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Long-Term Survival of *Toxoplasma gondii* Sporulated Oocysts in Seawater

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ABSTRACT: *Toxoplasma gondii* is now recognized as an important pathogen in costal marine mammals. Oocysts from cat feces are believed to be washed into seawater and serve as a source of infection via transport hosts. Experimentally, it has been demonstrated that *T. gondii* oocysts can sporulate in seawater and remain infectious for mice for up to 6 mo. The present study examined the long-term survival of *T. gondii* in seawater (15 ppt NaCl) kept at 4 C or at room temperature. Oocysts kept at 4 C for 24 mo were orally infectious for mice, while those kept at room temperature for 24 mo were not.

Cole et al. (2000) isolated *Toxoplasma gondii* from sea otters with encephalitis; they hypothesized that oocysts were making their way into the marine environment and that marine mammals were becoming infected by ingesting marine invertebrates, which were acting as phoretic hosts. This hypothesis is now widely accepted (see Conrad et al., 2005). We demonstrated that unsporulated oocysts of *T. gondii* would sporulate in seawater and that these sporulated oocysts were infectious to mice for up to a 6-mo storage in seawater (Lindsay et al., 2003). Lindsay et al. (2001) established that eastern oysters (*Crassostrea virginica*) could remove *T. gondii* oocysts from seawater and that sporulated *T. gondii* could remain viable in oysters for 85 days (Lindsay et al., 2004). Arkush et al. (2003) showed that mussels (*Mytilus galloprovincialis*) could remove *T. gondii* oocysts from seawater; they found that viable oocysts were present in mussels for 3 days and that *T. gondii* DNA could be detected by PCR for up to 21 days after exposure (Arkush et al., 2003). Recently, Miller et al. (2008) detected *T. gondii* DNA from 1 of 1,396 naturally exposed California mussels (*Mytilus californianus*) from Monterey Bay, California. Tissue from this mussel was repeatedly positive by PCR examinations, but it is not known if the oocysts present were infective because animal inoculation was not attempted (Miller et al., 2008). The purpose of the present study was to examine the long-term survival of sporulated *T. gondii* oocysts in seawater kept at 4 C or at room temperature (RT).

Oocysts of *T. gondii* collected from the feces of a cat fed tissues were from a naturally infected chicken from New England (Dubey et al., 2003) and had sporulated in seawater (15 ppt), as previously reported (Lindsay et al., 2003). Oocysts were counted in a hemocytometer, and the volume was adjusted so that 0.5 ml contained 5×10^5 oocysts for the inoculation of mice. Mice were given *T. gondii* oocysts orally using an animal feeding needle. These oocysts were from the same source as those previously demonstrated to be infectious for mice for 6 mo (Lindsay et al., 2003). Groups of 2 CD-1 female mice were housed together and fed a commercial laboratory diet and water, ad libitum. Impression smears were made from the lungs or liver of mice that died, and smears were examined, unstained, for tachyzoites of *T. gondii*. Surviving mice were killed 4–8 wk post-inoculation. Their brains were removed, and smears were made from both cerebral hemispheres of the brain and were examined, unstained, for *T. gondii* tissue cysts using a light microscope. The inoculation times post-storage, and the results of inoculations, are presented in Table I.

Oocysts of *T. gondii* remained infectious for mice when stored at 4 C for 24 mo in seawater (Table I). Clinical signs of toxoplasmosis (ruffled hair, labored breathing, lateral recumbence), and deaths, occurred in mice orally inoculated with oocysts that had been stored in seawater at 4 C during the entire study. Mice in all groups, except 1 of 2 mice inoculated with oocysts stored in seawater at 4 C for 15 mo, died or were killed because of acute toxoplasmosis. The infectivity of *T. gondii* oocysts kept at RT began to decrease at 15 to 18 mo of storage; both mice inoculated with oocysts stored for 15 mo survived and were clinically normal, and only 1 of 2 mice inoculated with oocysts stored for 18 mo became infected. *Toxoplasma gondii* oocysts did not survive for 24 mo in seawater kept at RT (Table I). Due to a refrigerator malfunction (freezing of samples), the infectivity of *T. gondii* oocysts kept in seawater at 4 C could not be evaluated after 2 yr.

TABLE I. Protocol and results of time of storage, and temperature, on the infectivity of sporulated *Toxoplasma gondii* oocysts* for mice.

Oocysts stored for	Mice fed oocysts stored at: 4 C†	Room temperature
1 yr	2/2	2/2
1 yr 3 mo	2/2	2/2
1 yr 6 mo	2/2	2/1
2 yr	2/2	2/0

* Oocysts orally administered, 5×10^5.
† Number of mice inoculated/number of mice positive.

Limited research has been done on the survival of *T. gondii* in the environment. Frenkel et al. (1975) demonstrated that sporulated *T. gondii* oocysts in soil could survive for 18 mo. Drinking unfiltered water is a risk factor for obtaining *T. gondii* infections (Bahia-Oliveira et al., 2003), and outbreaks of waterborne infections have been reported (see Dubey, 2004). Dubey (1998) examined the survival of sporulated *T. gondii* oocysts in water under defined conditions. In that study, sporulated *T. gondii* oocysts kept at 4 C were infectious in mice for 54 mo (Dubey, 1998). Oocysts kept in water at 20 C and 25 C did not loose infectivity after 6 mo of storage (Dubey, 1998). *Toxoplasma gondii* survived in water at RT for 18 mo (Hutchison, 1967).

The present study demonstrates that sporulated *T. gondii* oocysts will survive in seawater for at least 24 mo. Additional studies need to be conducted in costal marine environments to determine the phoretic hosts for *T. gondii* in these environments and to determine if *T. gondii* can be isolated from seawater in these locations.

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