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# ISOLATION OF *TOXOPLASMA GONDII* FROM BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*)

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ABSTRACT: *Toxoplasma gondii* infection in marine mammals is intriguing and indicative of contamination of the ocean environment and coastal waters with oocysts. In previous serological surveys, >90% of bottlenose dolphins (*Tursiops truncatus*) from the coasts of Florida, South Carolina, and California had antibodies to *T. gondii* by the modified agglutination test (MAT). In the present study, attempts were made to isolate *T. gondii* from dead *T. truncatus*. During 2005, 2006, and 2007, serum or blood clot, and tissues (brain, heart, skeletal muscle) of 52 *T. truncatus* stranded on the coasts of South Carolina were tested for *T. gondii*. Antibodies to *T. gondii* (MAT 1:25 or higher) were found in 26 (53%) of 49 dolphins; serum was not available from 3 animals. Tissues (heart, muscle, and sometimes brain) of 32 dolphins (26 seropositive, 3 seronegative, and 3 without accompanying sera) were bioassayed for *T. gondii* in mice, or cats, or both. Tissues of the recipient mice were examined for *T. gondii* stages. Feces of recipient cats were examined for shedding of *T. gondii* oocysts, but none excreted oocysts. *Toxoplasma gondii* was isolated from hearts of the 3 dolphins (2 with MAT titers of 1:200, and 1 without accompanied serum) by bioassay in mice. Genotyping of these 3 *T. gondii* isolates (designated TgDoUs1-3) with the use of 10 PCR-RFLP markers (SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico) revealed 2 genotypes. Two of the 3 isolates have Type II alleles at all loci and belong to the clonal Type II lineage. One isolate has a unique genotype. This is the first report of isolation of viable *T. gondii* from *T. truncatus*.

*Toxoplasma gondii* infections are widely prevalent in humans 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; Kreuder et al., 2003; Conrad et al., 2005; Thomas et al., 2007). A toxoplasmosis-like illness was reported in Atlantic bottlenose dolphins (*Tursiops truncatus*) from the United States (Migaki et al., 1977; Cruickshank et al., 1990; Inskeep et al., 1990; Shulman et al., 1997; Dubey et al., 2003), and Italy (Di Guardo, Agrimi et al., 1995; Di Guardo, Corradi et al., 1995). Serologic surveys indicated a very high prevalence of antibodies to *T. gondii* in *T. truncatus* from both coasts of the United States (Dubey et al., 2003, 2005). We report isolation of *T. gondii* from *T. truncatus*, the first time from this host.

## MATERIALS AND METHODS

### Naturally infected dolphins and sample collection

During 2005, 2006, and 2007, serum or blood clot, and tissues (brain, heart, skeletal muscle) from 52 stranded bottlenose dolphins were collected during necropsies conducted by the NOAA/National Ocean Service/Center for Coastal Environmental Health and Biomolecular Research as part of the Southeast U.S. Marine Mammal Stranding Network. The samples were shipped overnight with cold packs to the Animal Parasitic Diseases Laboratory (APDL), U.S. Department of Agriculture, Beltsville, Maryland, for *T. gondii* tests.

### Serological examination

Serum samples were available from 49 of 52 dolphins. Dolphin serum was tested for *T. gondii* antibodies with dilutions from 1:25 to 1:3,200 with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987).

### Bioassay for *T. gondii*

Tissues of 32 dolphins (26 seropositive, 3 seronegative, and 3 without accompanying sera) were bioassayed for *T. gondii* in mice, cats, or both. Samples (50 g) of brain, heart, and skeletal muscle were homogenized separately, digested in acid–pepsin (Dubey, 1998), and processed for inoculation into 5–15 outbred female Swiss Webster (SW) mice obtained from Taconic Farms, Germantown, New York, as described by Dubey et al. (2002).

For bioassay in cats, samples of heart and muscle of 13 dolphins (9 seropositive, 3 seronegative, 1 without serum) were fed separately to 10 *T. gondii*–free cats (Dubey et al., 2002). Feces of cats were examined for shedding of *T. gondii* oocysts 3–14 days after feeding dolphin tissues as previously described. Fecal floats were incubated in 2% sulfuric acid for 1 wk at room temperature on a shaker to allow sporulation of oocysts and were bioassayed by oral administration to mice (Dubey and Beattie, 1988).

Inoculated mice were examined for *T. gondii* infection. Tissue imprints of lungs and brains of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on Day 41 postinoculation (PI) and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 43 days 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.

### Genetic characterization

*Toxoplasma gondii* DNA was extracted from tissues of mice and strain typing was performed with genetic markers SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico (Dubey et al., 2006; Su et al., 2006).

## RESULTS

Antibodies to *T. gondii* were found in 27 of 52 dolphins with MAT titers of 1:25 in 2, 1:50 in 6, 1:100 in 3, 1:200 in 7, 1:400 in 3, 1:800 in 4, and 1:3,200 or higher in 2. *Toxoplasma gondii* was isolated from the heart of 3 dolphins (Table I). The strains from dolphins were designated TgDoUs1-3.

These isolates were grouped into 2 genotypes (Table II). TgDoUs1 has identical alleles to the reference strain MAS, except at the locus c22-8. TgDoUs2 and TgDoUs3 are identical to the clonal Type II strain at all 10 loci.

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TABLE I. Isolation of *Toxoplasma gondii* from stranded dolphins.

| Dolphin identifier | Date stranded | Modified agglutination test | Bioassay in mice* | | | Subpassage† | Strain designation |
|---|---|---|---|---|---|---|---|
| | | | Brain | Heart | Muscle | | |
| SC0507‡ | 25 March 2005 | No serum | 0/5 | 1/15 | 0/5 | 5/5 | TgDoUs1 |
| SC0708§ | 27 March 2007 | 200 | 0/5 | 1/5 | 0/5 | 2/2 | TgDoUs2 |
| SC0712‖ | 26 April 2007 | 200 | No sample | 1/5 | 0/5 | 2/2 | TgDoUs3 |

* Number of mice *T. gondii* positive/number of mice inoculated.
† Mice infected with *T. gondii*/number inoculated. These mice remained asymptomatic.
‡ Male, stranded on Daufuskie Island, Beaufort County, South Carolina (latitude 32.1229N; longitude 80.8401W).
§ Female, stranded on Toogoodoo Creek, Charleston County, South Carolina (latitude 32.65629N; longitude 80.26395W).
‖ Male, Daufuskie Island, Beaufort County, South Carolina (latitude 32.1229N; longitude 80.8401W).

## DISCUSSION

In the present study, *T. gondii* was isolated from heart tissue of only 3 dolphins. Tissues of 50% of dolphins had autolyzed. Additionally, brain tissue was not available from most of the animals because of the difficulties in removing brain under field conditions. It is of interest that the parasite was isolated only from the hearts of dolphins. Finding *T. gondii* in only 3 of 25 mice inoculated with heart tissue of infected dolphin indicates the presence of only a few viable *T. gondii* in each inoculum.

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, i.e., cold-blooded animals, or they are exclusively herbivorous; thus, ingestion of *T. gondii*–infected meat is unlikely. *Toxoplasma gondii* infection of dolphins is even more intriguing because they drink little or no water; their water requirements are derived from fish, squid, or other cold-blooded sea animals they consume (Elsner, 1999). Miller et al. (2002) presented evidence that land-based surface runoff was a 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 molluscs (Lindsay, Phelps et al., 2001; Arkush et al., 2003).

Among marine mammals, viable *T. gondii* has been isolated along the west coast of the United States from sea otters (Cole et al., 2000; Lindsay, Thomas et al., 2001; Miller et al., 2001, 2004; Conrad et al., 2005), a Pacific harbor seal (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 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). These observations suggest 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. *Toxoplasma gondii* isolate from a striped dolphin (*Stenella coeruleoalba*) from Costa Rica was Type II (Dubey et al., 2007).

Genotyping of the 3 *T. gondii* isolates from bottlenose dolphins in this study identified 2 genotypes, including the clonal

TABLE II. Genotyping of *Toxoplasma gondii* isolates from bottlenose dolphins.

| Genotypes | *T. gondii* isolate | Genetic markers | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SAG1* | (5′ + 3′) SAG2† | SAG2‡ | SAG3 | BTUB | GRA6 | c22-8 | c29-2 | L358 | PK1 | Apico |
| Reference | RH88 | I | I | I | I | I | I | I | I | I | I | I |
| Reference | PTG | II or III | II | II | II | II | II | II | II | II | II | II |
| Reference | CTG | II or III | III | III | III | III | III | III | III | III | III | III |
| Reference | COUGAR | I | II | II | III | II | II | II | u-1 | I | u-2 | I |
| Reference | MAS | u-1 | I | II | III | III | III | u-1 | I | I | III | I |
| Reference | TgCatBr5 | I | III | III | III | III | III | I | I | I | u-1 | I |
| | TgDoUs1 | u-1 | I | II | III | III | III | III | I | I | III | I |
| | TgDoUs2 | II or III | II | II | II | II | II | II | II | II | II | II |
| | TgDoUs3 | II or III | II | II | II | II | II | II | II | II | II | II |

* At SAG1 locus, Types II and III are indistinguishable.
† The SAG2 marker based on 5′- and 3′-end DNA sequence polymorphisms of SAG2 gene (Howe et al., 1997).
‡ The SAG2 marker developed recently based on 5′-end DNA sequence of SAG2 gene is able to identify additional alleles often seen in atypical *T. gondii* strains (Su et al., 2006).

Type II type and a unique genotype. The unique genotype is different from the genotype x identified in sea otters from California (Conrad et al., 2005). Recently, an isolate of *T. gondii* from a striped dolphin was isolated in the Pacific Coast of Costa Rica and it was a Type II strain (Dubey et al., 2007). In the current study, 2 of the 3 isolates were Type II strains, which further supports the observation that the clonal Type II lineage is widespread in marine mammals.

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## LITERATURE CITED

ARKUSH, K. D., M. A. MILLER, C. M. LEUTENEGGER, I. A. GARDNER, A. E. PACKHAM, A. R. HECKEROTH, A. M. TENTER, B. C. BARR, AND P. A. CONRAD. 2003. Molecular and bioassay-based detection of *Toxoplasma gondii* oocyst uptake by mussels (*Mytilus galloprovincialis*). International Journal for Parasitology **33:** 1087–1097.

COLE, R. A., D. S. LINDSAY, D. K. HOWE, C. L. RODERICK, J. P. DUBEY, N. J. THOMAS, AND L. A. BAETEN. 2000. Biological and molecular characterizations of *Toxoplasma gondii* strains obtained from southern sea otters (*Enhydra lutris nereis*). Journal of Parasitology **86:** 526–530.

CONRAD, P. A., M. A. MILLER, C. KREUDER, E. R. JAMES, J. MAZET, H. DABRITZ, D. A. JESSUP, F. GULLAND, AND M. E. GRIGG. 2005. Transmission of *Toxoplasma*: Clues from the study of sea otters as sentinels of *Toxoplasma gondii* flow into the marine environment. International Journal for Parasitology **35:** 1155–1168.

CRUICKSHANK, J. J., D. M. HAINES, N. C. PALMER, AND D. J. ST AUBIN. 1990. Cysts of *Toxoplasma*-like organisms in an Atlantic bottlenose dolphin. Canadian Veterinary Journal **31:** 213–215.

DI GUARDO, G., U. AGRIMI, L. MORELLI, G. CARDETI, S. TERRACCIANO, AND S. KENNEDY. 1995. Post mortem investigations on cetaceans found stranded on the coasts of Italy between 1990 and 1993. Veterinary Record **136:** 439–442.

———, A. CORRADI, U. AGRIMI, N. ZIZZO, L. MORELLI, L. PERILLO, L. KRAMER, E. CABASSI, AND S. KENNEDY. 1995. Neuropathological lesions in cetaceans found stranded from 1991 to 1993 on the coasts of Italy. European Journal of Veterinary Pathology **1:** 47–51.

DUBEY, J. P. 1998. Refinement of pepsin digestion method for isolation of *Toxoplasma gondii* from infected tissues. Veterinary Parasitology **74:** 75–77.

———, AND C. P. BEATTIE. 1988. Toxoplasmosis of animals and man. CRC Press, Boca Raton, Florida, 220 p.

———, AND G. DESMONTS. 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Veterinary Journal **19:** 337–339.

———, P. A. FAIR, G. D. BOSSART, D. HILL, R. FAYER, C. SREEKUMAR, O. C. H. KWOK, AND P. THULLIEZ. 2005. A comparison of four serologic tests to detect antibodies to *Toxoplasma gondii* in naturally-exposed bottlenose dolphins (*Tursiops truncatus*). Journal of Parasitology **91:** 1074–1081.

———, D. H. GRAHAM, C. R. BLACKSTON, T. LEHMANN, S. M. GENNARI, A. M. A. RAGOZO, S. M. NISHI, S. K. SHEN, O. C. H. KWOK, D. E. HILL, AND P. THULLIEZ. 2002. Biological and genetic characterisation of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from São Paulo, Brazil: Unexpected findings. International Journal for Parasitology **32:** 99–105.

———, J. A. MORALES, N. SUNDAR, G. V. VELMURUGAN, C. R. GONZÁLEZ-BARRIENTOS, G. HERNÁNDEZ-MORA, AND C. SU. 2007. Isolation and genetic characterization of *Toxoplasma gondii* from striped dolphin (*Stenella coeruleoalba*) from Costa Rica. Journal of Parasitology **93:** 710–711.

———, N. SUNDAR, N. PINEDA, N. C. KYVSGAARD, L. A. LUNA, E. RIMBAUD, J. B. OLIVEIRA, O. C. H. KWOK, Y. QI, AND C. SU. 2006. Biologic and genetic characteristics of *Toxoplasma gondii* isolates in free-range chickens from Nicaragua, Central America. Veterinary Parasitology **142:** 47–53.

———, R. ZARNKE, N. J. THOMAS, S. K. WONG, W. VAN BONN, M. BRIGGS, J. W. DAVIS, R. EWING, M. MENSEA, O. C. H. KWOK, S. ROMAND, AND P. THULLIEZ. 2003. *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona*, and *Sarcocystis canis*-like infections in marine mammals. Veterinary Parasitology **116:** 275–296.

ELSNER, R. 1999. Living in water: Solutions to physiological problems. *In*: Biology of marine mammals, J. E. Reynolds and S. A. Rommel (eds.). Smithsonian Institution Press, Washington, DC, p. 73–116.

HOWE, D. K., S. HONORÉ, F. DEROUIN, AND L. D. SIBLEY. 1997. Determination of genotypes of *Toxoplasma gondii* strains isolated from patients with toxoplasmosis. Journal of Clinical Microbiology **35:** 1411–1414.

INSKEEP, W., C. H. GARDINER, R. K. HARRIS, J. P. DUBEY, AND R. T. GOLDSTON. 1990. Toxoplasmosis in Atlantic bottle-nosed dolphins (*Tursiops truncatus*). Journal of Wildlife Diseases **26:** 377–382.

KREUDER, C., M. A. MILLER, D. A. JESSUP, L. J. LOWENSTINE, M. D. HARRIS, J. A. AMES, T. E. CARPENTER, P. A. CIBRAD, AND J. A. K. MAZET. 2003. Patterns of mortality in southern sea otters (*Enhydra lutris nesris*) from 1998–2001. Journal of Wildlife Diseases **39:** 495–509.

LINDSAY, D. S., K. K. PHELPS, S. A. SMITH, G. FLICK, S. S. SUMNER, AND J. P. DUBEY. 2001. Removal of *Toxoplasma gondii* oocyst from sea water by eastern oysters (*Crassostrea virginica*). Journal of Eukaryotic Microbiology **48**(Suppl.): 197S–198S.

———, N. J. THOMAS, A. C. ROSYPAL, AND J. P. DUBEY. 2001. Dual *Sarcocystis neurona* and *Toxoplasma gondii* infection in a Northern sea otter from Washington state, USA. Veterinary Parasitology **97:** 319–327.

MIGAKI, G., J. F. ALLEN, AND H. W. CASEY. 1977. Toxoplasmosis in a California sea lion (*Zalophus californianus*). American Journal of Veterinary Research **38:** 135–136.

MILLER, M. A., I. A. GARDNER, C. KREUDER, D. M. PARADIES, K. R. WORCESTER, D. A. JESSUP, E. DODD, M. D. HARRIS, J. A. AMES, A. E. PACKHAM, AND P. A. CONRAD. 2002. Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). International Journal for Parasitology **32:** 997–1006.

———, M. E. GRIGG, C. KREUDER, E. R. JAMES, A. C. MELLI, P. R. CROSBIE, D. A. JESSUP, J. C. BOOTHROOYD, D. BROWNSTEIN, AND P. A. CONRAD. 2004. An unusual genotype of *Toxoplasma gondii* is common in California sea otters (*Enhydra lutris nereis*) and is a cause of mortality. International Journal for Parasitology **34:** 275–284.

———, K. SVERLOW, P. R. CROSBIE, B. C. BARR, L. J. LOWENSTINE, F. M. GULLAND, A. PACKHAM, AND P. A. CONRAD. 2001. Isolation and characterization of two parasitic protozoa from a pacific harbor seal (*Phoca vitulina richardsi*) with meningoencephalomyelitis. Journal of Parasitology **87:** 816–822.

SHULMAN, F. Y., T. P. LIPSCOMB, D. MOFFETT, A. E. KRAFFT, J. H. LICHY, M. M. TSAI, J. K. TAUBENBERGER, AND S. KENNEDY. 1997. Histologic, immunohistological, and polymerase chain reaction studies of bottlenose dolphins from the 1987–1988 United States Atlantic coast epizootic. Veterinary Pathology **34:** 288–295.

SU, C., X. ZHANG, AND J. P. DUBEY. 2006. Genotyping of *Toxoplasma gondii* by multilocus PCR-RFLP markers: A high resolution and simple method for identification of parasites. International Journal for Parasitology **36:** 841–848.

THOMAS, N. J., J. P. DUBEY, D. S. LINDSAY, R. A. COLE, AND C. U. METEYER. 2007. Protozoal meningoencephalitis in sea otters (*Enhydra lutris*): A histopathological and immunohistochemical study of naturally-occurring cases. Journal of Comparative Pathology **137:** 102–121.