

## Review articles

# Are poikilothermic animals real hosts for *Toxoplasma gondii*?

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**ABSTRACT.** The protozoan *Toxoplasma gondii* is believed to be a common parasite of almost all endothermic animals and humans. However, recent reports of toxoplasmosis in marine mammals raise concern that cold-blooded animals may also be a potential source of *T. gondii* infection. The article discusses the presence of *T. gondii* in aquatic and terrestrial poikilothermic animals, which may be important elements in the transmission of the parasite.

**Key words:** *T. gondii*, poikilothermic hosts, sea water

*Toxoplasma gondii*, an important protozoan parasite of major public health importance, is found worldwide. The literature suggests it can infect virtually all warm-blooded vertebrates, including birds, livestock, marine mammals and humans [1]. Horizontal transmission of the parasite occurs through environmentally-resistant oocysts, tissue cysts containing bradyzoites and occasionally tachyzoites, which are also responsible for maternally-derived congenital toxoplasmosis or laboratory toxoplasmosis.

A specific PCR-based study of 3432 aquatic animals covering eight species of shellfish and fish in China revealed only four positive samples among 618 *Procambarus clarkii* crawdads and one positive in *Hypophthalmichthys molitrix* [2]. The results suggest that consumption of raw aquatic cold-blooded animals may pose a health threat. It is known that the brain is one of the preferential locations of *Toxoplasma gondii* during persistent infection and dormant bradyzoites enclosed in tissue cysts are located in a variety of brain regions. Nested PCR examination of the brains of 68 snakes from five species collected in various provinces of Iran found the *T. gondii* GRA6 gene to be present in 80.88% (55 samples) of the tested snakes [3].

Although *Toxoplasma gondii* infections have been reported in many marine mammals, it is not clear how these animals acquire the infection. A study using indirect fluorescent assay found that the eastern oyster *Crassostrea virginica* is able to

remove *Cryptosporidium parvum* oocysts from sea water [4]. In further experiments, performed under controlled laboratory conditions, Lindsay et al. [5] demonstrated that eastern oyster can remove oocysts of the *T. gondii* VEG strain. Hemolymph, gill washes and oyster tissue contained DNA of the parasite until day six post-infection.

Contaminated oysters may serve as vectors of *T. gondii* for marine animals and humans. The unique type X strain of *T. gondii* is responsible for 72% of otter infections [6]; however, the prevalence of type X in terrestrial animals and marine invertebrates has not yet been fully explored. A California mussel from an estuary draining into Monterey Bay and Estero Bay was found to be type X-positive, as indicated by multilocus PCR and DNA sequencing at the B1, SAG1 and GRA6 loci. Of 45 local carnivores, 15 also had PCR-confirmed infection. The findings confirm that fecal contamination was flowing to the sea through surface runoff, and that otters could be infected *via* the consumption of filter-feeding marine invertebrates.

The freshwater crustacean *Gammarus fossarum* was found to be able to bioaccumulate *T. gondii* oocysts proportionally to the ambient concentration, suggesting that it could be a potential effective bioindicator of protozoan contamination [7]. In addition, marine snails have also demonstrated an ability to concentrate *T. gondii* oocysts: parasite oocysts have been detected in snail feces and tissues after exposure to sea water [8]. Red abalone

(*Haliotis rufescens*) concentrated *T. gondii* surrogate microspheres following exposure, and the excretion of surrogates continued for 14 days post-exposure. This bioaccumulation and retention of such pathogens as *T. gondii* by benthic invertebrates seems to be a general mechanism of marine transmission of terrestrially-derived microorganisms [9].

A recent laboratory study confirmed that migratory filter-feeding fish (northern anchovies and Pacific sardines) can filter *T. gondii* oocysts from sea water. A mouse bioassay found that oocysts persist in the alimentary canals and are infective for at least eight hours post exposure [10]. However, a study on goldfish (*Carassius auratus*) provided ambiguous results. While the tachyzoites of two *T. gondii* strains (RH of genotype 1 and Beverley of genotype 2) penetrated into cells at six hours post inoculation and multiplied in cell culture at 37°C, many tachyzoites attached only to the cells with no multiplication observed at 24 hours post inoculation at 33°C. In contrast, while viable inoculated tachyzoites were seen locally on day three and then disappeared in *in vivo* experiments at 37°C, they were still present at the inoculation site at the lower temperature of 33°C [11]. The results suggest that under natural conditions, the parasite is not able to cause any infection but can persist for a longer time. The great majority of previous studies have been performed by molecular methods (DNA detection), and positive results were not confirmed by biological assays.

*T. gondii* infection is a significant cause of mortality among southern sea otters (*Enhydra lutris nereis*) and the exposure to the parasite has been linked with the consumption of marine turban snails. Mazillo et al. [12] hypothesize that water-borne *T. gondii* oocysts attach to exopolymer substances (EPS), forming a sticky matrix of biofilm on kelp and thus becoming available to snails. The authors confirmed the hypothesis under laboratory conditions in tanks spiked with *T. gondii* surrogate microspheres. Gelatinous polymers including EPS on macroalgae captured *T. gondii* from sea water, enabling uptake by marine invertebrates, such as snails, and transmission of the parasite to California sea otters and other animals [13].

In 2016, the first case of systemic toxoplasmosis in a New Zealand sea lion (*Phocartos hookeri*) was reported. The parasite was detected histologically and by immunochemistry in the brain, spinal cord, spinal nerves and pelvic muscles. Nested PCR and

sequencing revealed the presence of *T. gondii* DNA in uterine and lung tissue [14]. These findings confirm the cosmopolitan character of *T. gondii* infection, although the mode of transmission of the cosmopolitan parasite on these animals is not known.

The studies presented above suggest that many marine poikilothermic animals may serve as significant vectors which can transmit *Toxoplasma gondii* infection (oocysts), not only to other animals, but also to humans by the consumption of various sea organisms (shellfish, snails, prawns) and raw fish (sushi). These animals may be characterized as paratenic hosts or transport hosts for *T. gondii*. To date, there has been no evidence that the parasite multiplies in these animals.

Greater public awareness should be raised concerning these findings as they contradict the popular view that the threat of toxoplasmosis is connected only with cats and the contaminated raw meat of endothermic animals. In addition, evidence suggests that the infections caused by *T. gondii* oocysts in humans are clinically more severe than those acquired from tissue cysts [15]. Furthermore, from the ecological point of view, toxoplasmosis could play a role in the morbidity and mortality of many endangered species, particularly in those populations that are close to the feline source of *T. gondii* oocysts.

## References

- [1] Dubey J.P. 2010. Toxoplasmosis of animals and humans. 2nd edition, CRC Press.
- [2] Zhang M., Yang Z., Wang S., Tao L.F., Xu L.X., Yan R.F., Song X.K., Li X.R. 2014. Detection of *Toxoplasma gondii* in shellfish and fish in parts of China. *Veterinary Parasitology* 200: 85-89. <http://dx.doi.org/10.1016/j.vetpar.2013.10.022>
- [3] Nasiri V., Teymurzadeh S., Karimi G., Nasiri M. 2016. Molecular detection of *Toxoplasma gondii* in snakes. *Experimental Parasitology* 169: 102-106. <http://dx.doi.org/10.1016/j.exppara.2016.08.002>
- [4] Graczyk T.K., Farley C.A., Fayer R., Lewis E.J., Trout J.M. 1998. Detection of *Cryptosporidium* oocysts and *Giardia* cysts in the tissues of eastern oysters (*Crassostrea virginica*) carrying principal oyster infectious diseases. *The Journal of Parasitology* 84: 1039-1042. doi:10.2307/3284641
- [5] Lindsay D.S., Phelps K.K., Smith S.A., Flick G., Sumner S.S., Dubey J.P. 2001. Removal of *Toxoplasma gondii* oocysts from sea water by eastern oysters (*Crassostrea virginica*). *Journal of Eukaryotic Microbiology* 48: 197s-198s.

- doi:10.1111/j.1550-7408.2001.tb00517.x
- [6] Miller M.A., Miller W.A., Conrad P.A., James E.R., Melli A.C., Leutenegger C.M., Dabritz H.A., Packham A.E., Paradies D., Harris M., Ames J., Jessup D.A., Worcester K., Grigg M.E. 2008. Type X *Toxoplasma gondii* in a wild mussel and terrestrial carnivores from coastal California: new linkages between terrestrial mammals, runoff and toxoplasmosis of sea otters. *International Journal for Parasitology* 38: 1319-1328.  
<http://dx.doi.org/10.1016/j.ijpara.2008.02.005>
- [7] Bigot-Clivot A., Palos Ladeira M., Lepoutre A., Bastien F., Bonnard I., Dubey J.P., Villena I., Aubert D., Geffard O., François A., Geffard A. 2016. Bioaccumulation of *Toxoplasma* and by the freshwater crustacean *Gammarus fossarum*: involvement in biomonitoring surveys and trophic transfer. *Ecotoxicology and Environmental Safety* 133: 188-194. <http://dx.doi.org/10.1016/j.ecoenv.2016.07.006>
- [8] Krusor C., Smith W.A., Tinker M.T., Silver M., Conrad P.A., Shapiro K. 2015. Concentration and retention of *Toxoplasma gondii* oocysts by marine snails demonstrate a novel mechanism for transmission of terrestrial zoonotic pathogens in coastal ecosystems. *Environmental Microbiology* 17: 4527-4537. doi:10.1111/1462-2920.12927
- [9] Schott K.C., Krusor C., Tinker M.T., Moore J., Conrad P.A., Shapiro K. 2016. Concentration and retention of *Toxoplasma gondii* surrogates from sea water by red abalone (*Haliotis rufescens*). *Parasitology* 143: 1703-1712.  
<https://doi.org/10.1017/s0031182016001359>
- [10] 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. *Veterinary Parasitology* 169: 296-303.  
<http://dx.doi.org/10.1016/j.vetpar.2010.01.002>
- [11] Omata Y., Umeshita Y., Murao T., Kano R., Kamiya H., Kudo A., Masukata Y., Kobayashi Y., Maeda R., Saito A., Murata K. 2005. *Toxoplasma gondii* does not persist in goldfish (*Carassius auratus*). *The Journal of Parasitology* 91: 1496-1499.  
doi:10.1645/ge-3503rn.1
- [12] Mazzillo F.F.M., Shapiro K., Silver M.W. 2013. A new pathogen transmission mechanism in the ocean: the case of sea otter exposure to the land-parasite *Toxoplasma gondii*. *PLoS One* 8: e82477.  
<http://dx.doi.org/10.1371/journal.pone.0082477>
- [13] Shapiro K., Krusor C., Mazzillo F.F.M., Conrad P.A., Largier J.L., Mazet J.A.K., Silver M.W. 2014. Aquatic polymers can drive pathogen transmission in coastal ecosystems. *Proceedings of the Royal Society B: Biological Sciences* 281: 2014-1287.  
doi:10.1098/rspb.2014.1287
- [14] Roe W.D., Michael S., Fyfe J., Burrows E., Hunter S.A., Howe L. 2017. First report of systemic toxoplasmosis in a New Zealand sea lion (*Phocarctos hookeri*). *New Zealand Veterinary Journal* 65: 46-50.  
<http://dx.doi.org/10.1080/00480169.2016.1230526>
- [15] Dubey J.P. 2004. Toxoplasmosis – a waterborne zoonosis. *Veterinary Parasitology* 126: 57-72.  
<http://dx.doi.org/10.1016/j.vetpar.2004.09.005>

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