces of tentacle, macrohooks present on internal face; asymmetrical basal swelling of tentacle

present. Hook files 1(1′) widely separated, falciform; intercalary row(s) present proximal to each principal row; intercalary rows extend onto external surface to merge with band of hooks occupying midline of external surface of tentacle. Sheats very sinuous. Bulbs elongate. Pars postbulbosa present.

Callitetrarhynchus Pintner, 1931: *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931. Main morphological characteristics based on ten plerocerci: plerocercus with blastocist, with caudal extension. Scolex long, slender, feebly craspedote. Two patelliform bothria, notched on posterior margin. Pars vaginalis long, tentacle sheaths regularly sinuous, enlarged anteriorly. Numerous gland cells surround tentacle sheaths from bulbs through most of pars vaginalis. Bulbs relatively short. Retractor muscles originate in anterior third of bulb. Pars postbulbosa absent. Armature poecilacanthous, without basal armature or enlargement. Principal rows half-spiral, alternate, hooks, 6(6′), 7(7′), 8(8′) form distinct triad separate from chainette; the satellite hooks 7(7′) are almost twice longer than hooks 8(8′). Intercalary rows absent. Chainette simple, bases of elements without wings, consisting of hooks 9(9′) well separate and forming single file on middle of external face.

Callitetrarhynchus speciosus (Linton, 1897). Carvajal and Rego (1985). Main morphological characteristics based on two plerocerci: plerocercus has a longer scolex and different size and proportions of the scolex parts, almost twice than observed in specimens of *C. gracilis*.

The following morphological differences enabled us to separate the two species. *C. speciosus* has a longer scolex and different size and proportions of the scolex parts. Despite basic similarities in the arrangement and measurements of hooks, there are many differences in the oncotaxy. Hooks 1(1′) in *C. speciosus* are arranged in a parallel pattern without having their points convergent as in *C. gracilis*; hooks 2(2′) are much longer in *C. speciosus*; hooks 3(3′) are also larger and have bigger bases. All other hooks, with the exception of 5(5′) and 7(7′) are larger in *C. speciosus*. The satellite hooks 7(7′) and 8(8′) are different: in *C. speciosus* they are almost equal in size, throughout the length of the tentacle but in *C. gracilis* the satellite hooks 7(7′) are almost twice longer than hooks 8(8′) along much of tentacle. Furthermore, the chainette is distinct in two species, the hooks being sunken into the tegument in *C. speciosus*, whereas they are higher and have wider bases in *C. gracilis*. Additional differences were noted in the frontal glands which are widespread in *C. speciosus* and extend from the posterior border of the bothria to the posterior border of pars postbulbosa, whereas in *C. gracilis* the glands do not extend into anterior quarter of the pars vaginalis.

This is the first report of *Anisakis* sp., *Contracaecum* sp., *T. coryphaenae*, *P. crassicole*, *C. gracilis* and *C. speciosus* in *S. cavalla* from Brazil.

4. Discussion

The fact that anisakid larvae were found in the stomach serosa and/or mesentery of fishes represents a low risk of ingestion by consumers. However, the possibility of larval migration to the musculature, while fishes remain unevisacerated on board and/or in warehouses has always to be taken into account. Parasitological surveys of several fish species captured off the Brazilian coast have been reported aiming at a proper understanding of the role larval fish parasites play and their zoonotic potential, in order to supply data so that sanitarians can better detect the parasites and prevent an officially undescribed disease in our country so far (Tavares and Luque, 2006; Knoff et al., 2007).

The Trypanorhyncha recovered from specimens of *S. cavalla* were represented by *T. coryphaenae*, *C. gracilis*, *C. speciosus* and *P. crassicole*, the two latter referred for the first time in Brazilian scombrids. It was observed that these cestode species occur in several teleosteans of commercial importance in Brazil (São Clemente, 1986; São Clemente et al., 1995, 2004, 2007; Rego, 1987; Amato et al., 1990; Silva et al., 2000; Alves and Luque, 2006; Dias et al., 2009).

5. Conclusion

It must to been reinforced the necessity to better inform those in charge of the fish hygienic-sanitary control as well as the staffs dealing with mass production activities, from capture to final consumers about the ichthyoparasitoses and prophylactic approaches with the improvement of sanitary educational programs reaching different levels.

It is suggested the adoption of the Hazard Analysis and Critical Control Point (HACCP) during the steps of mass production, aiming the elimination, avoidance or risks reduction, in order to provide a qualified and safe final product and in this case, with fish evisceration on board, warehouses, industries or retail dealers with appropriate discharges.

Multidisciplinary researches are to be developed in the evaluation symptoms in patients with gastroenteric lesions and/or allergic reactions suggesting anisakiasis and their relation with the ingestion or manipulation of raw fish; also, investigations of Trypanorhyncha cestodes are to be carried out in order to evaluate their zoonotic potential considering the association between their released toxins and the settlement of allergies in humans.

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