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Isolation and Genetic Characterization of *Toxoplasma gondii* From Striped Dolphin (*Stenella coeruleoalba*) From Costa Rica

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ABSTRACT: *Toxoplasma gondii* infection in marine mammals is of interest because of mortality and mode of transmission. It has been suggested that marine mammals become infected with *T. gondii* oocysts washed from land to the sea. We report the isolation and genetic characterization of viable *T. gondii* from a striped dolphin (*Stenella coeruleoalba*), the first time from this host. An adult female dolphin was found stranded on the Pacific Coast of Costa Rica, and the animal died the next day. The dolphin had a high (1:6,400) antibody titer to *T. gondii* in the modified agglutination test. Severe nonsuppurative meningoencephalomyelitis was found in its brain and spinal cord, but *T. gondii* was not found in histological sections of the dolphin. Portions of its brain and the heart were bioassayed in mice for the isolation of *T. gondii*. Viable *T. gondii* was isolated from the brain, but not from the heart, of the dolphin. A cat fed mice infected with the dolphin isolate (designated TgSdCo1) shed oocysts. Genomic DNA from tachyzoites of this isolate was used for genotyping at 10 genetic loci, including SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico, and this TgSdCo1 isolate was found to be Type II.

Toxoplasma gondii infections are widely prevalent in human beings and other animals worldwide (Dubey and Beattie, 1988). Numerous studies reported the existence of *T. gondii* infections in marine mammals including sea otters, dolphins, seals, and whales (Dubey et al., 2003), and toxoplasmosis has been considered a cause of death in sea otters (Cole et al., 2000; Lindsay, Thomas et al., 2001; Dubey et al., 2003; Kreuder et al., 2003). A toxoplasmosis-like illness was reported in 8 stranded striped dolphins from Spain (Domingo et al., 1992) and Italy (Di Guardo, Agrimi et al., 1995; Di Guardo, Corradi et al., 1995); the diagnosis was based on finding *T. gondii*–like organisms in sections of brain. We report isolation of *T. gondii* from a striped dolphin (*Stenella coeruleoalba*) from Costa Rica, the first time from this host.

An adult female dolphin weighing 58 kg and 210 cm long was found alive, stranded on the Pacific coast of Costa Rica on 9 May 2006, and the animal died the next day. The dolphin was transported to the Departamento de Patología, Escuela Medicina Veterinaria, Universidad Nacional Autonoma, 3,000 Heredia, Costa Rica, where a necropsy examination was performed the same day.

Specimens of tissues were fixed in 10% buffered neutral formalin. For histological studies, paraffin-embedded sections were cut, stained with hematoxylin and eosin, and examined microscopically. Samples of serum, unfixed brain (37 g), and heart (57 g) were forwarded to the Animal Parasitic Diseases Laboratory, Beltsville, Maryland, for parasite examination. Deparaffinized sections of tissues were stained immunohistochemically with *T. gondii* and *Neospora caninum* polyclonal antibodies as described (Lindsay and Dubey, 1989).

Dolphin serum was tested for *T. gondii* antibodies using dilutions from 1:25 to 1:12,800 with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987).

Eight days elapsed between the day of death and bioassay of the dolphin tissues in mice. Brain and heart were homogenized, digested in acid–pepsin (Dubey, 1998), and processed for inoculation into mice. Brain homogenate was inoculated subcutaneously into 5 interferon gamma gene knock out (KO) mice (Dubey and Lindsay, 1998); homogenate of the heart was inoculated subcutaneously into 5 out-bred female Swiss Webster (SW) mice obtained from Taconic Farms, Germantown, New York, as described by Dubey et al. (2002). Tissue imprints of lungs and brain of the mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on day 49 postinoculation (PI) and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed on day 89 PI, and brains of all mice were examined for tissue cysts as described (Dubey and Beattie, 1988). The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues.

The 5 KO mice inoculated with the brain of the dolphin died (or killed when moribund) 24 or 25 days PI, and tachyzoites were found in their lungs. Tissues of the 2 KO mice that were killed on day 25 PI were fed to a *T. gondii*–free cat (287); the cat shed oocysts 6 days later. Oocysts were incubated in 2% sulfuric acid for 1 wk at room temperature on a shaker to allow sporulation. Sporulated oocysts were diluted 10-fold and aliquots were inoculated orally into 4 SW mice. The mice that were fed 100–100,000 counted oocysts died of acute toxoplasmosis 7–14 days PI, and tachyzoites were found in their mesenteric lymph nodes or lungs; mice inoculated with tachyzoites of this isolate remained asymptomatic, and tissue cysts were found in their brains 6 wk PI. The mice inoculated with the heart remained asymptomatic, and neither antibodies in their sera nor tissue cysts in their brain were found.

Toxoplasma gondii DNA was extracted from lung tissue of an infected mouse, and strain typing was performed using genetic markers SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico (Dubey, Sundar et al., 2006; Su et al., 2006) The isolate of *T. gondii* from the striped dolphin was genotype II based on all these markers and was designated TgSdCo1.

The dolphin had a severe nonsuppurative meningoencephalomyelitis, but *T. gondii* was not demonstrable histologically or immunohistochemically in sections of brain and spinal cord; *T. gondii* was not considered as the cause of death of the dolphin.

The ingestion of oocysts in contaminated food or water and the ingestion of *T. gondii*–infected tissues are the 2 main sources of postnatal *T. gondii* infection. The mechanism of *T. gondii* infection in marine mammals is most intriguing because most feed on fish or invertebrates, cold-blooded animals, or they are exclusively herbivorous, thus ingestion of *T. gondii*–infected meat is unlikely. Miller et al. (2002) presented evidence that land-based surface runoff was of significant risk for *T. gondii* infection in sea otters, so it is possible that *T. gondii* oocysts could be washed into the sea via runoff contaminated by cat excrement. The role of marine invertebrates in the life cycle of *T. gondii* is unknown. *Toxoplasma gondii* oocysts are extremely resistant to environmental influences and, therefore, likely to survive in the sea. *Toxoplasma gondii* does not parasitize any cold-blooded animals. However, molluscs can filter large quantities of water and may thus concentrate microbes from the water. Experimentally, *T. gondii* oocysts have been concentrated by mollusks (Lindsay, Phelps et al., 2001; Arkush et al., 2003).

Toxoplasma gondii infection of dolphins is intriguing because they drink little or no water, and their nutritional requirements are derived from fish, squid, or other cold-blooded sea animals that they consume. The prevalence of *T. gondii* antibodies in the bottle-nosed dolphin from the United States is very high (Dubey et al., 2003, 2005), but *T. gondii* has not been isolated from this host.

Among marine mammals, viable *T. gondii* has been isolated from sea otters (Cole et al., 2000; Miller et al., 2001), Pacific harbor seals (Miller et al., 2001), and a California sea lion (Conrad et al., 2005). Based on limited markers, all *T. gondii* sea otter isolates were identified as Type II (Cole et al., 2000). Based on *T. gondii* antigen loci B1, SAG1, SAG2,

SAG3, and GRA6, a new genotype X was proposed for the most of the sea otter *T. gondii* isolates (Miller et al., 2004). Thirty-eight of 50 isolates of *T. gondii* from sea otters from California and the isolate from the harbor seal and the California sea lion, were typed as genotype X, whereas 12 of 50 sea otter isolates were Type II (Conrad et al., 2005), suggesting that the type X genotype predominates in marine mammals in this particular geographical region, which is in contrast to Type II genotype that is widespread in North America and Europe. Our finding of a Type II isolate from dolphin in Costa Rica pacific coastal area suggests that Type II genotype may circulate in a variety of hosts globally.

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