OCURRENCE OF ANTIBODIES TO TOXOPLASMA GONDII AND LEPSTOSPIRA SPP. IN MANATEES (TRICHECHUS INUNGUIS) OF THE BRAZILIAN AMAZON


Abstract: The presence of Toxoplasma gondii and Leptospira spp. antibodies was investigated in 74 manatees (Trichechus inunguis [Mammalia: Sirenia]) kept in captivity in two rescue units in the northern region of Brazil. Antibodies to T. gondii were detected in 29 (39.2%) of 74 animals by using the modified agglutination test (titer, 1:25). For antibodies against Leptospira spp., sera were diluted 1:50 and tested against 24 strains of leptospires by microscopic agglutination microtechnique, and positive samples were end titrated. Twenty-three (31.1%) of 74 animals were reactive to four serovars (Patoc 21/23, Castellonis 2/23, Icterohaemorrhagiae 1/23, and Butembo 1/23), with titers ranging from 100 to 1,600. This is the first report of antibodies against T. gondii and Leptospira spp. in T. inunguis from the Brazilian Amazon.

Key words: Toxoplasma gondii, Leptospira spp., Trichechus inunguis, serology, Brazil.

INTRODUCTION

The manatee (Trichechus inunguis [Mammalia: Sirenia]) is distributed across the Amazon River basin. It is entirely aquatic and resides in calm waters of streams or lakes with floating vegetation. It is a herbivorous animal and can weigh up to 420 kg. Little is known of the causes of death or the prevalence of zoonotic organisms in manatees from Brazil; however, Toxoplasma gondii and Sarcocystis neurona, two related coccidians, have been reported to cause mortalities in marine mammals.

Toxoplasma gondii has a worldwide distribution, and in Brazil T. gondii antibodies were reported previously in 82.3% of free-living Amazon River dolphins (Inia geoffrensis) and 8.3% of captive T. manatus. Leptospira spp. are spirochetes that affect a wide variety of domestic and wild animals, including aquatic mammals such as pinnipeds, cetaceans, mustelids, and sirenians, in which cases of interstitial nephritis with clinical signs of renal failure (e.g., dehydration, polydipsia, vomiting, and depression) have been reported previously. The aim of this study was to evaluate the presence of T. gondii and Leptospira spp. antibodies in Amazonian manatees from two rescue units centers located in the Brazilian Amazon.

MATERIALS AND METHODS

Animals

Serum samples were collected from 74 Amazonian manatees (Trichechus inunguis) kept in captivity at two rehabilitation units; 70 of these manatees had been free-ranging animals that were taken into captivity and 4 were born in captivity. Of the 74 animals, 39 had been living at the National Institute of Amazonian Research (INPA) and 36 at the Aquatic Mammals Preservation and Research Center (CPPMA). INPA and CPPMA, 200 km distant from each other, are located in the cities of Manaus (3°6’0”S to 60°1’0”W) and Presidente Figueiredo, Balbina County (2°1’2”S to 60°1’30”W), respectively, both in the Amazon state, in northern Brazil.

The manatees were from 1 to 30 yr old (such ages represented the lifetime in captivity, because the animals arrived at these facilities as baby orphans). From these manatees, 25 were young (<5 yr old) and 49 were adults. The sex ratio was 36:38 (male:female).
Detection of antibodies

Detection of *T. gondii* antibodies was performed by the modified agglutination test (MAT) according to Dubey and Desmonts. The samples were examined at serum dilutions of 1:25, 1:50, and 1:500, and a titer of 1:25 was used as indicative of exposure to *T. gondii*. Negative and positive controls were used in all reactions.

The detection of anti-*Leptospira* spp. antibodies was performed by an agglutination test according to details in Faine et al. Sera were diluted at 1:50 and tested against 24 serovars. The titer of positive reactions was the reciprocal of the highest serum dilution in serum-antigen mixture that showed 50% of agglutinated leptospires per microscopic field.

Statistical analysis

The association of presence of antibodies against *T. gondii* and *Leptospira* spp. and place of sample collection (INPA or CPPMA), age (young or adult), and sex (male or female) was analyzed by chi-square and Fisher’s tests, and values were considered significant.

RESULTS

Antibodies to *T. gondii* were found in 29 (39.2%) of 74 manatees (Table 1), but only at a titer of 25. Seropositivity to *T. gondii* in manatees from INPA was 3.4 times (*P* < 0.05) higher than that in manatees from CPPMA. In both facilities, the age and sex of animals did not affect the occurrence of *T. gondii* antibodies (Table 1).

Seropositivity to *Leptospira* spp. was detected in 23 (31.1%) of 74 manatees, with titers of 100 to 1,600. The reactive serovars were as follows: Patoc (21/23), Castellonis (2/23), Icterohaemorrhagiae (1/23), and Butembo (1/23), and one manatee was positive to Patoc, Castellonis, and Icterohaemorrhagiae. The reactivity was approximately 6 times higher (*P* < 0.05) in manatees from INPA (51.3%) than those from CPPMA (8.6%). Seropositivity was higher in adult than young manatees (*P* < 0.05), but occurrences were unaffected by sex (*P* > 0.05) of the manatee (Table 1).

DISCUSSION

This study reports, for the first time, the presence of antibodies against *T. gondii* in *T. inunguis* born in captivity and from the wild. However, all positive manatees had only a titer of 25. The MAT used here is considered the most sensitive and specific test for the detection of *T. gondii* in animals. There is no reason to believe that an MAT titer of 25 is nonspecific, although the test has not been validated for the diagnosis of toxoplasmosis in manatees; the same test has been used in other species of aquatic mammals.

The ingestion of oocysts is probably the source of *T. gondii* in *T. inunguis* in this study, because these animals are herbivores. Whether manatees became infected in captivity is uncertain because these mammals had previously lived in rivers. Manatees inhabit the banks bordering of the main channels and deep lakes during the dry season and may have contact with sewage-polluted water. During floods, these animals are scattered in areas of flooded forest that can become infected by oocysts from the feces of wild cats living in the area.

At INPA, the facility that had the highest number of *T. gondii*-positive animals, domestic

### Table 1. Association between presence of anti-*T. gondii* antibodies and anti-*Leptospira* spp. in Amazonian manatees and variables: place of sample collection, age, and sex.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of animals examined</th>
<th><em>T. gondii</em></th>
<th></th>
<th></th>
<th>Leptospira spp.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td></td>
<td>Positive</td>
<td>P</td>
<td>Positive</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>INPA</td>
<td>39</td>
<td>23</td>
<td>58.9</td>
<td>20</td>
<td>51.3</td>
<td></td>
</tr>
<tr>
<td>CPPMA</td>
<td>35</td>
<td>6</td>
<td>17.1</td>
<td>3</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young (&lt;5)</td>
<td>25</td>
<td>9</td>
<td>36.0</td>
<td>4</td>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td>Adult (≥5)</td>
<td>49</td>
<td>20</td>
<td>40.8</td>
<td>19</td>
<td>38.8</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>36</td>
<td>17</td>
<td>47.2</td>
<td>13</td>
<td>36.1</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>38</td>
<td>12</td>
<td>31.6</td>
<td>10</td>
<td>26.3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>29</td>
<td>39.2</td>
<td>23</td>
<td>31.1</td>
<td></td>
</tr>
</tbody>
</table>

* INPA, National Institute of Amazonian Research, Manaus, Amazonas, Brazil.
* CPPMA, Aquatic Mammals Preservation and Research Center, Balbina County, Presidente Figueiredo, Amazonas, Brazil.
cats are present around the water tanks. The water that fills the tanks originate from artesian wells, and the grass, a major food source of these animals, is obtained from the periurban region. The remainder of the diet is composed of fruits and vegetables that are stored at the facilities for few days. At CPPMA, the facility that had the lower anti-\textit{T. gondii}-positive animals, the water used to fill the tanks was obtained by a nearby natural lake, and the grass was obtained from a rural environment. Fruits and vegetables are purchased and used on the same day. For both of these facilities, there is a lack of feline control. Hence, the first recommendation to decrease \textit{T. gondii} infection in the animals at these facilities should be the institution of a feline control program. Another recommendation would be to obtain a source of grass that might have no exposure to felines.

The present study also is the first report of \textit{T. inunguis} reactive for leptospires, and one third of manatees were exposed to one of the analyzed serovars of \textit{Leptospira}, and seropositivity was significantly higher in manatees at INPA. There also was a higher seropositivity in the older animals at INPA. This funding may indicate that the risk for contact with the bacteria and infection in tanks increases with increasing age.

There are many serovars of \textit{Leptospira} (≥250), and manatee sera were tested against 20 serogroups. A single animal can react simultaneously with more than one strain of \textit{Leptospira}. However, the serovar with the highest levels of antibodies observed is usually the cause of the infection. The Patoc serovar, a saprophyte, also causes cross-reactivity with pathogenic serovars; and because of its easy maintenance in laboratories, it is widely used as screening serovar in studies with leptospires.\textsuperscript{7} As a complementary diagnostic measure, the isolation of the bacteria followed by its molecular characterization is highly recommend-
ed.

In the Brazilian Amazon, several \textit{Leptospira} serovars have been reported in humans and in wild and domestic animals.\textsuperscript{8,20} However, the Icterohaemorrhagiae serovar has been identified as the main serovar responsible for clinical cases of human leptospirosis in that region.\textsuperscript{6} Despite the low occurrence of this serovar, it also was identified in one of the manatees we examined.

\textit{Leptospira} spp. are excreted in the urine of infected animals. The ingestion of contaminated water is the most likely source of infection for these manatees. The epidemiology of leptospirosis to a specific region can be summarized as a high infection frequency by serovars adapted to certain domestic species and a low infection frequency by serovars adapted to wild species, leading to accidental infection.\textsuperscript{16} It is very likely that the detection of antibody positive animals to leptospires was the result of contact with contaminated urine from terrestrial, domestic, and wild animals. These animals reside next to the facilities where the manatees are housed and thus serve as potential sources of leptospires for the aquatic environment.

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LITERATURE CITED


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