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## Pesquisa parasitária em urina de peixes-bois marinhos (*Trichechus manatus manatus*) mantidos em cativeiro no Brasil

## Parasitological research in urine from marine manatees (*Trichechus manatus manatus*) maintained in captivity in Brazil

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**Resumo.** O peixe-boi marinho (*Trichechus manatus*) é um dos mamíferos aquáticos mais ameaçados do Brasil, sendo atualmente classificado como vulnerável à extinção. Os principais riscos para a conservação da espécie são as de causas naturais, como a lenta taxa de natalidade, ações antrópicas e as doenças infecciosas. Dentre os principais objetivos do Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos, do Instituto Chico Mendes de Conservação da Biodiversidade (CMA/ICMBio) é promover pesquisas científicas e ações de manejo para a conservação e recuperação de espécies ameaçadas de mamíferos aquáticos, além de desenvolver e fomentar a reabilitação em cativeiro e a soltura em ambiente natural do peixe-boi marinho. A passagem destes indivíduos por cativeiro é de extrema importância para a conservação da espécie. A reabilitação em cativeiro de filhotes de peixes-bois marinho além de permitir a recuperação e possível devolução do animal ao ambiente natural, possibilita um maior conhecimento da espécie, seja referente a aspectos biológicos, de comportamento e clínico. Os estudos referentes ao parasitismo em peixe-boi marinho no Brasil ainda são poucos elucidados, sendo necessário mais pesquisas relacionadas ao tema, a fim de melhor compreender os aspectos patológicos e sanitários da espécie. O presente trabalho tem como objetivo realizar o isolamento de parasita em amostras de urina colhidas de peixe-boi marinho mantidos em processo de reabilitação. Todos os animais que fizeram parte do estudo são provenientes do Centro de reabilitação de animais silvestres CRAS/CMA/ICMBio, localizado na Ilha de Itamaracá, Estado de Pernambuco. Este se tratará da primeira descrição de parasitas de urina em peixe-boi marinho no Brasil e poderá subsidiar as ações de manejo a serem tomadas para garantir a saúde dos animais em reabilitação.  
Palavras-chave: sirênios, parasita, urina, *Trichechus manatus*.

**Abstract.** The marine manatee (*Trichechus manatus*) is one of the most endangered marine mammals in Brazil, and is currently classified as vulnerable to extinction. The main risks to the conservation of the species are from natural causes, such as the slow birth rate, human actions and infectious diseases. Among the main objectives of the National Centre for Research and Conservation of Aquatic Mammals, the Chico Mendes Institute for Biodiversity Conservation (CMA/ICMBio) is to promote scientific research and management actions for the conservation and recovery of endangered species of marine mammals, and develop and promote rehabilitation in captivity and release natural environment of the marine manatee. The passage of these individuals for captive is of utmost importance for the conservation of the species. The rehabilitation of captive cubs marine manatees and allow recovery and can return the animal to the natural environment, enables a greater knowledge of the species, referring to biological, behavioral and clinical. Studies on the parasitism of manatee in Brazil are few elucidated, more research related to the topic, it is necessary to better understand the disease and health aspects of the species. This work aims to realize the isolation of the parasite in urine samples of marine manatee kept in the rehabilitation process. All animals included in the study are from the rehabilitation center for wild animals CRAS/CMA/ICMBio, located in Itamaracá, State of Pernambuco. This deal is the first description of parasites in urine manatee in Brazil and can support management actions to be taken to ensure the health of animals in rehabilitation.

**Keywords :** Sirenia, parasites, urine, *Trichechus manatus*.

## Introduction

Brazil houses two of the four species of manatees in the Order Sirenia, belonging these two to the Family Trichechidae: the Amazonian manatee (Natterer, 1883) and the marine manatee (Lannaues, 1758). Both are classified as vulnerable to extinction (IUCN, 2010) and are protected under law since 1967, under the Protection of Fauna Federal Law (law nº 5.197/1967), under the alteration of the Protection of Fauna Federal Law (law nº 7.653/1987) and under the Environmental Crimes Law (Law nº 9.605/1998), (IBAMA, 2000)

The Family Trichechidae has three species: *Trichechus senegalensis* (Link, 1795), (African manatee), which is the less studied species, that inhabits the West coast of Africa, from Senegal to Angola and that its anatomical characteristics are similar to the marine manatees' (REYNOLDS, 2002); *Trichechus inunguis*, (Amazonian manatee) that resides in the Amazonian basin; and the *Trichechus manatus*, (marine manatee), that resides from the atlantic coast of the United States to the Northeast of Brazil (MONDOLFI, 1974; HUSAR, 1977a, 1978a,b).

The recent population knowledge in respect to the marine manatee in Brazil originated from researches accomplished by the Manatee Project in the beginning of the 90's, comprehending states from Sergipe to Amapá (LIMA et al., 1992; SOAVINSKI et al., 1992). LIMA (1997) estimated about 250 individuals for the region comprehended between the state of Alagoas and Piauí. More recently, LUNA (2001) proposed an estimation of 207 manatees for the band comprehended between the state of Maranhão and Amapá. As a result of these two works, the population estimation for the Brazilian coast is approximately of 500 individuals, distributed discontinuously along the North and Northeast of the country.

The historical processes of meat, oil and skin commercial exploration in long scale, were the principal factors responsible for such conservation status (FONSECA et al., 1994; LIMA, 1997; LUNA, 2001; ROSAS e PIMENTEL, 2001).

Despite the indiscriminate hunt, accidental death in nets (OLIVEIRA et al., 1994), the intense habitat degradation, silting of estuaries, collision with vessels, (PARENTE et al., 2004) the increase in the number of strands of calves in the northeastern coast, suggesting the lack of access of pregnant females into the sanctuaries for parturition (LIMA, 1997), and its' slow reproduction, promoted a reduction in the number of individuals (ROSAS, 1994; LIMA, 1997; LUNA, 2001). Such factors resulted in the inclusion of the Amazonian and the marine manatee in the Official List of Species of the Brazilian Fauna Threatened to Extinction (BRAZIL, 1989), and of the Appendix I of Conservation about the International Commerce of Wild Flora and Fauna Species in Risk of Extinction, CITES (2000). They are found in the category "vulnerable" to extinction in the IUCN "The World Conservation Union" (2013)

classification, that is, they present a great risk of extinction in natural habitat in mid-term. In Brazil, according to the Ministério do Meio Ambiente (2007), the marine manatee is classified as "In critical danger".

The presence of determined pathogenic agents as bacteria (VERGARA-PARENTE et al., 2003), viruses (BRACHT et al., 2006) and parasites (BORGES et al., 2005) can compromise animal sanity and cause death, in spite of the factors mentioned earlier.

Several parasitological agents compromising manatees have been described in the literature, such as infection caused by trematodes (BOEVER et al., 1977; DAILEY et al., 1988; BECK e FORRESTER, 1998; MULLINS et al., 2003), nematodes (MIGNUCCI-GIANNONI et al., 1999) and cetodes (BECK e FORRESTER, 1998), besides disturbances caused by coccidian, i.g *Eimeria manatus* (LAINSON et al., 1983), *Toxoplasma gondii* (BUERGELT e BONDE, 1983) e *Cryptosporidium* spp. (MARCONDES et al., 2002).

This study was proposed due to the lack of studies on the genitourinary tract parasitism in manatee (*Trichechus manatus manatus*), in order to contribute to conservation and knowledge that assist in the preservation of the species at issue. This study is very important for the investigation of endoparasites (eggs, larva, cists and oocysts) in the genitourinary tract, besides this study being the first description of parasites in urine of marine manatees in Brazil, which can provide management actions to be taken in order to guarantee the health of animals in rehabilitation.

## Methods

The animals are from the Rehabilitation Center of Wild Animals (CRAS/CMA/ICMBio) placed at Itamaracá Island, Pernambuco State.

From January of 2013 to January 2014 urines were collected from 10 captive animals, from both sexes and different ages groups from CMA/ICMBio during biometry procedures performed routinely in the institution. The histological layers utilized for the urine sample analyses were conceded by CMA/ICMBio biological material storage for the performance for this present study.

The asepsis in the genital region was performed with Clorexidine and sterile gauze after the containment of the animal for the biometry procedure. Then, urine collection was tried through an abdominal massage with warm potable water close to the region of the genital orifice, with the animal in lateral or dorsal decubitus. In cases, which urination hasn't been performed, a plastic receiver type *frisbee*® previously sterile was positioned in the genital region, in order to collect the urine sample during a management procedure.

After urine was collected, the material was stored in a sterile polietilene flask and taken to the CMA/ICMBio laboratory for sample separation and centrifugation.

The material was put in conical centrifuge tubes for five minutes at 1.500rpm. After sample centrifugation the supernatant was discarded and with a sterile pipette the sediment necessary was collected for the preparation of the histological layer.

Six layers were prepared for each animal, and in each layer three sample points were utilized, totalizing 18 sample points for each individual. After this procedure, the layers were maintained in a sterile box for approximately 24 hours, until it has dried, following Bonde, R.K instructions (*com. personal*). Subsequently, the materials were taken to the microscope for visualization of any parasitological state in the sample, besides the photo registration of all material observed, in spite of the presence of the parasite or not.

The lamina and the photo registrations were taken to the parasitological disease laboratory at FMU for identification of the parasites.

Animals are subjected to a series of bacterial, viral, fungal and parasitic agents in the ex situ marine habitat. In this study it was possible to observe that the animals, even under captive conditions, presented some type of larval stage in the urine samples collected, even the animals being maintained in pools or tanks with sea water being collected through physical method, filtration, chemical method, chlorination and being exchanged daily.

For this study, urine collection was performed in ten animals, and among the 10 animals that were studied and had its urine observed microscopically, all, without any exception, presented parasitic larvae (Image 1, 2, 3, 4, 5, 6, and 7) in its sample; The presence of two animals with a high number of larvae, possibly suggesting a possible parasitic infestation, is important to highlight.

### Results and discussion

**Image 1.** Parasite in the urine of a marine manatee. Font: Juliana Pires



**Image 2.** Parasite found in the urine of male manatee calf. Font: Juliana Pires



**Image 3.** Parasite in the urine of a female manatee. Font: Juliana Pires



**Image 4.** Parasite found in a calf that recently arrived at CMA. Font: Juliana Pires



**Image 5.** Parasite found in the urine of a young male. Fonte: Juliana Pires



**Image 6.** Urine parasite of an adult male. Font: Juliana Pires



**Image 7.** Parasite found in the urine of a young female. Font: Juliana Pires



The risk of sample contamination through external mean is low, although it exists. All material utilized for urine collection was sterile, with the exception of *frisbee*® utilized for urine collection of the animal, when the same was in lateral decubitus; however *frisbee*® was always cleaned and higienized with alcohol 70%.

Thinking about a possible contamination, we can suggest that the water from CMA utilized in the tanks may be contaminated, but as it was mentioned earlier, the water is captured from the sea and treated before the contact with the animal. Besides, feces and food leftovers are constantly being removed from the pools throughout the day and lastly, the water is exchanged daily.

It's important to highlight that one of the animals studied was a one month year-old calf, that recently arrived at the Aquatic Mammal Center. Its urine was collected at the moment of it arrival, without any previous contact with the water in the tanks of CMA. The results were positive for the parasitological test. Thinking about this question, we could discard external contamination, and point the hypothesis that these animals arrive at the Project already parasitized, and that this contamination occurs in the natural habitat.

According to Howard (1983), the source for parasitic infection often occurs through the ingestion of contaminated food, instead of being directly through the environment because most of the marine mammal parasites have indirect live cycles.

Some authors suggest that sirenian nematodes use crustaceans as intermediate hosts that many times are consumed by manatees by chance, through the habit of feeding vegetation. In natural environment, sirenian feed algae and campim-agulha (*Brachiaria humidicola*). On the other side, other authors propose that eggs can be ingested directly when hosts feed contaminated vegetation (BECK & FORRESTER, 1988).

According to Marigo (2009), the infection occurs commonly through diet, being mostly mollusks and fishes the intermediate hosts.

According to what Beck and Ferrester (1988) and suggested, and thinking about possible contamination causes, we can emphasize the presence of *Diocotophyuma renale*, a parasite exclusively from the urinary system, that can possibly be a source of infection, since can be found in its cycle the presence of an annelid that could have been ingested routinely and been passed unnoticed.

The knowledge of marine mammal parasites in Brazil is specially scarce; therefore it's important to highlight the lack of reports or studies towards the presence of parasites in the genitourinary tract of manatees and that this study will further contribute to conservation and preservation of the species studied.

In this study, it wasn't possible to identify the parasites specifically or genetically; however contacts have been already made for a future study, in order to possibly obtain a genetic sequence of the helminthes encountered.

### Final Considerations

Through the findings of helminthes in the urine of marine manatee maintained in captivity in Brazil and the lack of similar reports to this study, it's recommended for a new study the clarification of the phylogenetic classification of these microorganisms and also a effort to discover if such microorganisms affect or not the health of the animals in study.

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