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BIFFI GARCIA, CLAUDIA; MALHEIROS, ANTONIO FRANCISCO; GARCIA DAVILA, CARMEN ROSA  
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## DETECTION OF INFECTION WITH *Toxoplasma gondii* IN MANATEES (*Trichechus inunguis*) OF THE PERUVIAN AMAZON

### Detección de infección por *Toxoplasma gondii* en manatíes (*Trichechus inunguis*) de la Amazonía peruana

PATRICK MATHEWS DELGADO<sup>1</sup>, M.Sc.; NOFRE SANCHEZ PEREA<sup>2</sup>,  
D.V.M.; JONH PAUL MATHEWS DELGADO<sup>3</sup>, Ph. D.; CLAUDIA BIFFI  
GARCIA<sup>4</sup>, D.V.M.; ANTONIO FRANCISCO MALHEIROS<sup>5</sup>, Ph. D.;  
CARMEN ROSA GARCIA DAVILA<sup>6</sup>, Ph. D.

<sup>1</sup> Department of Parasitology, Institute of Biomedical Science,  
University of São Paulo, Rua do Matão, Travessa R, 187,  
CEP 05508-900, São Paulo, SP, Brazil. patrickmathews@usp.br

<sup>2</sup> Department of Parasitology, Tropical Veterinary Research Institute,  
Iquitos, 2350, Peru. nofresp@hotmail.com

<sup>3</sup> Department of Ecology, National Institute of Amazonian Research,  
Manaus, AM, 2936. Brazil. fedormath@hotmail.com

<sup>4</sup> Faculty of Veterinary Medicine, University Alas Peruanas,  
Lima, 6002, Peru. clabif@hotmail.com

<sup>5</sup> Department of Biological Science, University of Estado de Mato  
Grosso, Av. São João, S/n, Campus Universitário, Cáceres, Mato  
Grosso, MT, CEP 78200-000, Brazil. malheiros@unemat.br

<sup>6</sup> Department of Aquatic, Research Institute of the Peruvian Amazon,  
Iquitos, 6018, Peru. Cdavila19@yahoo.com  
Corresponding author: patrickmathews@usp.br

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### ABSTRACT

The Amazonian manatee (*Trichechus inunguis*) is an aquatic mammal that inhabits freshwater environments and is endemic to the Amazon Basin. The presence of *Toxoplasma gondii* antibodies was investigated in 19 manatees in one rescue unit in the northern region of Peru. Antibodies to *T. gondii* were detected in 12 (63.2 %) of 19 animals by using the modified agglutination test (titer, 1:25), and no association between sex and age of the animals and the presence of *T. gondii* antibodies was observed ( $p < 0.05$ ). The results suggest a contamination by *T. gondii* oocysts in the aquatic environment where these animals live.

**Keywords:** Amazonian manatee, Peruvian Amazon, serology, *Toxoplasma gondii*.

### RESUMEN

El manatí amazónico (*Trichechus inunguis*) es un mamífero acuático que habita en ambientes de agua dulce y es endémico de la cuenca del Amazonas. La presencia de

*Toxoplasma gondii* se investigó en 19 manatíes, en una unidad de rescate en la región norte del Perú. Los anticuerpos contra *T. gondii* fueron detectados en 12 (63,2 %), de 19 animales mediante el uso de la prueba de aglutinación modificada (título, 1:25). No fue observada asociación entre el sexo y edad de los animales con la presencia de anticuerpos de *T. gondii* ( $p < 0,05$ ). Los resultados sugieren la contaminación por ooquistes de *T. gondii* en el medio acuático donde viven estos animales.

**Palabras clave:** Amazonía peruana, manatí amazónico, serología, *Toxoplasma gondii*.

The Amazonian manatee (*Trichechus inunguis*) is an aquatic mammal (Family Trichechidae) that inhabits freshwater environments. It is endemic to the Amazon Basin, and occurs from Marajó Island (at the mouth of the Amazon River in Brazil) to the headwaters of the floodplain in Colombia, Peru, Guyana, Surinam and Ecuador. It is exclusively aquatic and resides in calm waters of streams or lakes with abundant floating vegetation. It is a herbivorous animal and can weigh up to 420 Kg (Rosas, 1994; Borges *et al.*, 2008; Amaral *et al.*, 2010). Currently, the Amazonian manatee is classified as a vulnerable species according to the list of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Reeves *et al.*, 2007). The pressure exerted by hunting, combined with the low rate of reproduction of this animal has drastically reduced the populations of the Amazonian manatee (Reeves *et al.*, 2007).

*Toxoplasma gondii* has been reported to cause mortalities in several species of marine mammals, including sea otters (Dubey *et al.*, 2003; Dubey, 2010). Freshwater runoff has been suggested as a risk factor for *T. gondii* infection in California sea otters (Miller *et al.*, 2002). It has been suggested that enough *T. gondii* oocysts to infect marine life can be excreted by felids on land and subsequently washed in to the sea to infect marine life (Dabritz *et al.*, 2007). Little is known of the causes of death or the prevalence of zoonotic organisms in manatees from Peruvian Amazon. However, in other countries *T. gondii* antibodies were reported in manatees (Silva *et al.*, 2001; Bossart *et al.*, 2012; Mathews *et al.*, 2012).

Some diseases such as toxoplasmosis, besides affecting marine mammals, may be of importance as a menace to human health (Dubey, 2010). However, the status of aquatic mammals in Peru in respect to these agents is largely unknown, mainly because of the difficulty in obtaining diagnostic samples from free-ranging aquatic mammals. Serum samples were collected from 19 Amazonian manatees (*Trichechus inunguis*) kept in captivity at one rehabilitation unit; all are free-living manatees, who were taken into captivity. Blood was obtained by venipuncture and the serum was kept at  $-20^{\circ}\text{C}$  until the completion of serological tests. The 19 animals were living in Aquatic Ecosystems Program (PEA) located in the city of Iquitos ( $3^{\circ} 48' 48.9'' \text{N}$ ,  $073^{\circ} 19' 18.2'' \text{W}$ ), Department of Loreto in northern Peru.

The manatees were from one to six years from these manatees, eight were young ( $<5$  year old) and 11 were adults. The sex ratio was 10:9 (male: female); these animals were all clinically healthy.

Detection of *T. gondii* antibodies was performed by the modified agglutination test (MAT) according to Dubey and Desmonts (1987). 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* (Dubey *et al.*, 2003; Cabezón *et al.*, 2004; Santos *et al.*, 2011). Negative and positive controls were used in all reactions.

For the statistical analysis of the variables gender (male and female) and age (young and adults) we used the Chi-square ( $\chi^2$ ) test with significance level at 5 %, using the program EPI INFO version 3.5.1.

*Toxoplasma gondii* is an important pathogen in aquatic mammals and its presence in these animals may indicate the water contamination of aquatic environment by oocysts. Antibodies to *T. gondii* were found in 12 (63.2 %) of 19 manatees, but only at a titer of 25. The age and sex of animals did not affect the occurrence of *T. gondii* antibodies. There was no significant variance with regard to gender ( $p = 0.12$ , five of 10 [50.0 %] males were seropositive, and seven of nine [77.7 %] females were seropositive) or age of manatees ( $p = 0.62$ , 62.5 % seropositivity in five young, 63.6 % seropositivity in seven adults) (Table 1).

Variable	N° of animals examined	Positive	%	p
<b>Location</b>				
PEA*	19	12	63.2	
Age (yr)				0.622
Young (<5)	8	5	62.5	
Adult ( $\geq 5$ )	11	7	63.6	
Gender				0.128
Male	0	5	50.0	
Female	9	7	77.7	
Total	19	12	63.2	

Table 1. Association between presence of anti-*T. gondii* antibodies in Amazonian manatees and variables: age and sex. \* PEA, Aquatic Ecosystems Program, Iquitos, Loreto, Perú.

This study reports, for the first time, the presence of antibodies against *T. gondii* in *T. inunguis* in the Peruvian Amazon. However, all positive manatees had only a titer of 25. The MAT used here is considered the most sensitive and specific test (99.8 %) for the detection of *T. gondii* in domestic and wild animals (Dubey *et al.*, 2003). The same test has been used in other species of aquatic mammals (Mikaelian *et al.*, 2000; Lambourn *et al.*, 2001; Measures *et al.*, 2004; Dubey *et al.*, 2009; Bossart *et al.*, 2012).

Waste from domestic and wild cats containing oocysts of *T. gondii* can be carried by the water from sewage and rain polluting the rivers, brooks and lakes (Bowater *et al.*, 2003). A cat may excrete millions of oocysts and oocysts can remain viable from 32 days to about a year (Dubey, 2010; Esmerini *et al.*, 2010). The climate in the region is tropical humid, favoring the viability of oocysts. The ingestion of oocysts is probably the source of *T. gondii* in *T. inunguis* described 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. 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.

Knowledge of infectious agents prevalent in this species may have relevance to conservation. Furthermore, this knowledge is critical during the process of reintroduction of captive animals kept in nature, in order not to incorporate new diseases into the wild, which could compromise the conservation of this species and other free-living animals (Bossart *et al.*, 2012; Sulzner *et al.*, 2012; Gonzales *et al.*, 2013). This approach can also lead to the identification of diseases occurring in populations of wild animals associated with contamination of ecosystems with pathogens from human or domestic animals. It is noteworthy that infection with *T. gondii* can interfere with the abundance of aquatic mammals by promoting high mortality in some species (Dubey *et al.*, 2003; Dubey, 2010). The results obtained by us reinforce the importance of ongoing studies for the detection of pathogens that can compromise the health of the Amazonian manatee and other species of aquatic mammals in the Peruvian Amazon.

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