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SEROPREVALENCE OF *TOXOPLASMA GONDII* IN CAPTIVE ANTILLEAN MANATEE (*TRICHECHUS MANATUS MANATUS*) IN BRAZIL

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Abstract: Antillean manatees (*Trichechus manatus manatus*) are aquatic mammals that inhabit marine waters from Central America to the northeastern region of Brazil, and they are an endangered species. Infection with *Toxoplasma gondii* through intake of water or food contaminated with oocysts has been reported among marine mammals. The present study aimed to evaluate the prevalence of antibodies to *T. gondii* in West Indian manatees living in captivity in northeastern Brazil. Serum samples from 55 West Indian manatees from three different captive groups were tested for *T. gondii* antibodies by means of the modified agglutination test using a cutoff of 1:25. The samples were screened at dilutions of 1:25, 1:50, and 1:500, and positive samples were end-titrated using twofold serial dilutions; antibodies were found in six Antillean manatees (10.9%) with titers of 1:50 in three, 1:500 in one, 1:3,200 in one, and 1:51,200 in one manatee. This study is the first report of *T. gondii* antibodies in captive Antillean manatees in Brazil.

Keywords: Antillean manatee, conservation medicine, marine mammals, modified agglutination test, *Toxoplasma gondii*, *Trichechus manatus manatus*.

INTRODUCTION

Manatees are marine mammals of the order Sirenia and Family Trichechidae. The subspecies known as the Antillean manatee (*Trichechus manatus manatus*) is distributed from Central America to the Caribbean islands and northeastern Brazil.^{15,17} The International Union for Conservation of Nature (IUCN) currently lists this species as endangered. The main cause of the population decline of this species in Brazil is loss of habitat, due to anthropogenic action that causes beaching of offspring, along with low genetic variability.¹⁷

Toxoplasma gondii infection in marine mammals is common and deserves special attention because these animals may serve as sentinels of environmental contamination by oocysts.^{2,3,9} Freshwater runoff has been suggested to be a risk factor for *T. gondii* infection among sea otters (*Enhydra lutris nereis*) in the United States.^{7,19} Oocysts excreted by felids on land can be washed out to sea and infect marine life.⁹

Although Antillean manatees live in saltwater, they drink freshwater coming from estuaries or from upwelling freshwater sources in the seas. When in captivity, freshwater from artesian wells or from the public water supply system is supplied to the animals. Freshwater may be an important route of transmission for

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T. gondii to these animals, both in nature and in captivity.

Although *T. gondii* antibodies have been found in several species of aquatic mammals, including the manatees, data from Brazil are limited.^{6,8,9,13,14,18,20} Fatal toxoplasmosis has also been reported in manatees.^{4,5,11} Here, we report seroprevalence of *T. gondii* antibodies in Antillean manatees in Brazil for the first time.

MATERIALS AND METHODS

Between August 2003 and June 2013, blood samples were collected from 55 Antillean manatees of both sexes and various ages, from three different captive groups: 38 Antillean manatees held in ex situ captivity at the Brazilian National Center of Research and Conservation of Aquatic Mammal (CMA)/Chico Mendes Institute for Biodiversity Conservation (ICMBio), Ilha de Itamaracá, state of Pernambuco (CMA/PE) (7°48'33.40"S, 34°50'16.36"W); 5 manatees held in situ captivity (natural environment at CMA, Barra de Mamanguape, state of Paraíba [CMA-PB]) (6°46'43.68"S, 34°55'50.88"W); and 12 manatees held in situ captivity at CMA-AL (9°13'05.47"S, 35°19'59.01"W), Porto de Pedras, state of Alagoas, all in northeastern Brazil.

Blood samples were collected by means of venipuncture in the brachial vascular bundle of the Antillean manatees. The blood was centrifuged, and the serum was separated, identified, and kept at -20°C until used for serologic tests. At the time of the sample collection, the animals' sex and age were ascertained. In relation to age, the animals were divided into the following: neonates (0–30 days), calves (31 days–2 yr), juveniles (2–6 yr), and adults (>6 yr).

The serum samples were assayed by means of the modified agglutination test (MAT).^{1,10} Sera were screened at dilutions of 1:25, 1:50, and 1:500, and positive samples were end-titrated using twofold serial dilutions. Positive and negative controls were included on each microplate. A titer of 1:25 was considered indicative of *T. gondii* infection.¹⁸ The sample collection had been authorized through a license for scientific purposes issued by SISBIO-ICMBio (number 20685).

RESULTS

Antibodies to *T. gondii* were found in 6 (10.9%) of the 55 Antillean manatees, with titers of 50 in 3, 500 in 1, 3,200 in 1, and 51,200 in 1 manatee. Three (10.7%) of the 28 males and 3 (11.1%) of the 27 females were seropositive. In relation to

age, the 10 neonates and 10 calves were negative, whereas 2 (7.1%) of the 28 juveniles and 4 (23.5%) of the 17 adults had antibodies to *T. gondii*. Regarding the origin, the prevalence at CMA/PE was 10.5% (4 of 38) and at CMA/AL was 16.7% (2 of 12). None of the five animals at CMA/PB presented antibodies to *T. gondii*. Two females at CMA/PE that had recently given birth were positive, but their calves did not present any *T. gondii* antibodies.

DISCUSSION

This is the first report of seroprevalence of *T. gondii* among captive Antillean manatees in Brazil. Seroprevalence of *T. gondii* infection similar to what was found in this study (10.9%) was also observed among free-living manatees in Belize (7%, 8 of 112).²¹

Most of the manatees examined were captive for 4.5–16 yr, increasing their chances for *T. gondii* exposure, possibly through intake of water or food contaminated by oocysts. Four of the six seropositive animals certainly became infected in captivity, because they have been born and had always lived at CMA/PE. The other two seropositive animals had arrived at CMA/PE while they were still neonates and lived there for 43 and 75 mo, respectively, before being sent to CMA/AL, which makes it impossible to state where the infection occurred.

In 2001, a latex agglutination test was performed as a control measure on the Antillean manatees kept at these centers at that time, to detect antibodies to *T. gondii*. On that occasion, only one female was positive (titer of 16,384). This was the same individual who, at the time of the present investigation, had the highest antibody titer (51,200) in the MAT. These data indicate that asymptomatic infected manatees can have high *T. gondii* agglutinating antibody titers.

With the exception of the female that was already positive in 2001, all serum samples tested up to the year 2009 were negative. These data indicate that the infection at these centers may have occurred after this date. The number of positive animals at CMA/PE increased from one to five animals subsequent to the evaluation in 2001.

The results indicate that, among captive Antillean manatees, there are risk factors for transmission of *T. gondii*. One important risk factor might be the presence of domestic cats and even small free-living Neotropical felids in areas surrounding the centers studied. Moreover, the manatees' diet

included lettuce, carrot, beetroot, and shoal grass (*Halodule wrightii*) that was acquired from local producers and also might have provided the transmission route for *T. gondii* oocysts.

In the present study, the young animals were seronegative, suggesting postnatal exposure in seropositive animals.

Toxoplasma gondii oocysts may sporulate in seawater and remain viable for many months.¹⁶ Because the enclosures of the Antillean manatees of this study were supplied with water pumped directly from the sea, this is a likely transmission route, given that feces from felids can easily reach seawater through sewage discharge or flooding.¹²

CONCLUSIONS

This report raises questions about *T. gondii* in captive manatees from Brazil, but the mechanism of transfer of parasite to the aquatic environment was not elucidated. Further studies are necessary to understand the health risks to endangered Antillean manatees such as the cycle and disease pathogenesis to *T. manatus manatus*.

Historically, Antillean manatees have been hunted for meat for human consumption among some riverbank populations in Brazil. Thus, ingestion of uncooked manatee meat could pose a public health concern.

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