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Short communication

Seroprevalence of *Toxoplasma gondii* in free-living Amazon River dolphins (*Inia geoffrensis*) from central Amazon, Brazil

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ABSTRACT

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. Serum samples from 95 free-living Amazon River dolphins (*Inia geoffrensis*) from the Mamirauá Sustainable Development Reserve (RDSM), Tefé, Amazonas, Central Amazon, Brazil were tested for *T. gondii* antibodies using the modified agglutination test (MAT). Antibodies (MAT ≥ 25) to *T. gondii* were found in 82 (86.3%) dolphins with titers of 1:25 in 24, 1:50 in 56, and 1:500 in 2. Results suggest a high level contamination of the aquatic environment of the home range of these animals.

1. Introduction

Toxoplasma gondii can cause mortality 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).

T. gondii infection in dolphins is biologically interesting because it can cause mortality in these animals (Dubey

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et al., 2003; Bowater et al., 2003; Dubey, 2010), and seroprevalence in these animals in Atlantic and Pacific ocean dolphins is very high (Dubey et al., 2003; Cabezón et al., 2004; Forman et al., 2009). This high seroprevalence is intriguing because dolphins drink little water (Dubey et al., 2003).

To our knowledge there is only one report of toxoplasmosis in an adult tucuxi (*Sotalia guianensis*) from Rio de Janeiro, Brazil (Bandoli and Oliveira, 1977). We report here for the first time prevalence of *T. gondii* antibodies in the Amazon River dolphin (*Inia geoffrensis*) or boto from Central Amazon, Brazil.

2. Materials and methods

Blood samples were collected from 95 Amazon River dolphins of both genders and various ages, free-living in the Mamiraua (64°45′W, 03°35′S) during capture/release expeditions of the Projeto Boto from 2001 to 2003. The capture and collection protocols for biological material are described in da Silva and Martin (2000). Blood was obtained by venipuncture and the serum was kept at −20 °C until the completion of serological tests.

Sera were assayed for antibodies to *T. gondii* by the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). Sera were screened in 1:25, 1:50, and 1:500 dilutions, and positive and negative controls were included in each run. A titer of 1:25 was considered indicative of *T. gondii* infection (Dubey et al., 2003; Cabezón et al., 2004).

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

3. Results

Antibodies to *T. gondii* were found in 82 of 95 (86.3%) botos with titers of 1:25 in 24 (29.3%), 50 in 56 (68.3%), and 500 in 2 (2.4%). There was no significant variance with regard to gender ($P = 0.93$, 45 of 52 [86.5%] males were seropositive, and 37 of 42 [88.1%] females were seropositive) or age of dolphins ($P = 0.6$, 85.7% seropositivity in 14 young, 87.0% seropositivity in 87 adults). Sixty-one dolphins were sampled more than once during the period; 42 dolphins were positive in all samplings; 5 animals were negative in all samplings; 13 dolphins that were seronegative in the first collection became positive in subsequent samplings; and 1 dolphin with a low MAT titer of 1:25 became negative in subsequent sampling.

4. Discussion

The high prevalence *T. gondii* antibodies in healthy Amazon River dolphins in the present study indicates that the infection by this pathogen is frequent. One dolphin with a low titer of 1:25 was seronegative in the second sampling; this could be due to test variability or due to transient *T. gondii* infection. Waste from domestic and wild cats containing oocysts of *T. gondii* can be carried by the water from sewage, agricultural waste and rain polluting the rivers, estuaries, coastal areas and beaches (Bowater et al., 2003).

The density of jaguars in the Mamiraua Reserve during the low water season is one of the highest reported for the species, reaching more than 10 individuals/100 km^2 (Ramalho, personal comm.). Thus, wild felids and domesticated cats may spread *T. gondii* oocysts in the environment. A cat may excrete millions of oocysts and oocysts can remain viable at 15–35 °C from 32 days to about a year (Dubey, 2010). The climate in the region is tropical humid, favoring the viability of oocysts. The Amazon River dolphin feeds on fish (Best and da Silva, 1993) and many of these fishes feed on shellfish. Although *T. gondii* does not multiply in cold blooded animals aquatic invertebrates and fish can be transport host at *T. gondii* oocysts (Lindsay et al., 2001; Arkush et al., 2003; Miller et al., 2008; Esmerini et al., 2010; Massie et al., 2010).

I. geoffrensis live in rivers where there is a significant seasonal variation in water level, with annual average amplitude of 10.6 m (Ramalho et al., 2009). The seasonal variation in water levels directly influences the habitat distribution and density of botos (Martin and da Silva, 2004a). Variations in the density of botos are substantially due to fish migration, dictated by changes in water level and concentrations of dissolved oxygen. These dolphins use preferably occupy the margins of main rivers, streams and lakes (Martin and da Silva, 2004a). None of the cities or riverside communities of the region have a sewage treatment system, facilitating the contact of these animals with the polluted waters, especially during the dry season when the water level is low and animals are more concentrated. During floods, dolphins are scattered in areas of flooded forests (Martin and da Silva, 2004b), which can become infected by oocysts from feces of wild cats living in the Reserve.

The Amazon River dolphin is a long-lived animal at the top of the food chain, and is therefore a sentinel of environmental contamination (Lailson-Brito et al., 2008). The species inescapably lives in close proximity to man, and consumes some of the same food. The results suggest a possible contamination by *T. gondii* oocysts in the aquatic environment where these animals live.

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References

Arkush, K.D., Miller, M.A., Leuteneggerd, C.M., Gardnerd, I.A., Packham, A.E., Heckeroth, A.R., Tenter, A.M., Barr, B.C., Conra, P.A., 2003. Molec-

ular and bioassay-based detection of *Toxoplasma gondii* oocyst uptake by mussels (*Mytilus galloprovincialis*). Int. J. Parasitol. 33, 1087–1097.
Bandoli, J., Oliveira, C., 1977. Toxoplasmose em *Sotalia guianensis* (Van Beneden, 1863), Cetacea-Delphinidae. Folha Med. 75, 459–468.
Best, R., da Silva, V.M.F., 1993. *Inia geoffrensis*. Mamm. Sp. 426, 1–8.
Bowater, R.O., Norton, J., Johnson, S., Hill, B., O'Donoghue, P., Prior, H., 2003. Toxoplasmosis in Indo-Pacific humpbacked dolphins (*Sousa chinensis*) from Queensland. Aust. Vet. J. 81, 627–632.
Cabezón, O., Resendes, A.R., Domingo, M.J.A., Agustí, C., Alegre, F., Mons, J.L., Dubey, J.P., Almería, S., 2004. Seroprevalence of *Toxoplasma gondii* antibodies in wild dolphins from the Spanish Mediterranean Coast. J. Parasitol. 90, 643–644.
da Silva, V.M.F., Martin, A.R., 2000. A Study of the Boto, or Amazon River Dolphin (*Inia geoffrensis*), in the Mamirauá Reserve, Brazil: Operation and Techniques. Occasional Paper of The IUCN Species Survival Commission 23, pp. 121–130.
Dabritz, H.A., Miller, M.A., Atwill, E.R., Gardner, I.A., Leutenegger, C.M., Melli, A.C., Conrad, P.A., 2007. Detection of *Toxoplasma gondii*-like oocysts in cat feces and estimates of the environmental oocyst burden. J. Am. Vet. Med. Assoc. 231, 1676–1684.
Dubey, J.P., 2010. Toxoplasmosis of Animals and Humans, 2nd edition. CRC Press, Boca Raton, FL, 313 pp.
Dubey, J.P., Desmonts, G., 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet. J. 19, 337–339.
Dubey, J.P., Zarnke, R., Thomas, N.J., Wong, S.K., Van Bonn, W., Briggs, M., Davis, J.W., Ewing, R., Mense, M., Kwok, O.C., Romand, S., Thulliez, P., 2003. *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona*, and *Sarcocystis canis*-like infections in marine mammals. Vet. Parasitol. 116, 275–296.
Esmerini, P.O., Gennari, S.M., Pena, H.F.J., 2010. Analysis of marine bivalve shellfish from the fish market in Santos city, São Paulo state, Brazil, for *Toxoplasma gondii*. Vet. Parasitol. 170, 8–13.
Forman, D., West, N., Francis, J., Guy, E., 2009. The sero-prevalence of *Toxoplasma gondii* in British marine mammals. Mem. Inst. Oswaldo Cruz 104, 296–298.
Lailson-Brito Jr., J., Dorneles, P.R., da Silva, V.M.F., Martin, A.R., Bastos, W.R., Azevedo-Silva, C.E., Azevedo, A.F., Torres, J.P.M., Malm, O., 2008. Dolphins as indicators of micropollutant trophic flow in Amazon basin. Oecol. Bras. 12, 531–541.
Lindsay, D.S., Phelps, K.K., Smith, S.A., Fuck, G., Sumner, S.S., Dubey, J.P., 2001. Removal of *Toxoplasma gondii* oocysts from sea water by eastern oysters (*Crassostrea virginica*). J. Eukaryot. Microbiol. 48, 197s–198s.
Martin, A.R., da Silva, V.M.F., 2004a. Number, seasonal movements, and residency characteristics of river dolphins in an Amazonian floodplain lake system. Can. J. Zool. 82, 1307–1315.
Martin, A.R., da Silva, V.M.F., 2004b. River dolphins and flooded forest: seasonal habitat use and sexual segregation of botos (*Inia geoffrensis*) in an extreme cetacean environment. J. Zool. 263, 295–305.
Massie, G.N., Ware, M.W., Villegas, E.N., Black, M.W., 2010. Uptake and transmission of *Toxoplasma gondii* oocysts by migratory, filter-feeding fish. Vet. Parasitol. 169, 296–303.
Miller, M.A., Gardner, I.A., Kreuder, C., Paradies, D.M., Worcester, K.R., Jessup, D.A., Dodd, E., Harris, M.D., Ames, J.A., Packham, A.E., Conrad, P.A., 2002. Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutrisnereis*). Int. J. Parasitol. 32, 997–1006.
Miller, M., Conrad, P., James, E.R., Packham, A., Toy-Choutka, S., Murray, M.J., Jessup, D., Grigg, M., 2008. Transplacental toxoplasmosis in a wild southern sea otter (*Enhydra lutris nereis*). Vet. Parasitol. 153, 12–18.
Ramalho, E.E., Macedo, J., Vieira, T.M., Valsecchi, J., Calvimontes, J., Marmontel, M., Queiroz, H.L., 2009. Ciclo hidrológico nos ambientes de várzea da Reserva de Desenvolvimento Sustentável Mamirauá – médio Rio Solimões, período de 1990 a 2008. UAKARI 5, 61–87.