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Molecules to modeling: *Toxoplasma gondii* oocysts at the human–animal–environment interface

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Abstract

Environmental transmission of extremely resistant *Toxoplasma gondii* oocysts has resulted in infection of diverse species around the world, leading to severe disease and deaths in human and animal populations. This review explores *T. gondii* oocyst shedding, survival, and transmission, emphasizing the importance of linking laboratory and landscape from molecular characterization of oocysts to watershed-level models of oocyst loading and transport in terrestrial and aquatic systems. Building on discipline-specific studies, a One Health approach incorporating tools and perspectives from diverse fields and stakeholders has contributed to an advanced understanding of *T. gondii* and is addressing transmission at the rapidly changing human–animal–environment interface.

Keywords

Toxoplasma gondii; One Health; Oocyst; Modeling; Transport; Molecular characterization; Climate change; Land use change; Zoonotic disease; Environmental resistance

1. Introduction

Understanding the ecology and epidemiology of disease in the context of ecosystems is critical for preserving human and animal population health. Over 7 billion people now depend upon the earth's resources [1], and global environmental change has made disease migration to new hosts and landscapes a reality rather than a potential threat [2,3]. Anthropogenic activities, in particular, reshape landscapes, climate, and species distributions and interactions across the globe, with significant potential to alter patterns of pathogen emergence and spread [4–7]. Habitat conversion, introduction of non-native species, and increased contact between human, domestic animal, and wildlife populations have been linked to emerging viral, bacterial, and parasitic diseases [8]. Climate change also has the potential to alter cycles of disease transmission by influencing vector and host ranges, pathogen survival, and dynamics of water-borne transmission [8,9]. Of the 1415 organisms documented as human pathogens, over 60% are believed to have come from domestic or

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wild animal reservoirs [10]. Examining animal, human, or environmental health alone ignores the vital links between these components.

Historical biological and geographic boundaries of disease transmission will likely continue to shift significantly with changing environmental conditions, making a more holistic, One Health approach to pathogen research and management essential. By linking diverse non-academic stakeholder communities and researchers from different disciplines, the One Health approach builds a synergistic base from which to study and address the unique health challenges emerging at the human–animal–environment interface in a changing global environment [11,12].

Toxoplasma gondii, a globally distributed, zoonotic, protozoan parasite capable of infecting a wide range of warm-blooded animals [13], provides a broadly applicable example of the complexity of pathogen transmission among diverse hosts and environments and illustrates the need for a One Health approach to better understand disease ecology and epidemiology. Although long-studied in terrestrial landscapes, *T. gondii* has also emerged as a significant aquatic pathogen linked to marine mammal infection and water-borne outbreaks of disease in humans around the world [14]. Oocysts, the exceptionally hardy free-living environmental stage of the parasite, play a key role in transmission of *T. gondii* to newly recognized hosts and ecosystems. As wild and domestic felids are the only known hosts capable of shedding *T. gondii* oocysts in their feces [15–17], infection of people and animals through contaminated terrestrial and aquatic sources emphasizes the need to jointly examine human, domestic animal, and wildlife populations. While parasitologists, physicians, veterinarians, ecologists, and molecular biologists have studied *T. gondii* independently, understanding how a traditionally terrestrial pathogen is emerging in new environments requires more integrated knowledge. For *T. gondii* and other pathogens, creating a more collaborative approach to research and management from molecular to landscape levels has enhanced our understanding of health at the human–animal–environment interface.

2. Importance of oocysts in transmission of *T. gondii* infections

Warm-blooded animals, including humans, are typically infected with *T. gondii* through one of three pathways: ingesting oocysts from the environment (through contaminated water, soil, or food), eating an infected intermediate host with *T. gondii* cysts in its tissues, or congenital transmission from infected mothers to offspring [13,18]. Additional potential routes of transmission, including *T. gondii* tachyzoite-contaminated sperm and unpasteurized milk, have been demonstrated, but are thought to be rare sources of infection [19–21]. Uncommon cases of human infection following blood transfusion or organ transplantation from *T. gondii*-infected donors have also been reported [22,23]. However, oocyst-induced infections are increasingly recognized as a significant route of *T. gondii* transmission (Fig. 1) [14,24].

A key feature of oocyst-borne infections is that the majority of human cases with clinical symptoms were reported in immunocompetent individuals, which contrasts with the traditional dogma that 90% of acquired cases of toxoplasmosis in healthy hosts are asymptomatic [25]. Clinical disease from acquired toxoplasmosis has been linked to strain virulence (as reviewed by [26]), and may be more severe when intermediate hosts acquire the infection through the oocyst versus tissue stages of *T. gondii* [27–29]. As reviewed by Grigg and Sundar [30], a number of studies link oocyst infections to symptomatic disease in immunocompetent individuals, with signs ranging from mild flu-like illness to more disseminated and severe outcomes, such as chorioretinitis, neurologic deficits, aborted fetuses, and even death. While outbreaks associated with consumption of bradyzoite tissue

cysts in undercooked meat have been reported, the numbers of individuals affected are minimal as compared with documented oocyst outbreaks [30].

2.1. Terrestrial oocyst transmission

Widespread infection in chickens, grazing livestock, and herbivorous wildlife species from diverse geographic locations provides evidence of environmental exposure to *T. gondii* oocysts [31,32]. Omnivorous animals may acquire *T. gondii* infection through ingestion of tissue cysts, but infection from oocyst-contaminated food or water has also been reported [33]. Additionally, human infection has been repeatedly linked to terrestrial environmental sources including oocysts in domestic cat (*Felis catus*) litter boxes and soil as well as ingestion of contaminated unwashed fruits and vegetables [34–36]. Oocysts have been recovered from soil under natural conditions [37,38], and epidemiological studies indicate that there is an increased risk of acquired *T. gondii* associated with soil exposure (Tables 1 and 2[39]). While differentiating routes of *T. gondii* acquisition has been historically difficult, a recently recognized oocyst-specific antigen [27,40,41] applied in a study of mothers of congenitally infected infants in the United States demonstrated that 78% of these women (59 of 76), had oocyst-acquired infections [41].

2.2. Water-borne oocyst transmission

Furthering the understanding of *T. gondii* epidemiology at a level that integrates environmental, human, and animal factors necessitates novel insight on the ecology of this parasite in water as well as terrestrial landscapes. Contamination of water with disease-causing microorganisms is a global health issue, with implications for human and animal health. Increasing reports of epidemic and endemic waterborne *T. gondii* infection in humans have emphasized the zoonotic potential for oocyst-based transmission [14,42]. Outbreaks of clinical toxoplasmosis in humans exposed to oocyst-contaminated water have been described in both developing and developed countries [43], with numerous additional reports identifying water as a risk factor for endemic *T. gondii* infection (Tables 1 and 2).

Some of the strongest support for the importance of oocyst-based transmission comes from *T. gondii* morbidity and mortality in aquatic mammals. Infection with *T. gondii* has been described for numerous marine mammal species living in near-shore and open ocean waters around the world, with clinical disease observed in seals, dolphins, whales, sea otters, and manatees [44–49]. Animals living in freshwater systems are also at risk; over 85% (82 of 95) of free-ranging Amazon River dolphins had antibodies to *T. gondii* [50]. Widespread *T. gondii* infection in aquatic mammals suggests that contamination of terrestrial watersheds with *T. gondii* is prevalent, and that sufficient numbers of oocysts are distributed in freshwater and marine ecosystems to infect and cause disease in both near-shore and pelagic mammals. *T. gondii* in marine mammal tissues also presents a public health concern, as widespread consumption of these animals, especially in indigenous communities, can provide an additional route of zoonotic transmission [51].

In California, *T. gondii* is a significant cause of mortality in threatened Southern sea otters (*Enhydra lutris nereis*) [52]. Because the only known definitive hosts of *T. gondii* are felids, otter infection supports land-to-sea transmission of this parasite through contaminated freshwater runoff [53]. Although a case of vertical transmission in sea otters has been reported [54], infection is more likely to occur through eating oocyst-contaminated invertebrate prey [55] or by direct exposure to oocysts in seawater. Southern sea otters, though they eat numerous marine prey species, have only rarely been observed to consume warm-blooded intermediate hosts of *T. gondii* [56,57]. Filter-feeding marine invertebrates, such as mussels and oysters, do not appear to become infected, but can concentrate viable *T. gondii* oocysts [58,59]. These invertebrates can then serve as transport hosts of the parasite

and a potential source of infection for otters and other marine predators as well as humans, underscoring the public health risks of *T. gondii* in the near-shore marine environment [60].

Marine filter-feeding fish, which are prey species for many pelagic marine mammals, may also be a source of *T. gondii* infection. Using PCR and mouse bioassay detection methods, anchovies and sardines were experimentally shown to filter viable, sporulated oocysts from *T. gondii* spiked seawater into their alimentary canals [61]. Whether this process occurs in nature is unknown, but if migratory filter-feeding fish serve as transport hosts of *Toxoplasma*, they could carry oocysts from coastal runoff to diverse marine environments.

3. Keys to oocyst success

Oocysts pose a serious threat to susceptible hosts because of their robust environmental resistance, low infectious dose for some species, and the lack of methods to reliably detect oocysts in water and other environmental substrates to identify sources of exposure. Multiple experiments evaluating the survival of oocysts in soils showed that they may remain viable for at least 1 year when covered and in cool temperatures (4°C) [38,62–64]. Under warm climate conditions in dry soils from Kansas, USA, oocysts remained viable for 18 months [63]. In fresh or marine waters, oocysts were shown to be viable for at least 4.5 and 2 years, respectively [65] (reviewed by [66]). Although *T. gondii* oocysts have been reported to be inactivated by exposure to >60 °C for 1 min [67], higher temperature and longer duration may be necessary for complete and reliable inactivation. Two chemicals commonly used to treat water, sodium hypochlorite (chlorine) and ozone, failed to inactivate all infective oocysts at concentrations well in excess of those used to treat both sewage and drinking water [68,69]. Furthermore, physical inactivation by ultraviolet (UV) irradiation at doses 500 mJ/cm², far exceeding doses commonly used to treat water, did not fully inactivate *T. gondii* oocysts [70].

Although nearly all warm-blooded animals are susceptible to oocyst-borne infections, the infectious dose for most species is unknown. Some intermediate hosts, including mice and pigs, are highly susceptible and become infected at doses as low as 1–10 *T. gondii* oocysts [71,72]. Even rats and horses, which appear resistant to clinical disease following infection with *T. gondii* oocysts, develop tissue cysts, indicating their potential to serve as sources of infection for other hosts [73,74].

4. How molecular characteristics contribute to oocyst success

Molecular description of the oocyst has been extremely limited for several reasons: (1) no method exists for maintaining or expanding oocysts in vitro, therefore, infection of the definitive felid host is required to produce oocysts; (2) oocysts are refractory to routine protocols used to isolate nucleic acids and proteins, thus requiring special equipment and procedures; and (3) live oocysts pose a biohazard risk as they are not readily killed by laboratory disinfectants [75–77]. However, recent molecular characterization of *T. gondii* oocysts provides preliminary clues to the critical proteins that comprise the oocyst wall and likely confer oocyst resistance to destruction [75,77]. Comprehensive mining of the recently published oocyst transcriptome and proteome revealed many genes/proteins that were found to be uniquely expressed in the oocyst stage and appear to be located in the oocyst wall or otherwise contribute to oocyst resistance. Among these genes/proteins were tyrosine-rich proteins, *T. gondii* oocyst wall proteins (OWPs), PAN-domain-containing proteins, and late embryogenesis abundant domain-containing proteins (LEAs) [75,77].

4.1. How the oocyst wall confers resistance

Evidence suggests that the oocyst wall is comprised of groups of highly cross-linked proteins that form a sturdy outer matrix contributing to the oocyst's resistance to destruction. The *T. gondii* oocyst wall is a bilayered structure with an outer electron dense layer and an inner electron lucent layer, both roughly 40 nm thick [78]. Oocyst wall formation takes place within feline intestinal epithelial cells as the macrogamete develops. The outer layer of the oocyst wall is reportedly composed primarily of proteins and carbohydrates, providing structural strength, and the inner wall is primarily lipid, providing protection from chemical insult [79,80]. Under UV excitation (330–385 nm) the oocyst wall is autofluorescent, and the wall retains its autofluorescence following bleach treatment and boiling [80]. Because molecular data on *T. gondii* oocysts were largely unavailable, predictions for oocyst wall formation and composition in *T. gondii* have been made based on what has been described in two closely related, extensively studied, and environmentally resistant protozoan genera: *Eimeria* and *Cryptosporidium*.

Similarities in oocyst characteristics, including: environmental stability; mechanism of wall formation; and autofluorescence of oocyst walls under UV excitation, indicate that the *T. gondii* oocyst wall is likely very similar to *Eimeria* spp. A unique feature of the identified proteins in the *Eimeria* oocyst wall is tyrosine-richness [81,82] with tyrosine-protein cross-linkages reported to be responsible for structural robustness and autofluorescence [82]. BLAST searches of the tyrosine-rich proteins identified in *Eimeria* failed to turn up any homologs in the predicted genome of *T. gondii* [75]. However, six tyrosine-rich proteins (>5% tyrosine) were found to be abundantly expressed in the proteome and transcriptome of *T. gondii* oocysts [75,77], suggesting that like *Eimeria*, *T. gondii* oocysts contain tyrosine-rich proteins. Cross-linking via dityrosine bonds has been described in a number of organisms and is associated with the formation of insoluble and physicochemical resistant matrices; examples include bacterial spores, fungal cell walls, sea urchin eggs, and coccidian oocysts [83–87]. The putative location of tyrosine-rich proteins in the walls of the oocysts and their ability to form bridges across tyrosine residues has yet to be experimentally investigated.

Oocyst wall proteins (OWPs) in *Cryptosporidium* spp. are cysteine-rich and predicted to be stabilized via disulfide bridges [88,89]. Seven homologs to the cysteine-rich family of OWPs identified in *Cryptosporidium* spp. are present in the *T. gondii* genome (designated TgOWPs 1–7) [89]. Three were recently confirmed in the *T. gondii* oocyst wall (TgOWPs 1–3), and all were identified in oocysts by mass spectrometry and/or microarray [75,77]. Also abundantly detected in *T. gondii* oocysts by mass spectrometry were PAN domain-containing proteins [77]. The structural conformation of PAN-domain containing proteins is also achieved through disulfide bridges that result in a pattern of folding that creates recognition and binding sites [90]. The role of these PAN domain-containing proteins has not yet been investigated in *T. gondii* oocysts, but they may be of structural significance in the oocyst wall given their large size and predicted extensive disulfide bridging.

4.2. Other molecules that confer resistance

While it has been presumed that the *T. gondii* oocyst achieves its resistance to environmental destruction through structures present in the wall, a group of proteins designated 'late embryogenesis abundant domain-containing proteins' (LEAs) may comprise another critical dimension to oocyst resistance. Four LEAs were discovered in oocysts by microarray and mass spectrometry [75,77]. While the function of these proteins in *T. gondii* is unknown, LEA proteins have been described in a number of other organisms including plants, invertebrates, and microorganisms [91]. There is significant diversity in the LEA families and their respective functions are still under investigation. However, a

commonly ascribed role is resistance to environmental stresses including drought, high salinity, and freezing [92].

Molecular characterizations of the oocyst will aid in developing targets for oocyst inactivation and development of reagents to concentrate and detect oocysts in water and other environmental substrates. Several methods have been evaluated for detection of *T. gondii* in water, soil, and felid feces, including molecular approaches, microscopy, and mouse bioassays as shown in Table 3 [66]. However, detection of oocysts in terrestrial and aquatic habitats remains challenging as sensitive, standardized techniques for large-scale environmental testing are not currently commercially available. The difficulty of detecting *T. gondii* oocysts in environmental sources has likely led to underestimation of the importance of this route of transmission and limited the ability to identify and address high-risk areas of oocyst exposure.

5. Oocysts at the animal–human–environment interface

Evaluating the role of oocysts in *T. gondii* transmission cycles requires a broad understanding that encompasses: the ecology of felids, aquatic mammals, and terrestrial and marine prey species; chemical and physical properties that determine oocyst resistance; human influences on domestic animal and wildlife populations; and the impact of environmental factors, such as land use, climate, and freshwater runoff. Considering the diverse factors that contribute to *T. gondii* oocyst loading, survival, and transport facilitates interdisciplinary research and management approaches to reduce environmental exposure.

5.1. Domestic and wild sources of oocysts

Experimentally, domestic cats typically shed millions to hundreds of millions of oocysts over a single 1- to 3-week period following initial infection with *T. gondii* bradyzoite cysts [76,93]. The route of infection, strain of *T. gondii*, and cat age may impact the time from infection to shedding, percentage of infected cats that shed *T. gondii*, and number of oocysts shed. Lower prevalence and delayed onset of shedding were reported in cats fed *T. gondii* oocysts compared to those infected with tachyzoites or bradyzoites [15]. Although experimental infection with certain strains of *T. gondii* yielded higher shedding prevalence and quantities of oocysts, the limited number of strains tested does not reflect the diverse *T. gondii* genotypes circulating in free-ranging populations [16,94–96]. Young cats may shed higher numbers of oocysts following primary infection, and higher prevalence of shedding was observed in outdoor pet and feral kittens compared to adults [18,97]. However, older cats may still play an important role in environmental loading, as oocyst shedding was detected in naturally infected adult domestic cats (1–18 years old) in diverse geographical locations [98–100]. Reported shedding prevalences of molecularly confirmed *T. gondii* oocysts in pet and feral domestic cat populations ranged from zero to nine percent, with higher shedding levels observed in feral cats than pet cats [14,99,100].

Even in experimental settings, few reports of prevalence, duration, and frequency of oocyst shedding in wild felids exist. The prevalence of oocyst shedding by bobcats (*Lynx rufus*) was lower than that in domestic cats experimentally infected with the same strain of *T. gondii* [15,16]. However, the strain used for infection was isolated from sheep, and the experimental response of bobcats may not have reflected natural exposure to strains circulating in wild populations. Asian leopard cats (*Prionailurus bengalensis*) exposed to *T. gondii* strains isolated from domestic and wild animal sources only shed oocysts following infection with the wild strain [16]. Although the number of oocysts shed by experimentally infected wild felids has not been quantified, a free-ranging mountain lion (*Puma concolor*) in Canada shed 1.25×10^6 oocysts per gram of feces, which is similar to quantities of oocysts shed by naturally infected domestic cats [14,101]. In the limited reports of *T. gondii*-

like oocyst shedding in naturally infected, free-ranging wild felids, shedding prevalences ranged from 0% to 33%, suggesting that shedding can vary widely among wild felid populations (Table 4).

Although discussions of oocyst shedding usually focus on fecal excretion following initial infection of felids, repeat or intermittent oocyst shedding may increase their contribution to parasite burden in the terrestrial environment. No evidence of persistent oocyst shedding exists for domestic or wild felids, but repeat shedding may occur under certain conditions. Chronically infected domestic cats have been experimentally shown to shed oocysts after being exposed to novel strains of *T. gondii*, as well as when receiving immunosuppressive doses of glucocorticoids or when co-infected with *Isospora felis*, a common feline parasite [14,94,102–104]. Interestingly, co-infection with immunosuppressive pathogens, such as feline immunodeficiency virus (FIV) has not been associated with repeat shedding [105]. Malnutrition was linked to repeat shedding in domestic cats, but similar results were obtained with well-nourished cats [97]. The repeat shedding observed in these cats may be due to novel strains of *T. gondii* used for reinfection rather than nourishment status. Natural repeat shedding of *T. gondii* oocysts has been observed in diverse species of captive wild felids kept in zoos and has been attributed to repeated exposures to the parasite in raw meat [106]. By impacting nutritional and immune status as well as exposure to *I. felis* and new strains of *T. gondii*, diet also has the potential to influence repeat shedding in free-ranging felids.

At this time, there is no commercially available vaccine or treatment that can be administered to felids to prevent or reduce oocyst shedding. Two candidate vaccines were successful in preventing oocyst shedding in domestic cats that were subsequently challenged with infective strains [107–110]. However, these vaccines are not feasible for widespread application because they utilized live tissue cysts maintained in chronically infected mice.

5.2. Environmental transmission – oocyst loading and transport from terrestrial to aquatic systems

Both anthropogenic and natural conditions strongly influence *T. gondii* oocyst loading in the terrestrial environment as well as transmission to freshwater, estuarine, and marine systems (Table 5). As *T. gondii* oocysts cannot replicate outside of felid hosts, spatial variation in the burden of oocysts on land reflects the distribution of *T. gondii* shedding by these populations. Native wild felids as well as introduced pet domestic cats and un-owned or feral domestic cats, have the potential to contribute massive quantities of oocysts to terrestrial and aquatic habitats [111,112]. The likelihood of oocyst shedding in a given location is influenced by felid distribution, availability of prey or other food resources, and human management of domestic and wild felid populations.

The distribution of pet cats and feral domestic cat colonies is closely linked to human households that supply food and shelter [113]. Even unmanaged, more solitary feral domestic cats not fed or cared for by humans may have home ranges linked to developed landscapes, inhabiting urban and agricultural lands in addition to less developed areas [113]. Human activities may also influence the distribution of wild felids, as many species were reported to be sensitive to habitat fragmentation and avoided areas of high human population density [114–117]. In protected natural areas with public access, abundance of bobcats declined with increasing human and domestic dog visitors [118], illustrating that even temporary human land use activities may influence felid distributions. However, some wild felids frequently used edge environments or riparian corridors of developed urban and agricultural areas [119]. Although many wild felids and unmanaged feral cats use a wide variety of habitats throughout their ranges, habitat choice may be influenced by prey availability, intra or inter-specific territorial interactions, competition with other predators for

access to prey, environmental stress, as well as vegetation and topography [113,120,121]. Research on domestic and wild felid ecology builds the understanding of and ability to predict loading of *T. gondii* oocysts in natural environments.

Even within a shared habitat, exposure of wild and domestic felids to *T. gondii* may increase or decrease based upon access to prey and dietary preferences. Pet and feral domestic cats showed different prey consumption patterns, with higher predation levels observed in feral cat populations [122,123]. Reports of specific prey preferences for both wild and domestic felids vary drastically both among felid species and populations of a single species living in different environments [124,125]. In regions where humans, livestock, and wildlife live in close contact, wild felid prey frequently included domestic livestock such as sheep, goats, and cattle as well as wildlife [126]. Prey availability and preferences of wild and domestic felids not only contribute to the distribution of these hosts in the terrestrial landscape, but may also influence their risk of infection and oocyst shedding, as *T. gondii* infection levels varied dramatically in sampled intermediate host species [32,127]. In a meta-analysis of studies on *T. gondii* infection in small mammals, reported seroprevalence of infection was significantly higher in large rodents and rabbits than smaller rodent species [127]. Additional information on local prey populations and dietary preferences of domestic and wild felids will help to determine the risk of *T. gondii* infection and oocyst shedding for diverse felids in a given region. Indirect measures of the abundance of intermediate hosts of *T. gondii*, including habitat distribution and precipitation patterns, may facilitate estimates of prey populations at landscape scales that would be impractical for field investigations [128–131].

Historic and current human land use and animal management strategies can directly and indirectly impact wild and domestic felid distribution, and *T. gondii* oocyst shedding and transport. Extensive and rapid land use change around the world has converted many natural habitats to developed urban and agricultural lands [132]. These environments are frequently used by both pet and feral domestic cats, whose populations will likely expand with increased development [113,133]. Changing land use may also alter unmanaged feral cat and wild felid distributions by shifting habitat and the numbers or types of prey in a given location. Individual pet owner decisions determine the amount of time pet cats spend outside the home, and therefore the likelihood that they will be exposed to infected intermediate hosts and defecate outdoors, contributing oocysts to the terrestrial environment. People feeding stray or feral cats influence the diet and behavior of these animals, and government-level management decisions also affect loading and transport of oocysts. City and regional pet and feral cat management policies, including trap-neuter-return programs and mandatory spay-neuter laws shape numbers and distribution of these felids [134]. National and regional policies on wildlife hunting and protection have also influenced population sizes of wild felids [135,136]. Additionally, the California legislature Bill AB2485 directly targeted oocyst loading and transport by requiring cat litter labels to direct owners to put used litter in landfills rather than depositing it outside or flushing it through the sewage system where it might eventually reach the ocean and sensitive marine wildlife [137]. Considering *T. gondii* environmental transmission in the context of felid distribution and oocyst shedding, as well as human landscape and animal management decisions, will facilitate identification and management of high-risk areas for parasite exposure.

Transport and potential accumulation of *T. gondii* oocysts in environmental matrices, such as soil or water, is determined by felid behavior, environmental attributes, and oocyst surface properties. In addition to affecting sporulation and survival, felid defecation behaviors can alter the number of oocysts that can be entrained and transported in freshwater flow. Fewer *T. gondii* oocysts may be mobilized from feces that are buried under the soil, and fecal burying behavior can differ greatly among different species and populations of

felids. Many free-ranging pet domestic cats bury feces, but kittens in Costa Rica, dominant individuals in feral cat colonies, and unmanaged feral cats left feces exposed [97,138]. In a zoo setting, mountain lions buried feces and occasionally defecated in water, whereas bobcats and tigers (*Panthera tigris*) frequently defecated in water (personal communication, Lynn Dowling, keeper Folsom Zoo Sanctuary). Semi-captive bobcats have also been reported to frequently defecate in running streams and/or shallow ponds, and free-ranging bobcats commonly leave their feces exposed at sites along roads or trails [138,139]. For some terrestrial carnivores, including mountain lions, the tendency to bury feces may also change with season or reproductive status [140,141]. Location of defecation can limit or enhance oocyst transport, with oocysts deposited near waterways more likely to be transported in freshwater runoff into aquatic environments. In some regions, mountain lions and bobcats commonly use riparian habitats [119,142], which could increase their contribution to the number of oocysts reaching aquatic systems. Understanding defecation behavior for wild and domestic felids in a given environment will help to predict oocyst deposition, survival, and transport.

Substrate, vegetation, and climate at the site where oocysts are deposited all affect transport. Permeable soils may allow oocysts and other pathogens to percolate vertically, reducing the number of oocysts mobilized and carried in freshwater flow to aquatic systems. In contrast, impervious surfaces, like asphalt and concrete in developed environments can lead to increased mobilization of contaminants into runoff [143]. Natural fresh and saltwater wetlands play a vital role in reducing the levels of these contaminants in runoff [144,145], and vegetation in constructed wetlands and terrestrial vegetation buffers was also effective in decreasing pathogens in effluent waters [146,147]. Physical attributes of watersheds and local climate characteristics also impact *T. gondii* environmental transmission. The slope of the watershed as well as its size can influence the amount of freshwater runoff and transported pathogens reaching aquatic systems where humans and animals can be exposed [148,149]. Freshwater runoff and particle transport are also directly influenced by storm patterns and the quantity, duration, intensity, and frequency of precipitation in the watershed [150,151]. Infections and deaths in marine mammals due to terrestrially derived fecal protozoa and bacteria have been temporally and spatially linked to increased precipitation or land-based runoff [152,153].

Surface properties of the environmental stages of protozoan pathogens are particularly relevant for driving their environmental transmission patterns [154]. The surface electrophoretic mobility (surface charge) and hydrophobicity (affinity to water) are two properties that influence the behavior of suspended particles in water. *T. gondii* oocysts were determined to have a negative charge in fresh water solutions, but their charge was neutralized in estuarine and seawater [155]. Oocysts were also determined to have a very low contact angle, indicating that they have a strong affinity to water molecules. The strongly hydrophilic nature and negative charge of *T. gondii* oocysts in freshwater could facilitate their widespread contamination in waterways. The loss of charge observed in saline waters suggests that enhanced flocculation and subsequent accumulation of *T. gondii* oocysts may occur in locations where fresh and marine waters mix, increasing the risk of exposure to humans and wildlife.

Aggregation studies further support the hypothesis that *T. gondii* oocysts are likely to concentrate in estuarine and marine ecosystems [156]. In laboratory studies, attachment of oocysts with aquatic macroaggregates (i.e. “marine snow”) was observed in fresh, estuarine, and marine water samples, but was greatest in waters with increased salinity. Concentration of *T. gondii* in estuarine or marine aggregates was enriched 3–4 orders of magnitude as compared with oocyst concentration in surrounding water. Aggregation of oocysts is significant because attached oocysts are likely to move through an aquatic habitat differently

and may have an altered ecological role as compared with unattached oocysts. Oocysts associated with aggregates have increased settling velocities and larger effective surface area that can facilitate their removal from the water column by emergent vegetation. Distinct high-risk sites of infection may thus form at locations where contaminated freshwater mixes with saline waters as aggregates deposit in the benthos or are trapped by vegetation. The high seroprevalence of *T. gondii* in California sea otters may be partially accounted for by the fact that otters spend the majority of their lives within 500 m of the coastline, exactly where oocyst concentration may be greatest. Furthermore, invertebrates are known to retain particles that are ingested within aggregates more readily than particles that are freely suspended in the water column [157]. Thus, the association of *T. gondii* with aquatic aggregates may facilitate their uptake into the marine food chain, with eventual ingestion by a susceptible warm-blooded host, such as a sea otter or human. For decades, oceanographers have recognized that aquatic aggregates are crucial vehicles for vertical and horizontal movement of materials in the ocean [158,159]. However, the role of aggregates in the transport of terrestrially derived zoonotic pathogens is only now starting to emerge, and future efforts are still needed to characterize when and where *T. gondii* and other zoonotic pathogens become a threat to human and animal health in aquatic habitats.

6. Land use and climate change – anthropogenic influences on oocyst transmission

Oocyst-borne infections with *T. gondii* may increase in animals and humans as climate and habitat changes reshape environments worldwide. As most of the human population and their domesticated animals are distributed along waterways, there has been an associated increase in the amount of fecal deposition within watersheds that drain into collecting freshwater bodies, estuaries, and coastlines. The physical forces that drive overland runoff events and mobilize transport of fecal matter are likely to increase with climatic factors that are forecasted to change in the coming decades. Across most latitudes, weather simulation models have projected reduced predictably of storms coupled with increased intensity of rainfall events, leading to precipitation patterns that are likely to result in a net increase of land-based runoff [160]. High amounts of rainfall that occur within a shorter duration of time would provide enhanced force for mobilizing overland runoff, which acts as a conduit of storm-driven pollutants, including fecal pathogens. In addition, increased runoff can indirectly exacerbate pollution by overcoming the ability of sewage treatment facilities to cope with large volumes, leading to treatment failures and discharge of untreated waste to receiving water bodies.

The potential for permeable soils and terrestrial and aquatic vegetation to reduce overland transport of fecal pathogens is increasingly threatened by conversion of natural habitats to developed environments. As one example, degradation of coastal wetlands has resulted in a net loss of nearly 67% of saltwater marshes in the United States, eliminating water-cleansing services in coastal regions where human development, and the associated production of fecal matter, is greatest [161]. Recent work that examined the effect of estuarine wetland degradation on transport of *T. gondii* revealed that erosion of wetlands to mudflats could result in 6 orders of magnitude greater flux of oocysts to coastal waters [144]. Coupled with wetland destruction, modification to historic flow of waterways through channelization and increased urban runoff through storm drains will likely increase pathogen transport in surface waters. Just as landscape change can exacerbate impacts of climate change on pollution, climate can also facilitate the speed of landscape change. Regions that are susceptible to sea level rise are predicted to suffer further loss of wetlands in areas where accretion cannot compensate submergence due the speed of rising sea levels, reduced delivery of sediment, or because higher grounds have already been converted to urbanized

or agricultural lands [162]. The combination of landscape change coupled with climate variability may therefore increase fecal pollution of waterways [163].

The presence of *T. gondii* oocysts in water is only a health concern if the parasite remains infectious to susceptible hosts. Persistence of *T. gondii* in terrestrial and aquatic environments is closely governed by climatic factors [66]. Humid environments and cooler temperatures are generally more favorable for oocyst survival [63]. Conversely, extremes in weather including freezing temperatures or hot and arid conditions are less likely to support prolonged oocyst viability [64,164]. In regions where long-term data are available, including the United States, a trend of increasing surface soil moisture was detected [165], a climate change that could prolong viability of *T. gondii* oocysts. In middle and higher latitudes, the duration of time the earth is covered by ice or snow is expected to decline, rendering those environments more favorable to oocyst survival. Higher latitude and mountainous regions that to date have reported comparatively lower prevalence of *T. gondii* infections in humans [166,167] may experience a rising infection rate if a warmer climate facilitates oocyst infectivity [168].

In addition to impacting the spatial distribution and potential viability of oocysts, landscape change may lead to alterations in *T. gondii* strain pathogenicity, or the likelihood that humans are exposed to more virulent strains. In French Guiana, virulent strains of *T. gondii* were associated with waterborne outbreaks of infection and even mortality in some immunocompetent adults [169–171]. Unique genotypes of *T. gondii* in French Guiana were identified in anthropogenically altered environments, which differed from *T. gondii* strains isolated from surrounding natural habitats [172]. Exposure of seronegative persons to a particularly virulent strain of *T. gondii* that emerged from strain hybridization may have led to fatal outbreaks of toxoplasmosis in local communities. The desperate need for livelihood improvement in many regions of the world will likely lead to increasing encroachment of people into undeveloped lands, resulting in greater exposure of humans to *T. gondii* strains that typically circulate within wild felid populations.

7. Why a One Health modeling approach enhances understanding of *T. gondii* oocyst transmission

Currently, there is a notable lack of literature on the relationship between *T. gondii* oocyst properties, felid shedding patterns, oocyst transport from terrestrial to aquatic systems, and climate and habitat change. This knowledge gap illustrates the vital need for a more integrative method to examine the impacts of environmental change on oocyst-based infection in human and animal populations. Uniting ecologists, veterinarians, physicians, epidemiologists, molecular biologists, and physical scientists including hydrologists, oceanographers, and engineers, as well as local stakeholders and policymakers is critical to developing models of *T. gondii* oocyst transmission and management in terrestrial and aquatic environments.

Previous efforts to model *T. gondii* transmission have focused predominantly on the interactions of felids and their prey in maintaining infection. Given the complexity of multiple predator-prey interactions in natural environments, these models were designed to simulate transmission in several small habitat patches or on a single farm, and they focused on a single or few prey species [173,174]. In reality, overlapping wild and domestic felid populations sharing an environment likely consume a wide diversity of prey species that would be difficult to incorporate into traditional transmission models. In a unique model including a domestic cat population, multiple prey species, and direction of hydrologic flow, water-borne transmission was identified as a critical component of *T. gondii* infection [175]. While these models provide important insight on parasite transmission dynamics, a broader

scale approach is still needed to investigate environmental transmission in areas with multiple felid populations. Watershed-level models integrating human, domestic animal, and wildlife ecology with oocyst properties, landscape structure, and hydrology offer a new direction for examining the impacts of environmental change on *T. gondii* oocyst loading and transport from terrestrial to aquatic systems. For *T. gondii* and many other pathogens, incorporating the tools and perspectives of diverse disciplines through a One Health approach will provide essential new insights on health from the molecular to landscape level.

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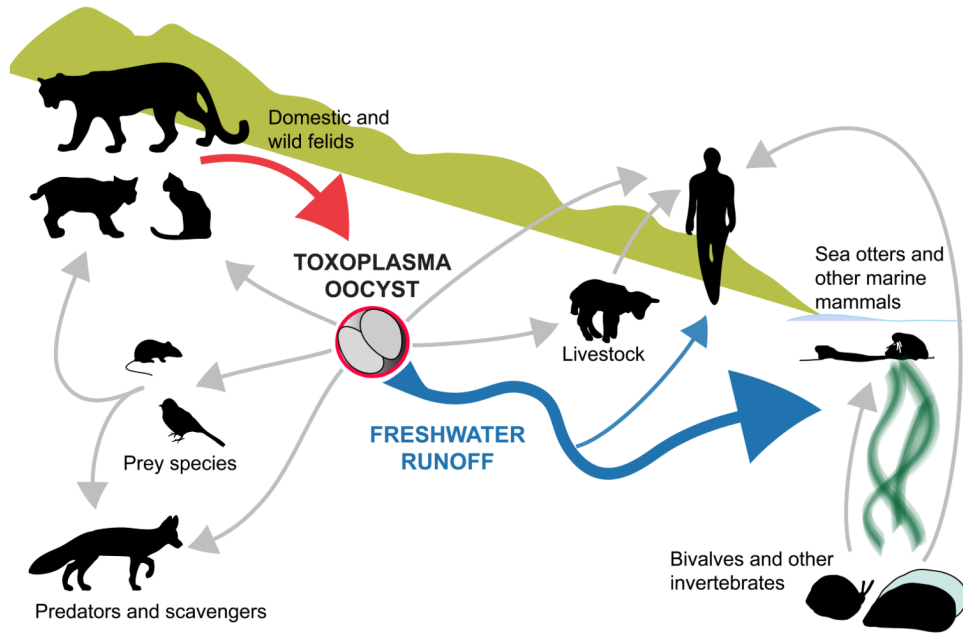


Fig. 1. Hardy, free-living *Toxoplasma gondii* oocysts, which can be transported in freshwater runoff (blue arrow), likely play a significant role in environmental transmission of *T. gondii* in terrestrial and aquatic systems. Domestic and wild felids are the only known source (red arrow) of *T. gondii* oocysts. Light gray arrows indicate possible routes of *T. gondii* transmission by exposure directly to oocysts or indirectly through food sources.

Table 1
Reported human clinical toxoplasmosis associated with oocysts in the environment

Reporting period	Location	Predominant habitat type	Implicated source	Cases	Reference
1972	Panama	Rain forest	River	32	[176]
1977	Georgia, USA	Horse stable	Soil	37	[177]
1980	Alabama, USA	Urban	Soil	10	[178]
1995	Victoria, Canada	Urban	Municipal water supply	100 (3000–7000 suspected)	[179]
1995–2002	French Guiana	Rain forest	Unknown	16	[169]
2001–2002	Santa Isabel do Ivaí, Brazil	Urban	Municipal water supply	426	[180]
2004–2005	Lublin, Poland	Farm	Well water	1 clinical (24 exposed)	[181]
2004–2005	Coimbatore City, India	Urban	Municipal drinking supply	248	[182,183]
1998–2006	French Guiana	Rain forest	Surface water	44	[169, 170, 184, 185]

Table 2
Epidemiological investigations linking *Toxoplasma gondii* infection with environmental exposure to oocysts

Reporting period	Location	Implicated source	No. infected (percent of tested)	Reference
1985	Ciego de Avila, Cuba	Non potable water	284(55.9)	[186]
1995	Grenada, West Indies	Soil or water	305(57)	[187]
1997–1999	Campos dos Goytacazes, Brazil	Unfiltered water	823(57.3)	[188]
2001	Iauareté, Brazil	Unfiltered water	191 (73.5)	[189]
2002	Rondonia, Brazil	Well or river water	195(73.3)	[189]
2003	Cascavel, Brazil	Homemade ice	161 (69.7)	[190]
2003	Guatamala	Well water	215(43)	[191]
2003–2004	Democratic Republic of São Tomé and Príncipe	Unboiled water	375(75.2)	[192]
2004	Aydin, Turkey	Non bottled water	185(30.1)	[193]
2004	Nunavik, Canada	Municipal and environmental waters	548(59.8)	[194]
2005	Fortaleza, Brazil	Homemade ice	666(69.1)	[195]
2005	Salvador, Brazil	Non-treated piped water	213(17.5)	[24]
2009–2010	Thailand	Pipe, tap, or rain water	181 (28.3)	[196]

Table 3
Published methods for detection of *Toxoplasma gondii* oocysts in water

Water tested	Laboratory or field study	Water concentration method	<i>T. gondii</i> detection assay(s) used	Quantitative, yes/no	Reference
Tap, fresh, marine	Laboratory	Ultrafiltration and capsule filtration	Microscopy, real time PCR, conventional PCR	Yes	[197]
Tap, fresh	Laboratory	Continuous separation channel centrifugation	Microscopy	Yes	[198]
Fresh	Laboratory	Capsule filtration	Real time PCR	Yes	[199]
Fresh	Laboratory and field	Capsule filtration	PCR, mouse bioassay	No	[200]
Fresh	Laboratory and field	Flocculation (Al ₂ (SO ₄) ₃)	Loop-mediated isothermal amplification	No	[201]
Tap, fresh	Laboratory ^a and field	Capsule filtration	Immunomagnetic separation	Yes	[202,203]
Tap, fresh, marine	Laboratory and field	Flocculation (Al ₂ (SO ₄) ₃)	Conventional PCR	No	[204]
Deionized, fresh	Laboratory and field	Capsule filtration	PCR, mouse bioassay	No	[205]
Fresh	Laboratory	Flocculation (Al ₂ (SO ₄) ₃ and Fe ₂ (SO ₄) ₃)	PCR	No	[206]
Deionized, tap	Laboratory	Centrifugation and Flocculation (Al ₂ (SO ₄) ₃ and Fe ₂ (SO ₄) ₃)	Microscopy	Yes	[207]
Tap	Laboratory ^a and field	Capsule filtration	Bioassay	No	[208]

^a *T. gondii* successfully detected only in spiked laboratory samples.

Table 4
Published reports of natural *Toxoplasma gondii* oocyst shedding in free-ranging wild felids. Reports of *T. gondii*-like oocysts (diagnosed by microscopy) and confirmed *T. gondii* oocysts (diagnosed by bioassay) are included

Region and felid	Sampling location	Diagnostic method	No. sampled (no. positive)	Shedding prevalence	Reference
North America					
Bobcat (<i>Lynx rufus</i>) ^a	New Mexico, Montana, USA	Microscopy and mouse bioassay	9 (3)	33.3%	[209]
Mountain lion (<i>Puma concolor</i>)	Montana, USA	Microscopy and mouse bioassay	5 (0)	0%	[209]
Mountain lion (<i>Puma concolor</i> ssp. <i>vancouverensis</i>)	Vancouver Island, British Columbia, Canada	Microscopy and mouse bioassay	23 (2) ^b	8.7%	[101,210]
Central/South America					
Ocelot (<i>Leopardus pardalis</i>)	Cockscomb Basin, Belize	Microscopy	8 (2)	25%	[211]
Jaguar (<i>Panthera onca</i>)	Cockscomb Basin, Belize	Microscopy	25 (1)	4%	[211]
Geoffrey's cat (<i>Leopardus geoffroyi</i>)	Pampas cat	Pig/mouse bioassay	73 (27) ^c	37%	[212]
(<i>Leopardus colocolo</i>)	Jaguarundi (<i>Puma yagouaroundi</i>)				
Asia					
Leopard (<i>Panthera pardus</i>)	Huai Kha Wildlife Sanctuary (HKWS), Thailand	Microscopy	54 (1)	1.9%	[213]
Tiger (<i>Panthera tigris</i>)	HKWS, Thailand	Microscopy	19 (0)	0%	[213]
Leopard cat (<i>Prionailurus bengalensis</i>)	HKWS, Thailand	Microscopy	3 (0)	0%	[213]
Iriomote cat (<i>Prionailurus iriomotensis</i>)	Iriomote-jima Island, Japan	Microscopy and mouse bioassay	45 (2) ^d	4.4%	[214]

^aFelid scientific names listed according to International Union for Conservation of Nature (IUCN) classifications [215]. Mountain lions (also commonly called pumas and cougars) identified as *Felis concolor* and *Felis concolor* in previous publications [14,39,101,209] all belong to a single species, *Puma concolor*.

^b16 fecal samples (1 positive) collected from dead mountain lions, 7 fecal samples (1 positive) collected in the environment from an unknown number of mountain lions. Overlap may exist between the sampled mountain lions and environmentally collected feces.

^cThe number and species of felids with confirmed shedding of *T. gondii* is unknown as *T. gondii*-like oocysts were pooled for bioassay.

^d45 fecal samples collected in the environment from an unknown number of Iriomote cats.

Table 5
Factors influencing *Toxoplasma gondii* oocyst-based transmission in terrestrial and aquatic environments

Oocyst loading	Oocyst survival	Transport of oocysts overland and in waterways
Distribution of wild and domestic felids <ul style="list-style-type: none"> • Human land use and available habitat • Habitat preferences • Distribution of prey species • Human management of felid populations 	Terrestrial conditions: <ul style="list-style-type: none"> • Soil chemistry and moisture • Air temperature and humidity Felid defecation behavior: <ul style="list-style-type: none"> • Depth of burying 	Felid defecation behavior: <ul style="list-style-type: none"> • Depth of burying • Proximity of oocyst deposition to waterway Chemical and physical surface properties of oocysts
Prevalence and frequency of oocyst shedding in wild and domestic cats <ul style="list-style-type: none"> • Felid diet (number and type of prey) • Prevalence of <i>T. gondii</i> infection in prey • Exposure to oocysts in the environment 	Aquatic conditions: <ul style="list-style-type: none"> • Water temperature 	Terrestrial conditions: <ul style="list-style-type: none"> • Watershed size and slope • Vegetation • Substrate (Permeable or impervious surfaces) Precipitation patterns: <ul style="list-style-type: none"> • Quantity, intensity, frequency, and duration
Duration and quantity of oocyst shedding by wild and domestic felids		Aquatic conditions: <ul style="list-style-type: none"> • Water chemistry and quality • Vegetation • Flow rate