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Short Communication

Serological evidence of *Toxoplasma gondii* infection in captive marine mammals in Mexico

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ABSTRACT

Toxoplasma gondii infection in marine mammals is important because they are considered as a sentinel for contamination of seas with *T. gondii* oocysts, and toxoplasmosis causes mortality in these animals, particularly sea otters. Serological evidence of *T. gondii* infection was determined in 75 captive marine mammals from four facilities in southern and central geographical regions in Mexico using the modified agglutination test (MAT). Antibodies (MAT, 1:25 or higher) to *T. gondii* were found in 55 (87.3%) of 63 Atlantic bottlenose dolphins (*Tursiops truncatus truncatus*), 3 of 3 Pacific bottlenose dolphins (*Tursiops truncatus gillii*), 2 of 4 California sea lions (*Zalophus californianus*), but not in 3 West Indian manatees (*Trichechus manatus*), and 2 Patagonian sea lions (*Otaria flavescens*). Seropositive marine mammals were found in all 4 (100%) facilities sampled. All marine mammals were healthy and there has not been any case of clinical toxoplasmosis in the facilities sampled for at least the last 15 years. The seroprevalence of *T. gondii* infection in marine mammals of the same species did not vary significantly with respect to sex and age. This is the first report on the detection of antibodies to *T. gondii* in marine mammals in Mexico.

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1. Introduction

Infections with *Toxoplasma gondii* occur in a variety of marine mammals (Dubey, 2010). Seroprevalence studies have revealed a wide distribution of *T. gondii* infections in wild and captive marine mammals in a number of countries in the American, European, and Asian continents (Dubey et al., 2003, 2005, 2009; Dubey, 2010). Infections with *T. gondii* cause morbidity and mortality in marine mammals (Dubey et al., 2003, 2007; Fayer et al., 2004; Dubey, 2010). Infection with *T. gondii* in marine mammals may result in disseminated disease in adults and occasionally in neonates (Dubey et al., 2009; Miller et al., 2008a; Dubey, 2010). Clinical toxoplasmosis in marine mammals has been reported in the USA, Europe and Australia (Dubey et al., 2003, 2009; Dubey, 2010) but we are not aware of any report from Mexico. Herein we report serological evidence for *T. gondii* infection in marine mammals raised in two geographical regions in Mexico.

2. Materials and methods

2.1. Marine mammals surveyed

Seventy five marine mammals were sampled from four marine mammal facilities in two geographical regions in Mexico (Table 1). Marine mammals were raised in captivity and were studied from January to December 2009. The number of marine mammals per facility ranged from 12 to 30 (median 16.5). Three facilities were located in the Mexican Caribbean in Quintana Roo State and one facility

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Table 1
Seroreactivity to *T. gondii* in marine mammals in four facilities in Mexico.

Species	Site								Total		
	S1		S2		S3		S4				
	No. of animals tested	No. positive	No. of animals tested	No. positive	No. of animals tested	No. positive	No. of animals tested	No. positive			
Tursiops truncatus truncatus	12	10	18	15	27	24	6	6	63	55	87
Tursiops truncatus gillii	0	0	0	0	0	0	3	3	3	3	100
Zalophus californianus	0	0	0	0	0	0	4	2	4	2	50
Trichechus manatus	0	0	0	0	3	0	0	0	3	0	0
Otaria flavecens	0	0	0	0	0	0	2	0	2	0	0

S1 = Cozumel; S2 = Isla Mujeres; S3 = Puerto Aventuras; S4 = Mexico City.

in Mexico City. The geographical coordinates of the sites surveyed are: Cozumel 20°25′N 86°55W, Isla Mujeres 21°14′10″N 86°44′08″W, Puerto Aventuras 20°30′42″N 87°14′03″W, and Mexico City 19°24′N 99°8″W. Marine mammals from the Caribbean ($n = 60$) included 57 adult Atlantic bottlenose dolphins (*Tursiops truncatus truncatus*), and 3 adult manatees (*Trichechus manatus*). Of these 57 dolphins, 31 were males and 26 females. Of the 3 manatees, one was male and two were females. Marine mammals from Mexico City (n = 15) included six Atlantic bottlenose dolphins (*Tursiops truncatus truncatus*), three Pacific bottlenose dolphins (*Tursiops truncatus gillii*), four California sea lions (*Zalophus californianus*), and two Patagonian sea lions (*Otaria flavescens*). Of the nine dolphins, three were adult males, four were adult females, and 2 were juvenile females. Of the four California sea lions, two were juvenile males, one adult male, and one adult female. All two Patagonian sea lions were adult males.

2.2. Serological examination

Sera were collected from whole blood by centrifugation and stored at −20 °C until tested. Marine mammal sera were tested for *T. gondii* antibodies using two-fold serial dilutions from 1:25 to 1: 3200 with the modified agglutination test (MAT, Dubey and Desmonts, 1987) using the reagents described in our earlier paper (Alvarado-Esquivel et al., 2011).

2.3. Statistical analysis

Statistical analysis was performed using Epi Info software version 3.5.1 (Centers for Disease Control and Prevention: http://wwwn.cdc.gov/epiinfo/). The Yates' corrected Chi square test was used for comparison of the frequencies among groups. A p-value of <0.05 was considered statistically significant.

3. Results

Antibodies to *T. gondii* were found in 55 (87.3%) of 63 Atlantic bottlenose dolphins (*Tursiops truncatus truncatus*), with titers of 1:25 in 14, 1:50 in 23, 1:100 in 11, 1:200 in two, 1:800 in one, 1:1600 in two, and 1:3200 or higher in two, in three of three *Tursiops truncatus gillii* (titers 1:100 in 1, and 1:200 in 2), in two (titer 1:25 in 1, and 1:100 in 1) of four California sea lions. Seropositive marine mammals were found in all four facilities sampled. The seroprevalence in mammals in the facility in Mexico City was 73.3%, while the seroprevalences in each of the three facilities in the Caribbean were 83.3%, 83.3% and 80% (Puerto Aventuras). Marine mammals from the Caribbean had a comparable seroprevalence of *T. gondii* infection to those from Mexico City ($p = 0.48$). The seroprevalence of *T. gondii* infection did not vary significantly in marine mammals of the same species with respect to sex and age.

4. Discussion

In this study, we found a wide distribution of *T. gondii* infection among marine mammals in Mexico. In fact,

seropositive marine mammals were found on all four facilities in central and southern regions in Mexico. The studied Mexican Caribbean is an important region for raising marine mammals in captivity in Mexico. In spite of the existence of seropositive mammals, all marine mammals studied were healthy and there has not been any case of clinical toxoplasmosis in the facilities sampled for at least the last 15 years. This suggests that *T. gondii* infection in marine mammals does not necessarily lead to disease.

Most of the animals in the present study were bottlenose dolphins. A very high *T. gondii* seroprevalence has been reported among free range and captive dolphins. In a study at a sea aquarium in Canada, antibodies to *T. gondii* were found in all seven captive bottlenose dolphins, and *T. gondii* was demonstrable in tissues of one dead dolphin and isolated from tissues of another diseased dolphin (Dubey et al., 2009). Seroprevalence of *T. gondii* in bottlenose dolphins on both coasts of the USA is very high (Dubey, 2010), but viable parasites have been rarely isolated. In a recent survey, *T. gondii* antibodies were found in only 16.8% of dead, stranded dolphins in South Carolina, and viable *T. gondii* was isolated from four of 18 dolphins (Dubey et al., 2011). This seroprevalence is much lower than in two previous studies conducted between 1999 and 2003 that found 96.8% of 94 dolphins from California and 100% of 47 dolphins from Florida were seropositive (Dubey et al., 2003). In a subsequent study from 2003 to 2004, 100% of 146 free-ranging dolphins from Florida and South Carolina were seropositive (Dubey et al., 2005). In an other survey, *T. gondii* antibodies were found in 51.9% of 52 dolphins from South Carolina that were stranded between 2005 and 2007, and viable *T. gondii* was isolated from three of 32 dolphins (Dubey et al., 2008). Reasons for differences in *T. gondii* seroprevalence in live versus dead stranded dolphins are unknown; data were obtained using standardized MAT test and all were analyzed by a single person (Dubey, 2010). The validity of the MAT for the detection of *T. gondii* antibodies in bottlenose dolphins has not been determined but a cut-off of 1:25 is widely used for the detection of *T. gondii* antibodies in animals and humans (Dubey, 2010). In one instance, a beluga whale (*Delphinapterus leucas*) that had histologically demonstrable *T. gondii* in tissues had only a low MAT titer of 1:25 (Mikaelian et al., 2000).

In the present study, seropositivity to *T. gondii* was found in both juvenile and adult marine mammals. How marine mammals might have contracted infection is not clear. Infections in marine mammals may result from contamination of sea water with *T. gondii* oocysts washed from land (Conrad et al., 2005; Dabritz et al., 2007; Miller et al., 2008b). An experimental *T. gondii* infection in grey seals has demonstrated that parasite oocysts may establish viable infection in seals and support the hypothesis that toxoplasmosis in marine mammals can be acquired from oocysts in surface water runoff and sewer discharge (Gajadhar et al., 2004). Therefore, feral cats shedding oocysts in the environment might have contributed to seropositivity. The importance of *T. gondii* infection by water has been examined by Jones and Dubey (2010). It has been demonstrated sporulation and long survival of *T. gondii* oocysts in seawater (Lindsay et al., 2003; Lindsay and Dubey, 2009). Infections with *T. gondii* in cetaceans are thought to interfere with population abundance, by inducing high mortalities, lowering reproductive success or by synergistically increasing the virulence of other diseases (Van Bressem et al., 2009).

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