PANAMJAS
Pan-American Journal of Aquatic Sciences

Contracaecum ogmorhini Johnston & Mawson, 1941 (Nematoda: Anisakidae) in southern sea lion, *Otaria flavescens* (Shaw, 1800) from southern Rio Grande do Sul State, Brazil

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Abstract: The objective of this study was to identify the nematodes from southern sea lion, *Otaria flavescens*, along the southern coast of Rio Grande do Sul State, Brazil. Twenty-nine southern sea lions were necropsied (23 males: 3 females: 3 individuals no gender determined).They were found dead on the coast of Rio Grande do Sul State, between Lagoa do Peixe and Arroio do Chuí, from June, 2010 to August, 2011.Gastrointestinal tract, liver,heart, lungs and kidneys were analyzed for helminthes. Parasitological indicators such as Prevalence (P%), Mean Abundance (MA) and Mean Intensity (MI) were estimate. Gastroliths were found in ten individuals. The only helminth species found comprised pre-adult and adult forms of *Contracaecum ogmorhini* (Nematoda: Anisakidae) mainly within the stomachal lumen. The parasite presented loads of P%: 10.34; MA: 34.34; MI: 332. There was no difference in mean abundance between sub-adults and adults hosts. In this first record of *C. ogmorhini* parasitizing *O. flavescens* specimens in in the South Atlantic, the data are different from those previously reported to Argentina and Uruguay coasts and those from sea lions sampled in the Pacific Coastof South America, indicating probably a tool for mark different stocks of this hosts.

Key words: parasite, nematode, mammalia, pinnipeds

Resumo. Contracaecum ogmorhini Johnston & Mawson, 1941 (Nematoda: Anisakidae) de leão-marinho-do-sul, *Otaria flavescens* (Shaw, 1800) do sul do Rio Grande do Sul, Brasil O objetivo deste estudo foi identificar os nematóides do leão-marinho-sul, *Otaria flavescens*, ao longo da costa sul do Rio Grande do Sul, Brasil. Vinte e nove leões-marinhos-do-sul foram necropsiados (23 machos: 3 fêmeas: 3 indivíduos sem gênero determinado). Animais foram encontrados mortos na costa do Rio Grande do Sul, entre Lagoa do Peixe e Arroio do Chuí, de Junho de 2010 a Agosto de 2011. O trato gastrointestinal, fígado, coração, pulmão e rins foram analisados para helmintos. Indicadores parasitológicos como Prevalência (P%), Abundância Média (MA) e Intensidade Média (MI) foram analisados. Gastrólitos foram encontrados em dez indivíduos. As espécies de helmintos encontrados compreendiam formas pré-adultas e adultas de *Contracaecum ogmorhini* (Nematoda: Anisakidae) principalmente no lúmen do estômago. O parasita apresentou cargas de P%: 10,34; MA: 34,34; MI:322. Não houve diferença na abundância média entre sub-adultos e adultos hospedeiros. Este é o primeiro registro de *C. ogmorhini* parasitando espécimes de *O. Flavescens* no Atlântico Sul, os dados são diferentes dos relatados anteriormente às costas da Argentina e do Uruguai e dos leões marinhos amostrados na Costa do Pacífico da América do Sul, indicando provavelmente uma ferramenta para marcar diferentes estoques desses hospedeiros.

Palavras-chave: parasita, nemátodos, mamíferos, pinnipedes.

Introduction

Parasitological study in pinnipeds has been developed more often in northern hemisphere, mainly in seals because of their greater abundance (Lehnert *et a*l. 2007). Concerning to otariids, in South America, the studies on this topic are conducted in animals inhabitants of Atlantic and Pacific coasts as well as in some islands (Dailey *et al*. 2005). These studies are mainly to record gastrointestinal and lung nematodes species (Morgades *et al*. 2006; Dailey, 2009), some of them identified only at genus status (Andrade *et al*. 1999). The study of the parasitic fauna contributes to understand the pinnipeds biological evolutionary origin, geographic distribution, as well as part of their deaths (Sepúlveda 1998).

The pinnipeds are mammals adapted to aquatic and terrestrial life (Pinedo *et al*. 1992). The southern sea lion,*Otaria flavescens*, is a species that utilizes predominantly marine habitats in depths less than 50m (Vaz- Ferreira 1981). Moreover it is widely distributed from northern Peru on the Pacific coast of South America to the south of Brazil on the Atlantic coast of this continent (Pinedo *et al.* 1992). The two high concentrations of this mammalian species on the Brazilian coastline are in Rio Grande do Sul State: Refuge of Terrestrial Life in the East pier of Lagoa dos Patos (São José do Norte city, and in the Ecological Reserve of Ilha dos Lobos, in Torres (Rosas *et al*. 1994). In South Atlantic, reproductive areas range from Coronilla (33◦56'S – Uruguay – Atlantic coast) to Lobos de Tierra Island – Peru - 6◦30'S) (Bastida e Rodríguez 2003).

In *O. flavescens*, the study of helminths was mainly developed on the Pacific coast. The presence of nematodes *Phocanema decipiens*, *Anisakis* sp., Uncinaria sp. and *Pseudoterranova cattani* is known in Peru and Chile (Cattan and Carvajal, 1980, Sepulveda, 1998, George-Nascimento & Urrutia, 2000). Reports are more scarce and incomplete on Atlantic coast. In Brazil, Andrade *et al.* (1999) cited the presence of only *Contracaecum* sp. Nematodes. In Uruguay, Morgades *et al*. (2006) reported the presence of *Uncinaria* sp. and *Contracaecum* sp.. In Argentina, Béron-Vera *et al*. (2004) identified the presence of *U. hamiltoni* in juvenile hosts captured in Punta León, Província de Chubut. Nematoda shows great diversity, infecting organisms from different *taxa*, and are known as causes of zoonoses (Cabrera & Trillo-Altamirano 2004). Two anisakids species are very common in marine mammals: *P. decipiens* e *A.simplex* (Bush *et al*. 2001). Nematode anisakids present a complex life cycle that includes invertebrates and vertebrates hosts of different trophic levels. They can be utilized as indicators of food chain integrity as well as of the general biodiversity of marine ecosystems (Mattiucci & Nascetti 2007).

This study presents the nematode species of *Otaria flavescens* from southern Brazil as well as its parasitological indices.

Material and Methods

From June, 2010 to September, 2011, 29 necropsies were performed on specimens of *O. flavescens* found stranded dead along the coastline of Rio Grande do Sul State, Brazil, in the area between Lagoa do Peixe (31°21'38''S) and Arroio do Chuí (33°44'35"S). The carcasses were necropsied and classified according to specific protocol of the IBAMA (2005). The animals collected were in the stages 2 and 3 of decomposition, according to Geraci & Lounsbury (2005) criterion. The identification of the species of the hosts, their genders, and their biometrics was performed according to Pinedo *et al.* (1992).

The hosts were classified into physical maturity groups according Rosa *et al*. (1994) and Silva (2004). Animals reaching total length less than 1.5m were considered as juvenile, sub-adults between 1.5 and 2.10m, and adults those in length longer than 2.10m. Sexual maturity was not considered in the present study.

The thoracic and abdominal cavities of the hosts were opened for the removal of the organs. Lungs and airways, heart and segments of the great vessels of the heart base, kidneys, and full gastrointestinal tract (including liver) were collected from 24 specimens. Additionally, five specimens had only the stomach sampled. The organs were placed into individual plastic bags, identified, numbered, and frozen for further analysis. The material was thawed and processed in the laboratory. The organs of the hosts were opened, washed on a sieve, and macroscopically inspected for attached helminths. The retained material was placed in Petri dishes, and observed under stereomicroscope (magnificances: 10-40x) to collect parasites. Nematodes were washed with distilled water, counted, and preserved in ethyl alcohol 70GL. Clarification was achieved with beechwood creosote (Dailey and Walker, 1978). Identification was performed by evaluation of the

morphological structures and morphometry of specimens on temporary slides under optic microscopy following specific bibliography (Fagerholm and Gibson, 1987). Quantitative descriptors such as Prevalence (P%), Mean Abundance (MA), and Mean Intensity of infection (MI) were estimated according to Bush *et al*. (1997). Comparison of MA between age groups was performed using the Wilcoxon test at 0.05 significant level and Action® software version 1.1.

Results

The average total length of the hosts was 2.14±0.31m (1.58 ≥TL≤ 2.64m), 13 sub-adults and 16 adults. None juvenile animals were sampled. For male hosts, the average of length was 2.18 m and the length amplitude was 1.75m≥TL≤ 2.64 m. For female hosts, the average of length was 1.70 m and the length amplitude was 1.58 m≥TL≤ 1.86 m. The three hosts with no determined genders showed 1.61≥TL≤ 1.72m in length amplitude.

There were stomachs without diet (or food) contents (n=10). In these cases, the stomachs were free from parasites, but gastroliths were present. In the 24 sea lions completely analyzed, helminthes were not found in lungs and airways, heart and segments of the great vessels of the heart base, kidneys, and liver.

Only one species of nematode, *Contracaecum ogmorhini* (Anisakidae), comprising a total of 996 specimens found in the gastrointestinal tract of *O. flavescens* (Figs. 1a-1d). The nematodes showed three large lips interspersed with three inter-labia (Fig. 1a), cuticule transversely striated, digestive tract consisted of a long esophagus with ventricular appendix, and simple intestine with ascending cecum (Fig. 1b), cervical papillae rounded, males with conical tail with pre- and post-cloacal papillae (Fig. 1c), and spicules with similar length (Fig. 1d).

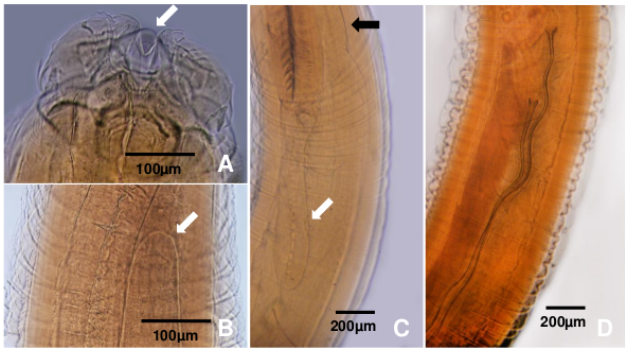

Figure 1.- *Contracaecum ogmorhini* from *Otaria flavescens*. A, Anterior end with 3 great lips and interlabia (arrow). B, Anterior end of the ascendent intestinal cecum (arrow). C, Ventricular appendix (write arrow) and intestinal cecum (black arrow). D, Spicula. Photogrpah: Eliane Pereira.

From the gastrointestinal tract of 29 sea lions sampled, only three contained *C.ogmorhini*. The prevalence was of 10.34%, intensity of infection was 332, and mean abundance 34.34 parasites. There was no significant difference between the infection of sub-adult (50.23±181.11) and adult hosts (21.44±76.21). Most species were found in the stomach (941 from 996 nematodes). Nematodes were found in others organs, such as small intestine (two nematodes/one host) and esophagus (53 nematodes/two hosts).

Contracaecum ogmorhini specimens were pre-adult (L4) and adult forms (L5), in the last case, including females with egg presence and males with developed spicules (Fig. 2).

Figure 2. Eggs of Contracaecum ogmorhini collected from *Otaria flavescens*. Photograph: E. Pereira.

Discussion

The presence of gastroliths in stomachs of southern sea lions is well known (Hamilton 1934, Vaz-Ferreira 1981, Drehmer & Oliveira 2003). Some hypotheses have been discussed about the reason of gastroliths presence in some animals. Among them is the one related to natural control of parasites in which it is believed that the gastrolith could crush the helminth adhered to gastric mucosa, relieving the burning sensation in stomach produced by parasitic spoliation (Wings, 2007). In this study, as well as in the Drehmer & Oliveira (2003), none of sea lions which contained gastroliths had gastric helminthes. Since the P% of *C. ogmorhini* was low even in animals which did not contain gastroliths, one cannot ensure that there is an association between their presence and parasitic levels, which

corroborates Koen Alonso *et al.* (2000). The currently and most accepted hypothesis is that the stones could influence the buoyancy of the animals, allowing a better control during the dives to capture prey (Taylor, 1993).

Contracaecum infections have been reported in a great diversity of organisms including fish and mammals (Martins *et al*. 2003). Due to economic, political, and pathological disruption, knowledge of anisakids infection, including those in *Otaria flavescens*, is important for human beings (Cabrera & Trillo-Altamirano 2004). Life cycles of these nematodes are, heteroxenous, including marine crustaceans as first intermediate hosts, and very often a variety of fish and cephalopods as secondary or paratenic hosts (Bush *et al*. 2001). Rokicki *et al*. (2009) identified three genera of nematode in fish collected in South Shetland Islands: *Anisakis* sp., *Contracaecum* spp. and *Pseudoterranova decipiens*. Human species is not normal host for any of these parasites, but by eating raw or undercooked fish, human being partakes of the food chain of the marine ecosystem could be infected by this nematode. Thus, humans assume a similar trophic position of marine mammals, and may become infected by consuming the flesh of a fish with infective larvae (Bush *et al*. 2001).

In marine mammals, infections by *Contracaecum* spp. are more frequent in pinnipeds than in cetaceans, in which the presence *Anisakis* spp. is common (Andrade *et al*. 1999). For Cetacea, Luque *et al*. (2010) reported the presence of de *Contracaecum* sp. in the stomach of the estuarine dolphin, *Sotalia guianensis*, collected on the southern coast of Espírito Santo State. *Contracaecum ogmorhini* seems to be an anisakid with specificity for otarids, since it has already been reported its infection in stomachs of Cape fur seals, *Arctocephalus pusillus pusillus*, collected on the southern coast of Africa (Stewardson & Fourie, 1998). Considering the southern Brazil coast, the South American fur seal, *Arctocephalus australis*, cohabits the studied area with an overlapping of niche including the diet (Pinedo & Rosas, 1982). *Contracaecum* infections in *A. australis* have been recently studied by Silva (2013) who detected that the infections are influenced by the gender and sexual maturity of the fur seals. Prevalence of *Contracaecum* in *A. australis* are higher than those found in the present study (61,1% versus 10,34%), although the abundance and mean intensity are discrepant. Low prevalence and high intensity of infection is a dispersion strategy of some helminthes, mainly those from the marine ecosystem where there no conspicuous barriers to segregate close phylogenetic hosts. This seems to be the case of *Contracaecum* infections in the south sea lion (*O. flavescens*) and south fur seal (*A. australis*) in the Brazilian coast. It is possible that due to the higher prevalence, *A. australis* to be more important in the dispersion of the nematodes than the *O. flavescens*. On the other hand, the relative low infections levels in the last host could be related to mechanism of defense to avoid the fixation of this parasite in the stomach. The infection site of the *Contracaecum* is the stomach, therefore, the presence of worms in the esophagus or intestine of *O. flavescens* observed in the present study probably is due to the *post mortem* reflux of the gastric contents and even parasite expulsion process, respectively.

In regard to nematode parasitic fauna of *O. flavescens*, the present data are different from those previously mentioned for specimens from Pacific coast of South America, which were recorded on Chilean coastline, *Phocanema decipiens* and *Anisakis* sp. (Cattan *et al*. 1976), and *Pseudoterranova cattani* (George-Nascimento & Urrutia, 2000). In the Atlantic waters, infections by *Uncinaria hamiltoni* in *O. flavescens* from the Northern Province of Patagonia, are known (Béron-Vera 2004). In the Uruguayan coast, there are also records of *Uncinari*a sp. Ancylostomatidae) and *Contracaecum* sp. (Anisakidae) in the small intestine and stomach of *O. flavescens*, respectively (Morgades *et a*l. 2006). Interestingly, *Uncinari*a sp. was not found in the animals from this study and this could be related to age of the animals sampled, that included only sub-adults and adults. In Argentina, the specimens studied by Béron-Vera *et al*. (2004) were 31 young infants (juveniles) which showed a 50% prevalence of this parasite. In Uruguay, Morgades *et al*. (2006) analyzed two pups, five sub-adults, and seven adults, of which only one of two pups were parasitized b*y Uncinari*a sp. In the same study, nematodes——Anisakidae, including *Contracaecu*m sp., were found in four of five sub-adults, and in four of seven adults sea lions . *Uncinaria* sp. was also registered in Falkland Islands (Baylis 1932), Uruguay (Botto & Mañe-Gazón 1975), and Chile (George-Nascimento *et al*. 1992). According to Lyons *et al*. (2001), Uncinaria is frequently found in the intestines of very young sea lions, and rarely in seals pups (George-Nascimento *et al*. 1992). Similarly to Ancylostomatidae congeneric, e.g., *Ancylostoma caninum*, it is believed that the infection of pup sea lions by

Uncinaria sp. may occur during the prenatal period when the larvae reach the fetus by the placenta, or by ingestion of colostrums. As occurs with adult dogs is possible that subadult and adult sea lion are not infected due to the development of specific immunity at this helminth (Pellon & Teixeira 1953, Fortes 2004).

The parasitic fauna of *O. flavescens* was little known until now on the southern coast of Brazil with only data described by Andrade *et al*. 1999, mentioning the presence of *Contracaecum* sp. This is the first record of *Contracaecum ogmorhini* parasitizing specimens of *O. flavescens*.

Conclusion

Adult and subadult specimens of *O. flavescens*, in special males, from the South Brazilian coast have only the stomach infected by helmiths. The assemblage of nematodes is very poor being represented by only a nematode species, C. *ogmorhini*, that shows relatively low infections levels.

Acknowlegments

We thank the team of Laboratório de Mamíferos e Tartarugas Marinhas do Instituto Oceanográfico, FURG, for their help in collecting, and necropsis of the sea lions. We also express our thanks to Campus Capão-do-Leão City Hall, UFPEL, for transporting material to the Instituto de Biologia. Eliane Machado Pereira appreciates CNPq for the master fellowship at Universidade Federal de Pelotas.

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Received: February 2013
Accepted: November 2016
Published:: September 2017