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Pulmonicola cochleotrema (Digenea: Opisthotrematidae) in Antillean manatees (*Trichechus manatus manatus*) from the North-eastern region of Brazil

JOÃO CARLOS GOMES BORGES[1,2], LARISSA MOLINARI JUNG[2], VITOR LUZ CARVALHO[3], ANDRÉ LUCAS DE OLIVEIRA MOREIRA[2], FERNANDA LÖFFLER NIEMEYER ATTADEMO[4], RAFAEL ANTONIO NASCIMENTO RAMOS[1] AND LEUCIO CÂMARA ALVES[1]

[1]Programa de Pós-Graduação em Ciência Animal Tropical, Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, CEP: 52.171-900, Recife, Pernambuco, Brasil, [2]Fundação Mamíferos Aquáticos - FMA, Avenida 17 de Agosto, n° 2001, Casa Forte, CEP: 52.061-540, Recife, Pernambuco, Brasil, [3]Associação de Pesquisa e Preservação de Ecossistemas Aquáticos – AQUASIS, SESC Iparana, rua José de Alencar, 150, CEP: 61.627-210, Caucaia, CE, Brasil, [4]Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos/Instituto Chico Mendes de Conservação da Biodiversidade, Estrada do Forte Orange, s/n, caixa postal n° 01, CEP: 53900-000, Itamaracá, Pernambuco, Brasil

Currently, little is known about the helminth fauna in sirenian species. Therefore, the objective of this study was to assess the frequency of infection by Pulmonicola cochleotrema *in Antillean manatees (*Trichechus manatus manatus*), in the North-eastern region of Brazil. Between the years of 1989 and 2014, 88 manatees found on the North-eastern Brazilian coast were clinically examined. They included animals that were found dead, animals maintained in captivity and specimens reintroduced into conservation areas. During their physical examination, helminths present in necropsied carcasses and in reintroduced animals were collected, as well as faecal samples. Parasites were detected in 7.95% (7/88) of the animals; all specimens collected being identified as* P. cochleotrema. *Only adult manatees were infected, and in two cases clinical signs were observed. This is the first report on the occurrence of* P. cochleotrema *in Antillean manatees in the states of Paraíba and Sergipe, in the North-eastern coast of Brazil.*

Keywords: Conservation, aquatic mammals, sirenians, helminth, trematode, parasite, parasitology, wildlife medicine, infection, clinical signs

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INTRODUCTION

Manatees are aquatic mammals that are considered vulnerable to extinction according to IUCN (2014), and endangered based on the Brazilian Red List (Mma, 2014). In Brazil, the presence of these animals has been reported on the coast and in estuary areas of all states from Amapá to Sergipe, with some discontinuous areas along the coast (Luna *et al.*, 2008; Lima *et al.*, 2011; Alves *et al.*, 2013).

For a long time, the indiscriminate hunting of animals was considered the major threat to manatee conservation in Brazil (Domning, 1982; Lima *et al.*, 2011). Moreover, other factors such as accidental death in fishing nets (Meirelles, 2008), the silting of estuaries, environmental degradation (Nishida *et al.*, 2008; Icmbio, 2011), indiscriminate use of motorized boats (Borges *et al.*, 2007), contamination of water resources (Anzolin *et al.*, 2012) and the stranding of manatee calves (Parente *et al.*, 2004; Meirelles, 2008) contributed to the reduction of the manatee population.

Corresponding author:
J.C. Borges
Email: jcgborges@hotmail.com

It is well known that the presence of certain pathogens such as bacteria (Vergara-Parente *et al.*, 2003), viruses (Ghim *et al.*, 2014) and parasites (Borges *et al.*, 2011; Bando *et al.*, 2014) may interfere in the health of these animals and in many cases have been associated with their mortality (Buergelt *et al.*, 1984; Beck & Forrester, 1988). So far, about 25 species of helminth parasites have been reported as infecting manatees (Mignucci-Giannoni *et al.*, 1999a; Lieven *et al.*, 2011; Bando *et al.*, 2014), however, clinical signs are associated with the parasitic infection in only a few of these cases (Beck & Forrester, 1988; Bossart, 2001).

From 1991 to 2003, a large copromicroscopic survey using different techniques (i.e. direct methods, flotation and sedimentation) was conducted in Brazil, which showed that all manatees studied were negative for the presence of helminths (Borges *et al.*, 2004). On the other hand, a research study carried out in the state of Ceará in 2009, detected *Pulmonicola cochleotrema* in the upper respiratory tract of free-living specimens (Carvalho *et al.*, 2009).

Therefore, in order to bridge the gap on the knowledge of helminths fauna in sirenian species, this study was to assess the frequency of infection by *Pulmonicola cochleotrema* in Antillean manatees (*Trichechus manatus manatus*), in the North-eastern region of Brazil.

MATERIALS AND METHODS

Between the years of 1989 and 2014, 88 manatees were studied, 39 specimens representing stranded animals found dead, rescued between the states of Sergipe (11°25′32S 37°19′18W) and Rio Grande do Norte (04°49′55S 37°15′09W). The carcasses were necropsied (Vergara-Parente, 2005) and careful examinations of the respiratory and digestive systems were performed (Marigo & Andrade, 2005).

In addition, considering the actions planned for the strategy of manatee conservation in Brazil (ICMBIO, 2011), 49 live specimens were monitored; some of them during their rehabilitation process and maintenance in pools (N = 23), some during the rehabilitation period in captivity, built in the natural environment (N = 5), and others after reintroduction (N = 21).

All animals in captivity and those reintroduced were physically examined and faecal samples were collected for laboratory analyses. The age of animals was estimated based on data previously published on Florida and Antillean manatees (Marmontel, 1993; Borges *et al.*, 2012). In the physical examination, three reintroduced animals eliminated parasites from their nostrils during breathing.

All faecal samples and helminths collected were fixed and preserved in a solution containing alcohol, formalin, glacial acetic acid and distilled water. Afterwards, the faecal material was analysed through sedimentation and flotation techniques (Bando *et al.*, 2014) and helminths were clarified in lactophenol, stained and mounted on microscope slides for morphological identification (Amato, 1985). Parasites were measured and identified using previous descriptions (Blair, 1981, 2005; Carvalho *et al.*, 2009).

In order to investigate the interaction between infection by *P. cochleotrema* and gender (male or female) and/or age (calf, young or adult) the log-linear method was used (Quinn & Keough, 2002). All analyses were performed by the glm() function of the MASS package (Venables & Ripley, 2002) of Software R (R Core Team, 2014).

All procedures herein performed were approved by the Sistema de Autorização e Informação em Biodiversidade (SISBIO) (licence number: 33.819–1) and by the Ethics Committee of Animal Experimentation (ECAE) of the Universidade Federal Rural de Pernambuco (licence number: 23082.011095/2013).

RESULTS

Out of 88 animals analysed, 7.95% (7/88) scored positive for the presence of *P. cochleotrema* in the nostrils (Fig. 1), trachea and/or bronchi. The trematodes herein found presented oval bodies with 6.6 mm in length and 4.83 mm in width. Morphologically they were concave ventrally and convex dorsally. In addition, they presented an oral sucker subterminal with transversal opening, branched intestinal caecum and multilobated and irregular ovaries. Testicles were rounded, multilobulated, and located in the posterior third of the body. All trematodes were identified as *P. cochleotrema* (Digenea: Opisthotrematidae).

Although a higher frequency of infection was observed in females (71.42%; 5/7), this gender did not present any correlation with the occurrence of the parasite (gl = 1; $F = 0$; $P = 1$). Conversely, age was correlated with the occurrence of infection by *P. cochleotrema* (gl = 2; $F = 4.6193$; $P < 0.001$), because all positive animals were adults. On the other hand, no correlation between the positivity and gender/age was observed.

Fig. 1. *Pulmonicola cochleotrema* (Digenea: Opisthotrematidae) found in the nostril of Antillean manatee.

The frequencies of infection in dead and live animals were 10.25% (4/39) and 6.12% (3/49), respectively. Positive results were obtained only in free-living animals, including native and reintroduced species. No parasites were detected in animals maintained in captivity.

In three reintroduced specimens (6.12%; 3/49), respiratory changes (i.e. atypical noises and nasal mucous secretion) were observed. In addition, clinical signs suggestive of bronchitis and bronchopneumonia were reported. Some specimens eliminated parasites from the nostrils during breathing. All clinical signs herein reported are compatible with infection by *P. cochleotrema*.

All analyses of faeces scored negative for the presence of eggs, larvae and/or oocysts.

DISCUSSION

The frequency of infection by *P. cochleotrema* observed in the present research study (i.e. 7.95%) was lower than that reported in a previous study in the USA, where a positivity of 38% was detected (Beck & Forrester, 1988), and in Puerto Rico (Mignucci-Giannoni *et al.*, 1999b) where 26% of manatees were parasitized by this trematode. Similarly, a study conducted in the state of Ceará, Brazil, reported a positivity of 26.7% (Carvalho *et al.*, 2009).

The findings of this study are relevant and expand the knowledge on the occurrence area (states of Paraíba and Sergipe) of *P. cochleotrema* in Antillean manatees in Brazil.

Special attention has been given to the factors that contributed to the occurrence of *P. cochleotrema* in manatees in the last years. Until 2013, no infection had been reported in Antillean manatees (free-living animals or in captivity) in the states of Sergipe, Alagoas, Pernambuco, Paraíba and Rio Grande do Norte. In addition, during a large copromicroscopic survey conducted from 1991 to 2003, and during the examination of manatee carcasses (Borges *et al.*, 2004), no trematode species were detected.

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In order to better understand the epidemiological factors associated with the infection by *P. cochleotrema* in sirenian species, as well as the host–parasite relationship, it is pivotal to know the biology of this trematode. Indeed, until now, information about its life cycle in manatees, as well as factors influencing its survival in environmental conditions and routes of infection remain unknown (Carvalho *et al.*, 2009).

In fact, it is believed that *P. cochleotrema* can use molluscs or crustaceans as intermediate hosts (Beck & Forrester, 1988). These invertebrates are found attached to algae and seagrasses, which are important sources of food for manatees (Borges *et al.*, 2008). Therefore, it is likely that the infection occurs after ingestion of these (Blair, 1981; Beck & Forrester, 1988; Bando *et al.*, 2014). In addition, it has been proved in other studies that environmental factors such as salinity and water temperature can influence the occurrence of *P. cochleotrema* (Beck & Forrester, 1988; Carvalho *et al.*, 2009).

The infection by *P. cochleotrema* was observed only in native and reintroduced species. Therefore, it is likely that intrinsic factors related to the condition of free-living animals may play an important role in the susceptibility to infection of the Antillean manatees herein studied. Moreover, it has been demonstrated that manatees living in the Florida Peninsula were more exposed to the infection due to their displacements and consequent diversity of food resources ingested by them (Bando *et al.*, 2014). In Brazil, this sirenian species may also move over a large area, which makes them vulnerable to infection by these parasites (Lima *et al.*, 2012; Normande *et al.*, 2014).

Most likely, the lack of infections by this trematode in animals maintained in captivity, especially in pools with physical and chemical water treatment, occurs due to the absence of intermediate hosts.

Only adult animals were found infected with *P. cochleotrema*. This finding is similar to another study previously reported, in which only older animals were affected, probably because these animals use a large area of habitat during their life-time (Beck & Forrester, 1988).

The presence of atypical noises and nasal mucous secretion in animals naturally infected by *P. cochleotrema* has already been reported (Beck & Forrester, 1988; Mignucci-Giannoni *et al.*, 1999b). In fact, clinical signs related to the infection by this trematode are seldom recorded (Bossart, 2001), but when they occur the animal may present chronic rhinitis, pulmonary oedema, pneumonia, and in some cases these may lead to death (Beck & Forrester, 1988).

The absence of helminth eggs and larvae in the faeces herein analysed is similar to the findings previously reported by Borges *et al.* (2004). Therefore, it is important to adopt new tools with higher sensitivity in order to improve diagnoses of parasite infections.

In conclusion, the displacement ability of Antillean manatees along the Brazilian coast might be an important factor for the spreading of *P. cochleotrema*. In addition, it is important to note that this is the first report of manatee infection by this trematode in the states of Paraiba and Sergipe from the North-eastern region of Brazil.

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Correspondence should be addressed to:
J.C.G. Borges
Programa de Pós-Graduação em Ciência Animal Tropical, Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, CEP: 52.171-900, Recife, Pernambuco, Brasil
email: jcgborges@hotmail.com

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