Recommendations on the control of *Toxoplasma gondii* infection in southern sea otters (*Enhydra lutris* nereis)

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Abstract

The southern sea otter, a culturally, economically, and ecologically important species, has been slow to recover from overexploitation by the fur trade in the 1800s. Studies suggest that this delayed recovery is partly due to mortality caused by the intracellular, apicomplexan parasite *Toxoplasma gondii*. While *T. gondii* is a terrestrial parasite whose life cycle requires ingestion by feline hosts and their prey, it can infect all endothermic animals, and its eggs (oocytes) are reaching southern sea otter habitat via feline feces in freshwater runoff. The objective of this report is to propose control measures to mitigate the exposure of southern sea otters to *T. gondii.* The proposed measures are UV irradiation of stormwater to kill *T. gondii* oocysts before they reach sea otters; vaccination or medication of sea otters to prevent or treat infection by *T. gondii;* and a public education campaign to raise awareness, fund the proposed control measures, and encourage a reduction of housecat feces in runoff. The anticipated outcomes of these measures are reduced sea otter mortality related to *T. gondii,* an increased population growth rate, and healthier, more biodiverse coastal ecosystems.

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Background

Toxoplasma gondii, a unicellular eukaryote in the phylum Apicomplexa, is an intracellular parasite that infects a wide range of endotherms, both in terrestrial and aquatic systems. Its most well-studied life cycle involves a rodent intermediate host and feline definitive host: felines release *T. gondii* oocysts in their feces, which are ingested by rats or mice. The rodent becomes infected, and tissue cysts form throughout their body (Hill et al. 2005). Additional pathology can occur in the brains of rodents, including necrotic lesions, inflammatory nodules, and granulomas (Ferguson et al. 1991). *T. gondii* then facilitates trophic transmission to its feline definitive host by altering its rodent host's behavior such that it is more active, less cautious, and attracted to feline urine, thus making the rodent more susceptible to predation and increasing the likelihood of the parasite reaching its definitive host (Webster 2007). *T. gondii* has three infective life stages (tachyzoites, bradyzoites, and sporozoites) that can each infect *T. gondii*'s intermediate hosts (Webster 2007). Feline definitive hosts can be infected only by ingestion of infected tissues (tachyzoites or bradyzoites; Webster 2007). Once a feline is infected, it may have *T. gondii* in its intestinal epithelial cells for the rest of its life (Hill et al. 2005).

While felines are the only known definitive host for *T. gondii*, nearly any endothermic animal, including humans, can act as an intermediate host (Frenkel et al. 1970). Prevalence is as high as 60% in Pennsylvanian white-tailed deer (Humphreys et al. 1995), 84% in North Carolinian black bears (Nutter et al. 1998), and 77% in southern river otters in Southern Chile (Barros et al. 2018), for example. Most clinical disease caused by *T. gondii* occurs in

immunocompromised hosts, though the strain of *T. gondii* and the host species can also influence the severity of pathology, and serious reactions or death can result (Hill et al. 2005).

Though *T. gondii* evolved as a terrestrial parasite in felines and their prey (Hill et al. 2005), it has also been detected in marine mammals, such as sea otters. *T. gondii* has been found in high prevalence in sea otters from Washington State and California in recent years (Verma et al. 2018, Condrad et al. 2005). In two independent studies, a combination of otters found dead or euthanized were sampled for the presence of *T. gondii* or its antibodies. Seroprevalence in northern sea otters (*Enhydra lutris kenyoni*) sampled from Washington was 93 percent (Verma et al. 2018), while seroprevalence in Californian southern sea otters (*E. lutris nereis*) was 52 percent in animals found dead and 38 percent in those sampled alive (Conrad et al. 2005). While infection is found most frequently in adults, it has been reported in juveniles and pups as well (Verma et al. 2018, Conrad et al. 2005) and is believed to be vertically transmitted from mother to pup (Miller et al. 2008).

Unlike in other intermediate host species, *T. gondii* is associated with significant sea otter mortality, particularly in southern sea otter populations. *T.* gondii was identified as the primary cause of death in 16 percent of sampled southern sea otters and a factor contributing to mortality in another 11 percent; it was second only to acanthocephalan infection in number of fatalities caused (Kreuder et al. 2003). Kreuder et al. (2003) also reported that sea otters with moderate to severe *Toxoplasma*-induced encephalitis were 3.7 times more likely to be killed by sharks, presumably due to the errant behavior and seizures induced by the encephalitis, and were also more prone to heart disease. Correlation between encephalitis and *T. gondii* infection was also reported by Thomas et al. (2007).

Risk of *T. gondii* infection has been linked to freshwater runoff from urbanized areas and individual diet choice. Burgess et al. (2018) found significant correlation between sea otter infection and high human population densities, as well as anthropogenic land modifications. Miller et al. (2002) reported a link between infection and coastal runoff. Considering these findings, along with the knowledge of *T. gondii*'s life history, it is hypothesized that freshwater runoff transports *T. gondii* oocysts in the feces of stray and outdoor cats to the marine environment, where they can infect sea otters. Risk of infection has also been correlated with diet, suggesting that oocysts are sequestered in marine snails upon which some sea otters prey (Johnson et al. 2009, Burgess et al. 2018). On local scales, risk of *T. gondii* infection is heterogenous, likely due to small-scale variation in land development and marine snail abundance (Burgess et al. 2018).

Relevance

Sea otters are well-known and well-loved by the general public; one need only look to popular conservation movements, advertising for zoos and aquaria, pop and historical culture, media and entertainment, and any coastal gift shop to see it. Sea otters are a valuable tourist attraction and an icon of the California coast (Conrad et al. 2005).

In addition to being everyone's favorite charismatic marine mammal, sea otters also play important ecological roles as a keystone species (Estes and Palmisano 1974). A keystone species is a species with a disproportionate effect on its environment relative to its abundance (Paine 1995). In nearshore ecosystems where otters are present, otters control sea urchin abundance, which in turn allows kelp and its associated fauna to grow in greater abundance (Estes and

Palmisano 1974). In the absence of sea otters, sea urchins abound, increasing grazing on kelp and reducing the abundance of species which use it, such as fish, birds and other marine mammals (Estes and Palmisano 1974). Sea otters thus help to maintain local biodiversity, an important feature of a healthy ecosystem (Figge 2004).

The current status of California's southern sea otter populations gives conservationists reason for concern. Following severe overharvesting by the fur trade in the 1800s, *E. lutris nereis* was nearly driven to extinction. While northern sea otter populations (*E. lutris kenyoni*) have recovered steadily, southern sea otters have not seen the same growth, despite forty years of federal protection. Estes et al. (2003) suggest that, based on the high proportion of infectious disease-related mortality observed in the latter half of the twentieth century, parasites may be contributing to the slow population recovery. Taken with Kreuder et al.'s (2003) findings of *T. gondii* being the second most prominent cause of death in sampled otters (discussed above), evidence suggests that *T. gondii* is a significant hindrance to the recovery of southern sea otter populations. In addition to causing death via encephalitis and increased risk of shark attack or heart disease (Kreuder et al. 2003), the evidence of vertical transfer of *T. gondii* (Miller et al. 2008) also presents a greater risk of birth defects or miscarried pups, lowering otter reproductive success and contributing to slow population recovery. In order to improve the health and prevent the decline of southern sea otter populations, we must take action to mitigate *T. gondii* infection in this species.

Control Measures

Eradicating *Toxoplasma gondii* is not currently feasible, given the ubiquitousness of felids (its definitive host) and its large suite of reservoir hosts. While domestic cats are more numerous than wildcats in California, *T. gondii* prevalence is much higher in coastal Californian wildcats, and they are more likely to shed oocysts than managed domestic cats (VanWormer et al. 2013). Eradication of *T. gondii* from the coastal region of California is therefore not only unfeasible, but also ecologically unfavorable, as it may cause conflicts between the conservation of sea otters and of wildcats. Thus, measures against *T. gondii* infection in southern sea otters should be aimed at controlling its abundance and adverse effects. Three proposed control measures – UV stormwater irradiation, sea otter vaccination or medication, and a public education campaign – are discussed below.

UV Stormwater Treatment

Ultraviolet (UV) irradiation is an effective and widely-used water disinfection strategy (Guo et al. 2013) that has potential to reduce the prevalence of *T. gondii* in freshwater runoff (Le Goff et al. 2015, King et al. 2010). UV treatment has been effectively used to reduce infection by oocysts of parasites in the closely-related genus *Cryptosporidium* in laboratory experiments (Le Goff et al. 2015, King et al. 2010), suggesting that UV irradiation may have the same effect on *T. gondii* oocysts.

As the primary source of *T. gondii* oocysts appears to be from freshwater runoff (Conrad et al. 2005, Miller et al. 2002), treating terrestrial effluent before it reaches the sea could reduce the number of viable oocysts with which sea otters come in contact. Since storms drains serve as concentrated places of water collection, water treatment focusing near or in them may be

effective. Filters for large debris are used commonly near the mouths of storm drains (Jarvis 2016, Kluge 2007, Harms et al. 1999) and could be used as attachment points for UV lights. By placing them on filters that are, by necessity, accessible and maintained, UV lights would not require substantial additional work and could reduce the rates of *T. gondii* infection and related mortality.

I propose further laboratory experiments to assess the efficacy of UV light installation with storm drain filters on the virulence of *T. gondii* oocysts. Replicas of commonly used storm drain and filter designs could be assembled in a laboratory, and UV lights could be attached (this would also provide an opportunity to develop protocol for the installation of the lights). A series of experiments could be conducted, testing the efficacy of UV irradiation on combinations of *T. gondii* oocyst densities, water volume and flow rate, and debris (leaves, trash, etc.) density. Specific levels for these factors should be based on the range of conditions normally seen in coastal Californian storm drains. Water with a known concentration of active oocytes can be passed by the light under the predetermined series of conditions, and the concentration of active oocytes can be measured again after irradiation. This experiment would approximate the efficacy of UV irradiation in the sterilizing of oocysts under a range of runoff conditions. Assuming UV irradiation is effective, implementation of UV lights on storm drain filters can commence using the installation protocol established in this experiment.

Sea Otter Vaccination or Medication

Sea otter-specific *T. gondii* vaccines have not yet been discovered; however, such a vaccine would prevent new infections in sea otters and reduce *T. gondii*-associated mortality in vaccinated individuals. Currently, the only approved vaccine for *T. gondii* (Toxovax) is for sheep

(Hiszczynska-Sawicka et al. 2014), and most current *T. gondii* vaccine research focuses on vaccine development for humans, livestock, or cats (Garcia et al. 2014). While current toxoplasma vaccine research is not intended for sea otters, the vaccines being developed (or variants of them) may also prove effective in sea otters. Otters – like humans, livestock, and cats – are mammals, and thus are relatively physiologically similar. Evidence for *T. gondii* vaccines effectively immunizing species for which they were not intended has been documented by Verma and Khanna (2013), who found that the sheep vaccine Toxovax also reduced oocyst shedding in cats.

Toxovax (and other prospective vaccines under development [Fox and Bzik 2015, Verma and Khanna 2013]) could be experimentally tested first on northern sea otters (*Enhydra lutris kenyoni*), a genetically similar but unendangered species, to prevent further endangerment of the struggling southern sea otter populations. A subpopulation of northern sea otters can be given the vaccine. Using historical prevalence of *T. gondii* in northern sea otter beach-cast carcasses (Verma et al. 2018) as a baseline, the prevalence of *T. gondii* in beach-cast carcasses in the years following the vaccine administration can be recorded and compared. If *T. gondii* prevalence and *T. gondii*-related mortality are significantly reduced following vaccination, the vaccine could be considered for southern sea otters.

Though a vaccine would be ideal, as it would need only be administered once to each individual, drugs for treating *T. gondii* infection may be a more viable short-term option. Drugs such as pyrimethamine, sulfadiazine, and levamisole have been used to inhibit the growth of *T. gondii* and increase the survival rate of mouse hosts, though these drugs have several adverse side effects (Köksal et al. 2016). Alternatives to these drugs have been explored, such as

artesunate (a malarial drug; Gomes et al. 2012), haloperidol and valproic acid (an antipsychotic drug and a mood stabilizer, respectively; Jones-Brando et al. 2003), and the compounds tanshinone IIA and hydroxyzine (Murata et al. 2017), all of which are considered to cause fewer adverse effects. Tanshinone IIA and hydroxyzine appear particularly effective at treating not only acute *T. gondii* infection, but also chronic infection due to latent bradyzoites in tissue cysts, with no effects on host cell viability (Murata et al. 2017). These findings are promising for the development of drugs that cure both acute and chronic *T. gondii* infection and could potentially be applied to sea otters. While a drug would not prevent future infection, if employed jointly with UV irradiation of stormwater, continual drug administration may not be necessary; simply clearing current infections and reducing sea otter exposure to *T. gondii* oocysts by treating stormwater may be sufficient.

To test the effectiveness of such drugs in sea otters, they could be administered to sea otters in captivity (in zoos, for example) that are known to be infected with *T. gondii* (through testing for seropositivity). Testing the drug first on sea otters in captivity would be more financially feasible than testing on wild otters, and the subjects could be monitored under controlled conditions. If the drug effectively cleared the sea otter subjects of *T. gondii* infection, it could then be given to a subpopulation of southern (or northern) sea otters. Finally, if a decrease is observed in the percentage of sea otter mortality attributable to *T. gondii* in beach-cast carcasses from the studied subpopulation, the drug could be administered to the remainder of southern sea otters.

These control measures (vaccination and medication) would be feasible to deploy in wild populations. Given that sea otters are relatively large and enumerated animals that live near to

shore, the administration of a vaccine or drug to most individuals would likely be achievable in their natural habitat by teams in small, minimally-intrusive vessels.

Public Education Campaign

In order to raise awareness, support, and control measure funding for toxoplasma infections in southern sea otters, I finally propose a campaign to educate the public. Examples of methods of public outreach are posters, fliers, presentations, endorsement by prominent figures, or documentaries promoted by local aquaria, the California Department of Fish and Wildlife, or other conservation groups. By informing the public of what *Toxoplasma gondii* is, how it spreads, and its effects on local sea otters, otter enthusiasts can own a sense of responsibility for their local waters. Cat owners can be equipped with simple and specific ways to help their favorite charismatic marine mammals: for example, cats can be kept inside or outdoor litter boxes can be maintained, litter boxes should be cleaned frequently, cat waste should be disposed of carefully (sealed in bags that prevent feces from leaking), and outdoor cats should be spayed and neutered so as to reduce the number of kittens born as strays. Non-cat owners could reduce the number of stray cats in their neighborhood by reporting them to humane societies, and could promote natural runoff filtration by preserving unpaved surfaces on their property. These measures could reduce the number of *T. gondii* oocysts that reach coastal waters, and could garner financial support (via donations) for UV light installation and vaccine/medication research and implementation. These measures would thus reduce the risk of sea otter infection and promote the recovery of their struggling populations.

Anticipated Results

The expected outcomes of these measures are 1) a reduction in the exposure of southern sea otters (*E. lutris nereis*) to infectious *T. gondii* oocysts via UV treatment of stormwater; 2) a reduction in *T. gondii* pathology and associated mortality in currently infected sea otters (with the potential of future immunity conferred by a vaccine); and 3) a reduction in the abundance of infectious oocytes reaching coastal waters via human behavioral modifications. Overall, these outcomes will lead to fewer *T. gondii*-related deaths and less vertical transmission, aiding in the recovery of this endangered species. Population growth rates should be expected to increase in the years following implementation of these measures. This will additionally promote a biodiverse coastal ecosystem as this keystone species is replenished.

Additional Co-benefits

In addition to the anticipated outcomes listed above, reductions in other harmful pathogens may result from UV irradiation of stormwater or medical intervention in sea otters. UV irradiation could also kill pathogens such as fecal coliforms, which abound along the coast of California (Lewis et al. 2011). This would benefit coastal wildlife and humans using the waters, as well as improve the aroma and aesthetic of areas near effluent pipes. By medicating or vaccinating sea otters, other related parasites may also be targeted, such as *Sarcocystis neurona*, another apicomplexan parasite that commonly coinfects with *T. gondii* (Gibson et al. 2011). Even if *S. neurona* is not associated with serious mortality in sea otters, its removal may improve their health and quality of life.

Literature Cited

Barros, M., Cabezón, O., Dubey, J.P., Almería, S., Ribas, M.P., Escobar, L.E., Ramos, B., Medina-Vogel, G. 2018. Toxoplasma gondii infection in wild mustelids and cats across an urban-rural gradient. *PLoS One* **13(6):** e0199085.

Burgess, T.L., Tinker, M.T., Miller, M.A., Bodkin, J.L., Murray, M.J., Saarinen, J.A., Nichol, L.M., Larson, S., Conrad, P.A., Johnson, C.K. 2018. Defining the risk landscape in the context of pathogen pollution: *Toxoplasma gondii* in sea otters along the Pacific Rim. *Royal Society Open Publishing* **5**: 17118.

Conrad, PA., Miller, M.A., Kreuder, C., James, E.R., Mazet, J., Dabritz, H., Jessup, D.A., Gulland, F., Grigg, M.E. 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.

Estes, J.A., Hatfield, B.B., Ralls, K., Ames, J., 2003. Causes of mortality in California sea otters during periods of population growth and decline. *Marine Mammal Science* **19**: 198–216.

Estes, J.A., Palmisano, J.F. 1974. Sea Otters: Their Role in Structuring Nearshore Communities. *Science* **185**: 1058-1060.

Ferguson, D.J.P., Graham, D.I., Hutchinson, W.M. 1991. Pathological changes in the brains of mice infected with *Toxoplasma gondii*: a histological, immunocytochemical and ultrastructural study. *International Journal of Experimental Pathology* **72**: 463-474.

Figge, F. 2004. Bio-folio: applying portfolio theory to biodiversity. *Biodiversity and Conservation* **13:** 827-849.

Fox, B.A., Bzik, D.J. 2015. Nonreplicating, cycst-defective Type II Toxoplasma gondii vaccine strains stimulate protective immunity against acute and chronic infection. Infection and Immunity 83: 2148-2155.

Frenkel, J.K., Dubey, J.P., Miller, N.L. 1970. *Toxoplasma gondii* in cats: fecal stages identified as coccidian oocysts. *Science* **167:** 893-896.

Garcia, J.L., Innes, E.A., Katzer, F. 2014. Current progress toward vaccines against Toxoplasma gondii. *Vaccine: Development and Therapy* **4:**23-37.

Gibson, A.K., Raverty, S., Lambourn, D.M., Huggins, J., Magargal S.L., Grigg, M.E. 2011. Polyparasitism is associated with increased disease severity in *Toxoplasma gondii*-infected marine sentinel species. *PLoS Neglected Tropical Diseases* **5(5)**: e1142.

Gomes, C.T., de Andrade Júnior, H.F., Lescano, S.A.Z., Amato-Neto, V. 2012. *In vitro* action of antiparasitic drugs, especially artesunate, against *Toxoplasma gondii. Revista de Sociedade Brasiliera de Medicina Tropical* **45(4)**: 485-490.

Harms, R.C., Stiles, P.W., Preuss, G.E. 1999. US Grant US5980740A. Civitas Environmental Products, Inc.

Hill, D.E., Chirukandoth, S., Dubey, J.P. 2005. Biology and epidemiology of *Toxoplasma gondii* in man and animals. *Animal Health Research Reviews* **6(1):** 41–61.

Hiszczynska-Sawicka, E., Gatkowska, J.M., Grzybowski, M.M., Dlugonska, H. 2014. Veterinary vaccines against toxoplasmosis. *Parasitology* **141(11):** 1365-1378.

Humphreys, J.G. , Stewart, R.L. , Dubey, J.P. 1995. Prevalence of *Toxoplasma gondii* antibodies in sera of hunter-killed white-tailed deer in Pennsylvania. *American Journal of Veterinary Research* **56(2)**:172-173.

Jarvis, E. 2016. US Grant US9322155B2. BioClean Environmental Services, Inc.

Johnson, C.K., Tinker, M.T., Estes, J.A., Conrad, P.A., Staedler, M., Miller, M.A., Jessup, D.A., Mazet, J.A.K. Prey choice and habitat use drive sea otter pathogen exposure in a resource-limited coastal system. *PNAS* **106(7):** 2242-2247.

Jones-Brando, L., Torrey, E.F., Yolken, R. 2003. Drugs used in the treatment of schizophrenia and bipolar disorder inhibit the replication of *Toxoplasma gondii. Schizophrenia Research* **62:** 237-244.

King, B.J., Hoefel, D., Wong, P.E., Monis, P.T. 2010. Solar Radiation Induces Non-Nuclear Perturbations and a False Start to Regulated Exocytosis in *Cryptosporidium parvum. PLoS ONE* **5(7)**: e11773.

Kluge, R. 2005. US Application US20050199537A1. Modular Wetland Systems, Inc.

Köksal, Z.S., Yanik, K., Bilgin, K., Yilmaz, E.M., Hokelek, M. 2016. *In vivo* efficacy of drugs against *Toxoplasma gondii* combined with immunomodulators. *Japanese Journal of Infectious Diseases* **69:** 113-117.

Kreuder, C., Miller, M.A., Jessup, D.A., Lowenstine, L.J., Harris, M.D., Ames, J.A., Carpenter, T.E., Conrad, P.A., Mazet, J.A.K. 2003. Patterns of mortality in southern sea otters (*Enhydra lutris nereis*) from 1998-2001. *Journal of Wildlife Diseases* **39(3)**: 495-509.

Le Goff, L., Hubert, B., Favennec, L., Villena, I., Ballet, J.J., Agoulon, A., Orange, N., Gargala, G. 2015. Pilot-scale pulsed UV light irradiation of experimentally infected raspberries suppresses *Cryptosporidium parvum* infectivity in immunocompetent suckling mice. *Journal of Food Protection* **78(12)**: 2247-2252.

Lewis, D.J., Atwill, E.R., Pereira, M.G.C., Bond, R. 2011. Spatial and temporal dynamics of fecal coliform and *Escherichia coli* associated with suspended solids and water within five northern California estuaries. *Journal of Environmental Quality* **42(1):** 229-238.

Miller, M., Conrad, P., James, E.R., Packham, A., Toy-Choutka, S., Murray, M.J., Jessup, D., Grigg, M. 2008. Transplacental toxoplasmosis in a wild southern sea otter (*Enhydra lutris nereis*). *Veterinary Parasitology* **153:** 12-18.

Miller, M.A., Gardner, I.A., Kreuder, C., Paradies, D.M., Worcester, K.R., Jessup, D.A., Dodd, E., Harris, M.D., Ames, J.A., Packham, A.E., Conrad, P.A. 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.

Nutter, F.B., Levine, J.F., Stoskopf, M.K., Gamble, H.R., Dubey, J.P. 1998. Seroprevalence of *Toxoplasma gondii* and *Trichinella spiralis* in North Carolina Black Bears (*Ursus americanus*). *Journal of Parasitology* **84(5):** 1048-1050.

Murata, Y., Sugi, T., Weiss, L.M., Kato, K. 2017. Identification of compounds that suppress *Toxoplasma gondii* tachyzoites and bradyzoites. *PLoS ONE* **12(6):** e0178203.

Paine, R.T. 1995. A conversation on refining the concept of keystone species. *Conservation Biology* **9(4):** 962-964.

Thomas, N.J., Dubey, J.P., Lindsay, D.S., Cole, R.A., Meteyer, C.U. 2007. Protozoal meningoencephalitis in sea otters (*Enhydra lutris*): a histopathological and immunochemical study of naturally occurring cases. *Journal of Comparative Pathology* **137(2-3):** 102-121.

Webster, J.A. 2007. The effect of *Toxoplasma gondii* on animal behavior: playing cat and mouse. *Schizophrenia Bulletin* **33(3)**: 752-756.

Verma, R., Khanna, P. 2013. Development of Toxoplasma gondii vaccine: a global challenge. Human Vaccines and *Immunotherapeutics* **9(2):**291-293.

Verma, S.K., Knowles, S., Cerquiera-Cézar, C.K., Kwok, O.C., Jiang, T., Su, C., Dubey, J.P. 2018. An update on *Toxoplasma gondii* infections in northern sea otters (*Enhydra lutris kenyoni*) from Washington State, USA. *Veterinary Parasitology* **258**:133-137.