



Detection of infection with *Leptospira* spp. in manatees (*Trichechus inunguis*) of the Peruvian Amazon

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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 and Ecuador. It is exclusively aquatic and resides in calm waters of streams or lakes with abundant floating vegetation. It is an herbivorous animal and can weigh up to 420kg (Rosas, 1994; Amaral *et al.*, 2010). Little is known of the causes of death or the prevalence of zoonotic organisms in manatees from Peru; however, *Leptospira* spp. have been reported to cause mortalities in marine mammals (Visser *et al.*, 1991; Higgins, 2000; Mackereth *et al.*, 2005; Bossart *et al.*, 2012; Sulzner *et al.*, 2012).

Leptospira spp. are spirochetes that affect a wide variety of domestic and wild animals, including aquatic mammals such as pinnipeds, cetaceans, mustelids, and sirenians¹ (Colegrove *et al.*, 2005; Sulzner *et al.*, 2012). Here we evaluated the presence of *Leptospira* spp. antibodies in Amazonian manatees. To our knowledge, this is the first report of presence of antibodies against *Leptospira* spp. in aquatic mammals maintained in captivity in the Peruvian Amazon.

In January 2011, serum samples were collected from 19 Amazonian manatees which were previously free-ranging animals but then taken into captivity. The 19 animals were living at the Aquatic Ecosystems Program (PEA), a rescue center located in the city of Iquitos (03°48'48.9"N,

073°19'18.2"W), department of Loreto in northern Peru. The manatees ranged from one to five years old (such ages represented the lifetime in captivity, because the animals arrived at these facilities as baby orphans). Eight manatees were young (one to four years old) and 11 adults (five years old). The sex ratio was 10:9 (male:female) and all the animals were clinically healthy. Residence time of the animals in the rescue center was determined by their health conditions upon arrival at the facility, and regulatory approvals for reintroduction into the wild.

The detection of anti-*Leptospira* spp. antibodies was performed by a microscopic agglutination test (MAT) according to Lambourne *et al.* (1998) and Mackereth *et al.* (2005). The association of presence of antibodies against *Leptospira* spp. and age (young or adult), and sex (male or female) was analyzed by Chi-square and Fisher's tests, and $p \leq 0.05$ values were considered significant.

Seropositivity to *Leptospira* spp. was detected in 15 (78.9%) of 19 manatees, with titers of 1:600 to 3:400. The reactive *L. interrogans* serogroups were as follows: Patoc 2/15 (13.3%), Icterohaemorrhagiae 7/15 (46.6%), Iquitos 4/15 (26.7%), Cynopteri 1/15 (6.7%), and Canicola 1/15 (6.7%) and one manatee was positive to Patoc, Canicola, and Icterohaemorrhagiae. Seropositivity was higher in adults than young manatees ($p < 0.05$), but occurrences were unaffected by sex ($p > 0.05$) of the manatee (Table 1).

Leptospirosis is one of the most commonly encountered zoonoses worldwide. In the Peruvian Amazon, this disease has been identified as a menace to public health (Bharti *et al.*, 2003). Antibodies against leptospires were reported previously in captivity and wild populations of manatees

¹Faine, S. (1999) *Leptospira* and Leptospirosis. MediSci, Melbourne, Australia.

Table 1. Association between presence of anti-*Leptospira* spp. antibodies in Amazonian manatees and variables: age and sex. 2011. Significant values are in bold.

Variable	N° of animals examined	Positive	%	<i>p</i>
Location				
PEA*	19	15	78.9	
Age (yr)				0.032
Young (< 5)	8	5	62.5	
Adult (≥ 5)	11	10	90.9	
Gender				0.468
Male	10	8	80.0	
Female	9	7	77.7	
Total	19	15	78.9	

* PEA, Aquatic Ecosystems Program, Iquitos, Loreto, Peru.

in other countries² (Mathews *et al.*, 2012). In the Peruvian Amazon region, several mammal species of rodents, marsupials and bats have been confirmed as carrier hosts (Jori *et al.*, 2001; Matthias *et al.*, 2005) being able to infect other feral and domesticated animals (Bunnell *et al.*, 2000). This study reports, for the first time, the presence of antibodies against *Leptospira* spp. in *T. inunguis* in captivity in the Peruvian Amazon.

There are many serovars of *Leptospira* (≤ 250), and manatee sera were tested against 20 serogroups. A single animal can react simultaneously with more than one strain of *Leptospira*. 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 a screening serovar in studies with leptospires (Cullen *et al.*, 2004). As a complementary diagnostic measure, the isolation of the bacteria followed by its molecular characterization is highly recommended.

The MAT method used here is considered the 'gold standard' test for the serological diagnosis of leptospirosis¹. However, antibodies cross-reaction is common with the MAT and it is not unusual to see antibodies mounted against multiple serovars when there is only one infecting strain (Turner, 1968). In addition, the serovar against which the highest titer is mounted is not always the infecting strain. The only way to assess strain is through culture and isolation of the organism (Levett, 2003). PCR of leptospires shed in the urine or colonizing the kidneys can be used to identify the infecting organism to the serovar level (Ahmed *et al.*, 2012).

All serogroups reported in this study are of clinical

importance for human patients (Bharti *et al.*, 2003; Matthias *et al.*, 2005). However, the Icterohaemorrhagiae serovar has been identified as the main serovar responsible for clinical cases of human leptospirosis in that region (Johnson *et al.*, 2004). Despite the Icterohaemorrhagiae serogroup was the most prevalent with high titers in four out of the seven positive individuals, serogroup Iquitos was present in almost 26.7% of the sampled population.

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 (Radostits *et al.*, 2000). 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 to the aquatic environment.

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). It is also classified as threatened in the Red Book of Endangered Peruvian Fauna, Ministry of Environment (Pulido, 1991; 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). 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 back to nature, in order to not incorporate new diseases into the wild, which

²Silva, J.C.R., Marvulo, M.F.V., Picanço, M.C., Lima, R.P., Vergara-Parente, J.E., Marcondes, M.C.C., Ferreira, P.M., Morais, Z.M., Ogassawara, S., Vasconcellos, S.A. and Ferreira-Neto, J.S. (2001) Pesquisa de anticorpos anti-*Toxoplasma gondii*, *Leptospira interrogans* e *Brucella abortus* em peixes-bois-marinhos (*Trichechus manatus manatus*) mantidos em cativeiro. In Anais, X Congresso da Associação Brasileira de Veterinários De Animais Selvagens, 2000, ABRAVAS, São Paulo, Brazil.

could compromise the conservation of this species and other free-living animals. It is noteworthy that infection with *Leptospira* spp. can interfere with the abundance of aquatic mammals by promoting high mortality in some species or causing a decrease in fertility. 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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