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Original Contribution

Toxoplasma gondii, Source to Sea: Higher Contribution of Domestic Felids to Terrestrial Parasite Loading Despite Lower Infection Prevalence

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Abstract: Environmental transmission of *Toxoplasma gondii*, a global zoonotic parasite, adversely impacts human and animal health. *Toxoplasma* is a significant cause of mortality in threatened Southern sea otters, which serve as sentinels for disease threats to people and animals in coastal environments. As wild and domestic felids are the only recognized hosts capable of shedding *Toxoplasma* oocysts into the environment, otter infection suggests land-to-sea pathogen transmission. To assess relative contributions to terrestrial parasite loading, we evaluated infection and shedding among managed and unmanaged feral domestic cats, mountain lions, and bobcats in coastal California, USA. Infection prevalence differed among sympatric felids, with a significantly lower prevalence for managed feral cats (17%) than mountain lions, bobcats, or unmanaged feral cats subsisting on wild prey (73–81%). A geographic hotspot of infection in felids was identified near Monterey Bay, bordering a high-risk site for otter infection. Increased odds of oocyst shedding were detected in bobcats and unmanaged feral cats. Due to their large populations, pet and feral domestic cats likely contribute more oocysts to lands bordering the sea otter range than native wild felids. Continued coastal development may influence felid numbers and distribution, increase terrestrial pathogens in freshwater runoff, and alter disease dynamics at the human–animal–environment interface.

Keywords: *Toxoplasma gondii*, feral cats, mountain lions, bobcats, pathogen pollution, zoonotic disease

Introduction

The importance of health at the human–animal–environment interface is increasingly recognized worldwide as new zoonotic diseases emerge from animal reservoirs; known pathogens continue to be shared among people, domestic animals, and wildlife; and environmental change alters disease transmission ecology (Daszak et al. 2000; Patz et al. 2000; Jessup et al. 2007; Plowright et al. 2008). *Toxoplasma gondii*, a global, zoonotic protozoan parasite capable of infecting a wide range of warm-blooded animals (Tenter et al. 2000), illustrates the complexity of disease transmission among diverse hosts and environments.

Although long studied in urban and rural terrestrial landscapes, *T. gondii* has emerged as a significant aquatic

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pathogen, causing freshwater and marine mammal infection and water-borne outbreaks of disease in humans around the world (Miller 2008; Jones and Dubey 2010; Santos et al. 2011). In the early 2000s, reports of high levels of mortality due to *T. gondii* in threatened Southern sea otters (*Enhydra lutris nereis*) along the central California coast, USA, drew significant attention from researchers, wildlife managers, and citizens (Kreuder et al. 2003; Conrad et al. 2005). *Toxoplasma*-related deaths and high levels of infection in the sea otter population sparked concern due to the critical role of these animals in near-shore marine ecosystems. Sea otters facilitate kelp forest function and serve as important sentinels for chemical and biological pollution (Jessup et al. 2004; Conrad et al. 2005).

Wild and domestic felids are the only known hosts capable of shedding *T. gondii* oocysts, the environmentally-resistant, free-living stage of the parasite (Hutchison et al. 1969; Dubey et al. 1970; Miller et al. 1972). As felids are the definitive host of *T. gondii*, infection in sea otters provided evidence for land-to-sea pathogen transmission. Miller and colleagues (2002) observed a link between coastal areas with increased freshwater runoff and higher risk of sea otter infection. This association suggests that the extremely hardy oocysts shed in felid feces could be transported overland and in surface waterways to estuarine and marine waters, where sea otters were exposed. Oocyst persistence increases the potential for transport, as oocysts can remain viable for more than 1 year in soil, freshwater, and seawater (Frenkel et al. 1975; Dubey 1998; Dumetre and Darde 2003; Lindsay and Dubey 2009). *T. gondii*'s broad host range, diverse transmission routes, and environmental durability contribute to its potential to accumulate and persist in terrestrial and aquatic environments, thus threatening human and animal health.

Oocysts in coastal environments play a key role in land-to-sea transmission of *T. gondii*. Three main routes of *T. gondii* infection have been described for warm-blooded animals, including humans: eating an infected intermediate host with *T. gondii* in its tissues, ingesting oocysts (through contaminated water, soil, or food), and congenital transmission (Dubey and Beattie 1988; Tenter et al. 2000). As sea otters rarely consume known intermediate hosts of *T. gondii* (Riedman and Estes 1988; Estes et al. 2003), transmission may occur through direct exposure to oocysts in seawater or, more likely, through eating contaminated invertebrate prey (Conrad et al. 2005; Johnson et al. 2009). Filter feeding marine invertebrates, such as mussels, do not appear to become infected, but can retain and bioconcentrate viable *T. gondii* oocysts (Arkush et al. 2003; Lindsay et al. 2004). As transport hosts for *T. gondii*, these invertebrates are a potential source of infection for marine predators as well as humans (Jones et al. 2009).

Sites of increased risk for sea otter infection have been identified in California's coastal waters, but the distribution of *T. gondii* oocysts in the terrestrial environment is not well characterized. Molecular tests exist for detecting *T. gondii* in soil and water (Dumetre and Darde 2003; Lelu et al. 2011; VanWormer et al., 2013), but rapid, cost-effective, reliable, and sensitive diagnostics are not yet available for environmental assessment. Infection and oocyst shedding patterns in felids offer a targeted proxy for direct environmental testing, providing insight into terrestrial *T. gondii* loading. We investigated *T. gondii* infection and shedding in sympatric feral domestic and wild felids in coastal California. Concurrent sampling of diverse felids sharing the coast offers important perspective on their relative contributions to terrestrial oocyst load. We hypothesized that prevalence and risk factors for infection and oocyst shedding would differ among the groups of felids sampled, as host biology and behavior may influence *T. gondii* transmission dynamics. We also hypothesized that contributions to the total number of oocysts in the terrestrial environment vary among felid groups, based on levels of infection and oocyst shedding, as well as their population sizes in coastal California. Finally, we hypothesized that geographic clusters of infection and oocyst shedding could be detected. These clusters would identify regions with an increased parasite burden that could pose a higher risk of exposure for terrestrial populations and downstream wildlife.

Methods

Feral domestic cats (*Felis catus*), bobcats (*Lynx rufus*), and mountain lions (*Puma concolor*) were opportunistically sampled along the central California coast, USA, from 2006 to 2009. Felids were sampled along the sea otter range within 80 km of Moss Landing (36°48′15.28″N, 121°47′13.09″W) and Morro Bay (35°22′16.83″N, 120°51′25.00″W). Marine sites bordering these locations and the coast north of Morro Bay to San Simeon were previously identified as areas of increased risk for sea otter infection with *T. gondii* (Fig. 1, Miller et al. 2002; Kreuder et al. 2003; Conrad et al. 2005; Jessup et al. 2007; Johnson et al. 2009).

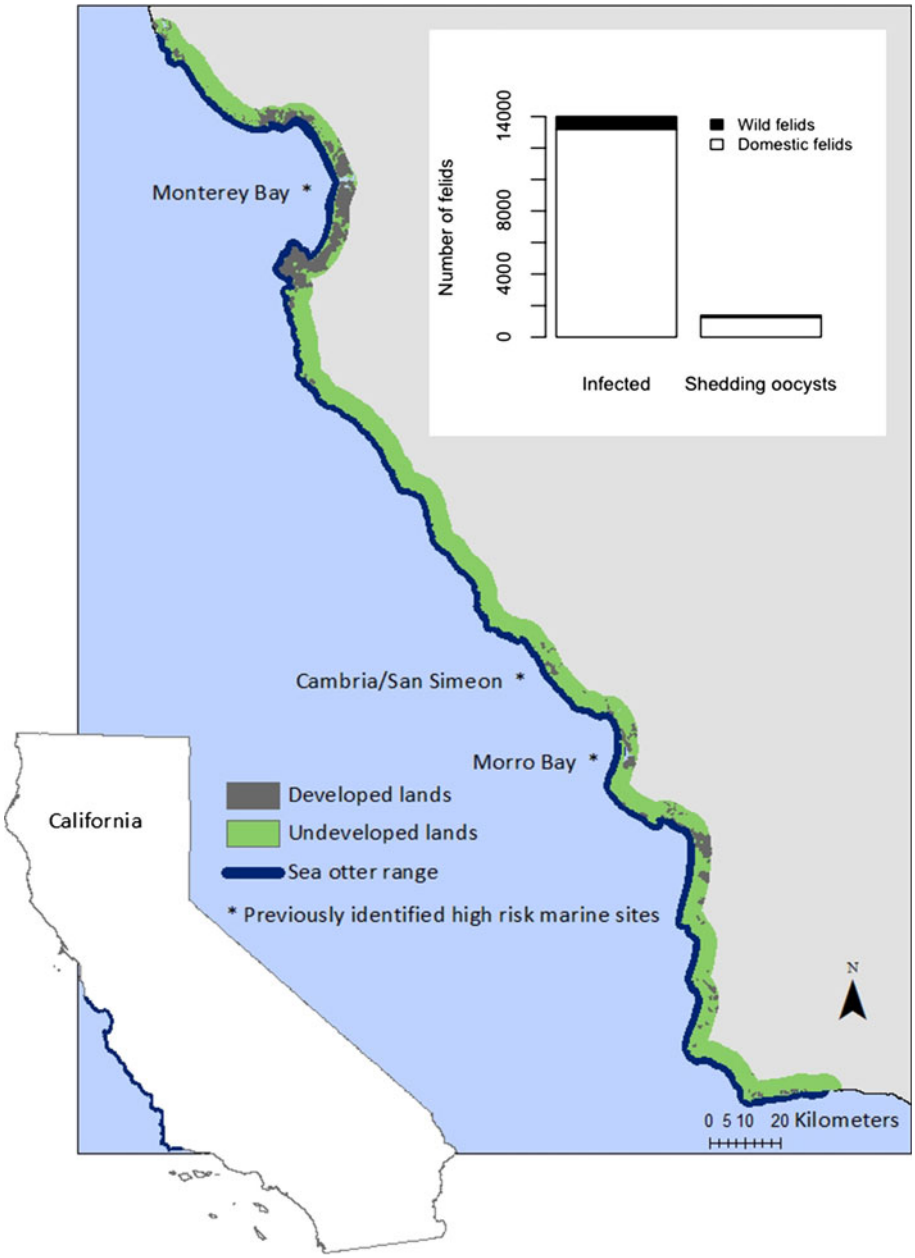

Figure 1. Land-use and estimated felid *T. gondii* infection and oocyst shedding in a 5-km-wide terrestrial buffer of the California sea otter range. Domestic felid populations (outdoor pet cats and managed feral cats) are closely associated with humans and developed lands, whereas wild felids (mountain lions and bobcats) more commonly use undeveloped habitats. Due to the higher numbers of infected and shedding domestic cats relative to wild felids, the developed urban and agricultural lands likely represent areas of increased oocyst loading and risk of terrestrial exposure to *T. gondii*. Three previously identified high-risk marine sites for *T. gondii* infection in sea otters are bordered by developed lands, highlighting the potential for land-to-sea pathogen transmission from these terrestrial areas. Unmanaged feral cats were excluded due to the uncertainty in estimating their distribution and population sizes.

Live Sampling

Blood and feces were collected from anesthetized feral domestic cats during regional trap-neuter-return programs. Age (juvenile vs. adult [>12 months]), sex, and nutritional status based on body condition score (rated 1–5 on an ordinal scale modified from Burkholder 2000) were assessed.

Postmortem Sampling

No animals were euthanized for the purpose of this study. Domestic cats were sampled through collaborations with regional animal shelters and federal wildlife protection programs. As part of a conservation program, wildlife specialists humanely euthanized feral cats found in critical nesting habitat for threatened and endangered shorebirds. Additional sampled domestic cats identified as feral by county residents or through behavioral testing were humanely euthanized by shelters for population control. Sampled wild felids included bobcats and mountain lions killed by vehicles, bobcats that died or were euthanized at animal shelters, and mountain lions euthanized by law enforcement personnel due to public safety concerns or predation on domestic animals. Felids were submitted to the California Department of Fish and Wildlife, and

demographic data (age, sex, and species), blood, and feces were collected during postmortem examination.

Serological Analysis

Sera were analyzed for antibodies against *T. gondii* using an indirect fluorescent antibody test (IFAT), as previously described (Dabritz et al. 2007b). All felid sera were tested using fluorescein isothiocyanate-labeled, goat anti-cat IgG secondary antibodies (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland, USA). The reciprocal end-point titer for each sample was the highest serum dilution with distinct, outline fluorescence of fixed *T. gondii* tachyzoites observed by fluorescent microscopy (Carl Zeiss, Oberkochen, West Germany). Based on an IFAT validation study in domestic cats (Dabritz et al. 2007b), a titer of $\geq$1:160 serum dilution was considered positive, indicating *T. gondii* infection.

Infection with feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) can adversely impact immune function in domestic or wild felids (Roelke et al. 2006; Hartmann 2011). Exposure to these viruses was used as a marker to evaluate association of immunosuppression with *T. gondii* shedding. Commercially available SNAP® FIV/FeLV Combo tests (Idexx Laboratories, Westbrook, Maine, USA) were performed according to the manufacturer's instructions to test sera for FeLV antigen and antibodies to FIV.

Fecal Analysis

Fecal samples were prepared by double centrifugation and flotation in zinc sulfate ($ZnSO_4$) and examined by light microscopy for the presence of *T. gondii*-like oocysts, as previously described (Dabritz et al. 2007a). DNA extraction, PCR amplification, and sequencing were performed on all samples containing oocysts that were morphologically consistent with *T. gondii.* Due to the challenges of detecting *T. gondii* DNA in feces, including low levels of oocyst shedding and the presence of PCR inhibitors (Dabritz et al. 2007a; Salant et al. 2007; Schares et al. 2008), not all of the *T. gondii*-like oocysts identified by microscopy could be confirmed as *T. gondii* using molecular methods.

Following microscopy, *T. gondii*-like oocysts were harvested from feces using a modified double centrifugation technique (Dabritz et al. 2007a) with 10 g of feces and 0.1% Tween-80. Following storage at −20°C, thawed oocyst pellets were resuspended in 50 microliters (μL) of ATL lysis buffer (Qiagen, Valencia, California, USA), frozen for 4 min in liquid nitrogen, and then immersed in boiling water for 4 min to break open oocyst walls. DNA was extracted using DNeasy® extraction kits (Qiagen) with the following modifications to the manufacturer's protocol: Samples were digested with 40 μL of proteinase K and a 1:10 dilution of AE buffer heated to 95°C was used in the final elution. Nested PCR reactions were performed using B1 primers (external: Pml/S1, Pml/S2; internal: Pml/AS1, Pml/AS2, Grigg and Boothroyd 2001) and ITS1 primers (external: ITS1DF, ITS1DR; internal: ITS1diF, ITS1diR, Rejmanek et al. 2009) following established reaction and thermocycler conditions (Rejmanek et al. 2009). Positive and negative controls (*T. gondii* tachyzoite DNA and sterile water, respectively) were included in each round. PCR products were separated electrophoretically on a 2% agarose gel stained with ethidium bromide. Gels were observed under UV light, and products consistent with *T. gondii* controls were prepared with ExoSAP-IT® (Affymetrix, Santa Clara, California, USA), and sequenced at University of California, Davis, Division of Biological Sciences DNA sequencing facility.

Statistical Analyses

Sampled felids were grouped by species, and feral domestic cats were further characterized as managed or unmanaged, based on their association with humans. The term "feral" is commonly applied to diverse groups of unowned domestic cats ranging from "stray" cats or cat colonies given food and shelter by people to more truly feral cats with little connection to humans. Here, we used "feral" to describe all sampled free-ranging, unowned domestic cats, and then grouped them according to human management. Live-sampled and euthanized feral cats from animal shelters were pooled as "managed feral cats". The majority of these cats were collected from small to large colonies in close proximity to people, where they had access to provided food sources (e.g., commercial cat food or discarded human foods). Animal control staff and local residents captured these cats in developed coastal areas. Cats removed from critical shorebird habitat by specialists through intensive trapping were characterized as "unmanaged feral cats". These solitary, feral domestic cats living in undeveloped landscapes likely subsisted primarily on wild prey and had minimal association with humans. All felids that tested positive on fecal flotation were classified as "*T. gondii*-like" shedders, and those animals additionally testing positive by

molecular methods (PCR and sequencing) were also identified as "confirmed *T. gondii*" shedders.

Seroprevalence of *T. gondii* infection, *T. gondii*-like oocyst-shedding prevalence, confirmed *T. gondii* oocyst-shedding prevalence, and respective 95% exact confidence intervals were estimated for felid groups. Differences in infection and oocyst-shedding levels among groups were tested using univariable logistic regression (LR) models. Due to non-independent sampling of many managed feral cats (i.e., multiple cats from the same colony), risk factors for *T. gondii* infection in these hosts were evaluated using mixed effects LR. Capture location was chosen as the cluster variable to account for unmeasured correlation among multiple cats from one site. Based on similarities in feeding behavior and infection prevalence, data for independently sampled unmanaged feral cats, mountain lions, and bobcats were combined. Risk factors for *T. gondii* infection in these animals were assessed with LR models. Due to low shedding prevalences, LR models for all felid data combined were used to examine risk factors for *T. gondii*-like and confirmed *T. gondii* oocyst shedding.

Demographic variables evaluated for association with infection and shedding included age (juvenile vs. adult), sex, nutritional condition, and felid type. Season (wet vs. dry), FIV exposure, and FeLV exposure were also evaluated as risk factors for *T. gondii* shedding. The majority of annual rainfall in California typically occurs from late October through March. However, given spatial and interannual variation in local rainfall patterns, season of sample collection was determined for each felid using daily rainfall records from the nearest California Irrigation Management Information System (CIMIS) gauge station (CIMIS 2011). Samples collected on dates falling between the first and last rainfall events recorded in the period from October to May were classified as wet season samples.

Odds ratios and 95% confidence intervals were estimated in univariable models. Demographic, environmental, and health variables associated with infection or shedding ($P \leq 0.20$) were incorporated into multivariable LR models. Final parsimonious models were selected by comparing Akaike's Information Criterion (AIC) among competing nested and non-nested models. Final model fit was evaluated by goodness of fit tests and graphical residual diagnostics. For multivariable models, adjusted odds ratios with 95% confidence intervals were estimated to assess the strength of the association between each risk factor and infection or shedding. Statistical tests were performed in R (R Core Development Team 2011), using the glmmML package (Broström and Holmberg 2011) for mixed effects models.

Locations of death or capture for each felid were manually geocoded using Google Earth version 5.2 (Google Inc., Mountain View, California, USA). Animals lacking location information (27 managed feral cats and four bobcats) were excluded from spatial analyses. Due to low levels of oocyst shedding in study felids, *T. gondii* infection was used to examine the spatial distribution of terrestrial oocyst load. Because bobcats and feral cats have small home ranges that reflect *T. gondii* transmission and shedding in a limited geographic area, infection in these felids was used as a proxy for local oocyst loading. In southern coastal California, average bobcat home ranges were 1.3–4 km^2 (Riley et al. 2003). Average feral cat home ranges varied from <1 km^2 for group-living feral cats in an urban area to 6.2 km^2 for solitary, unmanaged domestic cats in a rural environment (reviewed by Liberg et al. 2000). Mountain lions were excluded from spatial analyses, as their larger home ranges make it challenging to identify areas where they were exposed to and shed *T. gondii*. Average mountain lion home range size varied seasonally from 90–100 km^2 for females to 300–350 km^2 for males in California's coastal mountain ranges (Grigione et al. 2002).

Geographical clustering of *T. gondii* infection was evaluated using a Bernoulli model elliptical scanning window with a medium non-compactness penalty in SatScan version 9.0 (Kulldorff and Nagarwalla 1995; Kulldorff et al. 2006). Maximum spatial cluster size of 50% of the population at risk was used. Overlapping clusters were not permitted. The spatial analysis was adjusted for felid type in order to identify clusters reflecting a higher risk of *T. gondii* loading rather than differences in distribution of felid groups (Kulldorff et al. 2007). The analysis also controlled for spatial differences in animal sampling intensity along the coast. Sampling locations and spatial clusters were mapped using ArcGIS® version 10 (ESRI, Redlands, California, USA). A significance level of $\alpha = 0.05$ was used for all analyses.

Population Size and Oocyst Shedding

To compare relative contributions to *T. gondii* oocyst load, coastal felid population sizes were considered in addition to infection and shedding prevalence. Population sizes were estimated for study felids as well as outdoor pet domestic cats living in the coastal lands immediately bordering the California sea otter range (Fig. 1). Using ArcGIS, the otter

Table 1. *Toxoplasma gondii* percent seroprevalence of infection (SP) and *T. gondii*-like oocyst and confirmed *T. gondii* oocyst percent shedding prevalence (ShP) estimates with 95% confidence intervals (CI) for domestic and wild felids from central coastal California, 2006–2009.

	Serum samples	*T. gondii* antibodies [a]		Fecal samples	*T. gondii*-like oocysts [b]		Confirmed *T. gondii* oocysts[b]	
		Positive	SP (95% CI)		Positive	ShP (95% CI)	Positive	ShP (95% CI)
Managed feral cats	*720*	*121*	*16.8 (14.2–19.7)*	*435*	*8*	*1.8 (0.8–3.6)*	*2*	*0.5 (0.1–1.7)*
Unmanaged feral cats	16	13	81.3 (54.4–96.0)	17	2	11.8 (1.5–36.4)	1	5.9 (0.2–28.7)
Bobcats	22	16	72.7 (49.8–89.3)	16	2	12.5 (1.5–38.4)	1	6.3 (0.2–30.2)
Mountain lions	72	58	80.6 (69.5–88.9)	51	2	3.9 (0.7–13.0)	1	2.0 (0.1–9.7)

Levels of infection and oocyst shedding were lowest in managed feral cats (italicized).

[a]A serologic titer of $\geq$1:160 on indirect fluorescent antibody test was considered positive.

[b]The *T. gondii*-like oocyst-shedding prevalence includes all animals shedding oocysts consistent with *T. gondii* morphology by light microscopy. The confirmed *T. gondii* oocyst-shedding prevalence includes all animals shedding oocysts identified as *T. gondii* by PCR and DNA sequence analyses.

range (Tinker 2010) was buffered by 5 km to create the terrestrial border. The area of habitat (undeveloped lands) available to unmanaged feral cats, mountain lions, and bobcats was estimated by intersecting the terrestrial border with a land cover layer (FMMP 2006) that excluded developed lands and wetlands. Available habitat (km^2) was multiplied by published felid density estimates to approximate numbers of unmanaged feral cats and wild felids present in the 5-km-wide terrestrial border (Table 3). Outdoor pet cat and managed feral cat population sizes were estimated by multiplying total identified households in the border by survey-generated, cat-per-household values for the central California coast (Table 3). The number of households was determined by intersecting the terrestrial border with public census block data (FRAP 2011). Population sizes were multiplied by seroprevalences and *T. gondii*-like oocyst-shedding prevalences to estimate the numbers of infected and actively shedding felids within the terrestrial border. As outdoor pet cats were not sampled, published estimates of seroprevalence (15%; IFAT survey, Dabritz et al. 2007b) and *T. gondii*-like oocyst shedding (1.3%, Dabritz et al. 2007a) in owned domestic cats from central coastal California were used.

Results

From 2006 to 2009, 722 managed feral cats, 17 unmanaged feral cats, 73 mountain lions, and 26 bobcats were sampled along the central California coast. Seroprevalence of *T. gondii* infection differed among sympatric felid groups ($P < 0.01$), with a significantly lower seroprevalence observed in managed feral cats (Table 1). Infection prevalence did not differ significantly among unmanaged feral cats, mountain lions, and bobcats. Age and sex were not significantly associated with *T. gondii* infection in unmanaged feral cats and wild felids. However, the odds of infection were two times higher in adult managed feral cats than juveniles (odds ratio = 2.1, 95% CI (1.1–3.8), $P = 0.02$). Sex and nutritional condition were not significantly ($P > 0.05$) associated with *T. gondii* infection in managed feral cats.

Oocysts morphologically consistent with *T. gondii* were observed in the feces of 15 felids, but only five samples (from two managed feral cats, one unmanaged feral cat, one bobcat, and one mountain lion) were confirmed as *T. gondii* using molecular methods. One of the 15 samples, from a managed feral cat, was identified as *Hammondia hammondi* based on PCR and sequencing results, and was excluded from prevalence estimates and statistical analyses. DNA from *T. gondii* or other related parasites was not detected in the remaining nine suspect-positive samples.

The odds of shedding *T. gondii*-like oocysts were more than seven times higher in unmanaged feral cats and bobcats than managed feral cats ($P = 0.02$; Table 2). Unmanaged feral cats and bobcats were over 13 times more likely to be shedding confirmed *T. gondii* oocysts than managed feral cats ($P = 0.04$). For mountain lions, unlike other felids subsisting on wild prey, the odds of oocyst shedding did not differ significantly from those

Table 2. Significant risk factors for *T. gondii*-like and confirmed *T. gondii* oocyst shedding in wild and feral domestic felids from the central California coast, 2006–2009 (univariable logistic regression models).

		T. gondii-like oocysts[a]			Confirmed *T. gondii* oocysts[a]		
Risk factor	*n*	OR	95% CI	*P* value	OR	95% CI	*P* value
Type of felid							
Managed feral cat	435	1.0	–	–	1.0	–	–
Unmanaged feral cat	17	7.1	(1.4, 36.4)	0.02	13.5	(1.2, 157.1)	0.04
Bobcat	16	7.6	(1.5, 39.2)	0.02	14.4	(1.2, 168.1)	0.03
Mountain lion	51	2.2	(0.5, 10.6)	0.33	4.3	(0.4, 48.6)	0.24
T. gondii serologic status							
Negative	383	1.0	–	–	1.0	–	–
Positive	132	4.1	(1.4, 11.9)	0.01	11.9	(1.3, 107.8)	0.03

[a]*T. gondii*-like oocysts were consistent with *T. gondii* morphology by light microscopy. Confirmed *T. gondii* oocysts were identified as *T. gondii* by PCR and DNA sequence analyses.

Table 3. Estimated population sizes and numbers of domestic and wild felids infected with and actively shedding *T. gondii* oocysts in a 5-km-wide strip of coastal urban, agricultural, and undeveloped lands bordering the central California sea otter range.

Type of Felid	Felids/Household or Felids/km^{2a}	References	Estimated population size	Infected felids	Actively shedding felids
Outdoor pet cats[b]	0.32/Household	Calculated from Dabritz et al. (2006)	47,520–50,720	7,128–7,608	618–659
Managed feral cats	0.21/Household	Calculated from Dabritz et al. (2006)	31,185–33,285	5,239–5,592	561–599
Unmanaged feral cats	0.90–3.50/km^2	Reviewed by Liberg et al. (2000)	1,264–6,048	1,264–4,917	184–714
Bobcats	0.25–0.60/km^2	Larrucea et al. (2007), Ruell et al. (2009)	432–1,037	314–754	54–130
Mountain lions	0.01–0.04/km^2	Pierce et al. (1999), Laundré and Clark (2003) reviewed by Quigley and Hornocker (2009)	17–69	14–56	1–3

[a]153,500 (± 5,000) human households and 1,728 km^2 of undeveloped habitat were identified in the terrestrial border for domestic and wild felid population size calculations.

[b]For outdoor pet cats, infection (15%) and *T. gondii*-like oocyst-shedding (1.3%) prevalences were adapted from Dabritz et al. (2007a), (b). Infection and *T. gondii*-like oocyst-shedding prevalences listed in Table 1 were used for all other felid groups.

observed in managed feral cats. In addition to felid type, testing seropositive for *T. gondii* was significantly associated with *T. gondii*-like and confirmed *T. gondii* oocyst shedding. Feral domestic and wild felids with detectable IgG antibodies to *T. gondii* were four times more likely to be shedding *T. gondii*-like oocysts than seronegative cats ($P = 0.01$). Oocyst shedding was not associated with sex or exposure to potentially immunosuppressive viruses (FeLV or FIV). Age and season were not significantly associated with shedding. However, four of five confirmed *T. gondii* oocyst samples were from adult felids, and all confirmed *T. gondii* oocyst samples were obtained during the wet season. Estimated felid group oocyst-shedding prevalences are provided in Table 1.

As a component of population size estimates for outdoor pet cats and managed feral cats, 153,500 (±5,000) households were counted in the 5-km-wide terrestrial border along the sea otter range. In addition, 1,728 km^2 of undeveloped habitat were identified for density-based population estimates of unmanaged feral cats, mountain lions, and bobcats. Approximately 78,700–84,000 outdoor pet cats and managed feral cats were estimated to be living in the terrestrial border (Table 3). Much smaller populations of unmanaged feral cats (1,264–6,048), bobcats (432–1,037), and mountain lions (17–69) were estimated in this area. Numbers of infected and actively shedding cats (based on *T. gondii*-like oocyst-shedding prevalence) followed similar trends. Consistently higher contributions to terrestrial

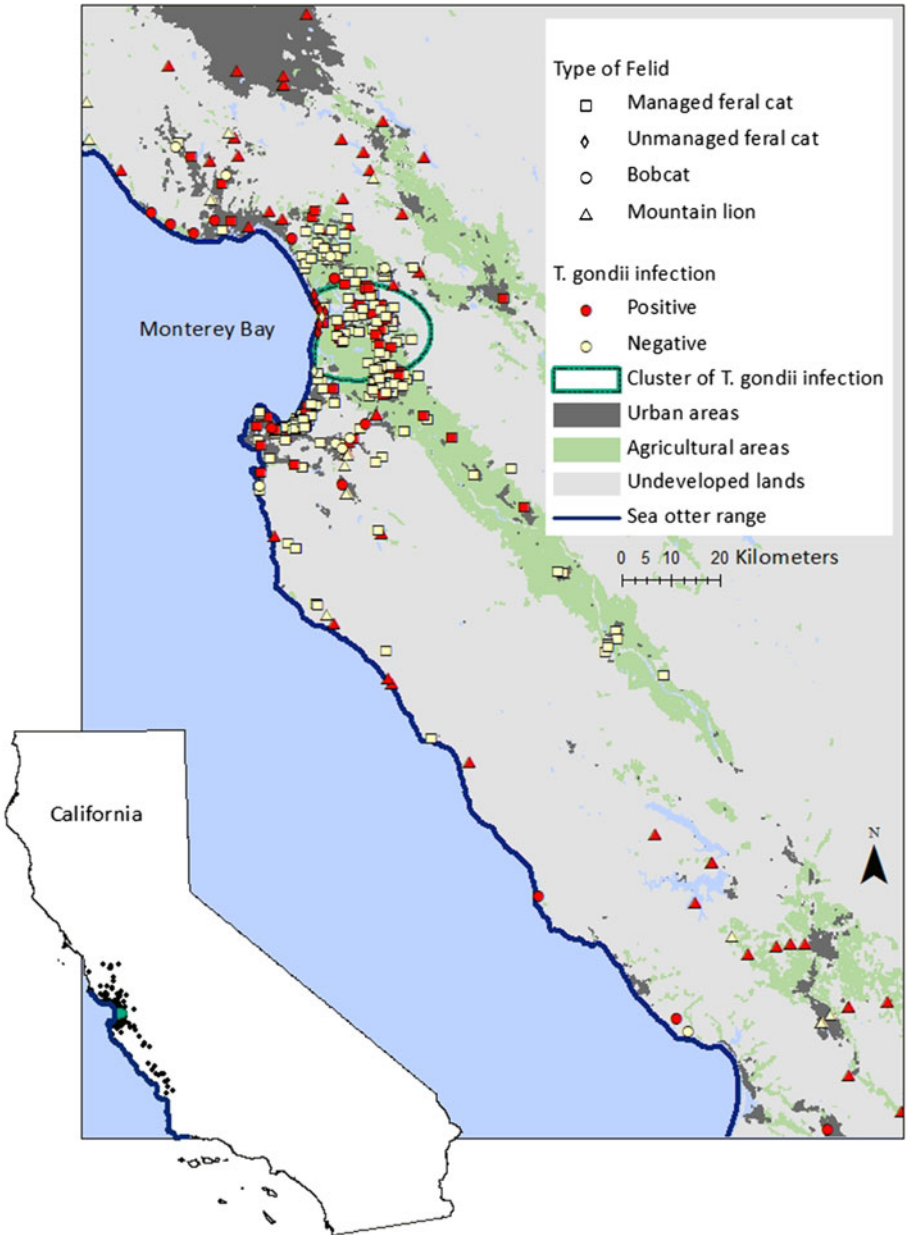

Figure 2. Locations of domestic and wild felids sampled along the central California coast, USA, 2006–2009. The distribution of *T. gondii* infection in felids with small home ranges (feral cats and bobcats) was used as a proxy to detect the geographic cluster of locally increased risk of *T. gondii* oocyst loading in the terrestrial environment. This hotspot of infection borders Monterey Bay, a previously identified high-risk site for sea otter infection with *T. gondii*.

oocyst load were estimated from outdoor pet cats and feral domestic cats than wild felids. Using infection in bobcats and feral cats as a proxy for local terrestrial loading, a cluster of increased local *T. gondii* oocyst environmental contamination was detected near Monterey Bay, California (Fig. 2, $P < 0.01$).

Discussion

Understanding the roles of domestic and wild felids in land-to-sea transmission of *T. gondii* is an important step in developing research and management strategies to protect the health of humans, threatened wildlife species like sea otters, and other animals sharing the coastal environment. This study is the first to investigate patterns of both *T. gondii* infection and oocyst shedding in overlapping populations of feral domestic and wild felids and to estimate their relative contributions to coastal oocyst loading. In addition, we provide critical baseline information for wild felid populations. Limited reports exist on *T. gondii* shedding in many free-ranging felids, including mountain lions and bobcats (as reviewed by VanWormer et al. 2013).

Patterns of *T. gondii* Infection and Oocyst Shedding Among Domestic and Wild Felids

Differences in infection prevalences among feral domestic and wild felids in coastal California are likely linked to diet. Unmanaged feral cats, mountain lions, and bobcats, which subsist primarily on wild prey, exhibited high levels of *T. gondii* exposure (72.7–81.3%), consistent with reports of infection in other free-ranging populations (Miller et al. 2008; Jones and Dubey 2010; Bevins et al. 2012). The lower seroprevalence (16.8%) and odds of infection detected in managed feral cats likely reflect their access to alternative food sources (e.g., commercial cat food or scraps scavenged from or distributed by people), which pose a lower risk of exposure to *T. gondii* tissue cysts. Though well-fed domestic cats may sometimes instinctively hunt (Fitzgerald and Turner 2000; Winter 2004), low predation and low seroprevalences of *T. gondii* have been observed in managed feral cat colonies (Levy and Crawford 2004; Afonso et al. 2006). The theory that managed cats predate only occasionally, with the risk of exposure to *T. gondii* increasing over a lifetime was supported by our finding that adult managed feral cats were more likely to be infected with *T. gondii* than juveniles. There was no association between age and infection in unmanaged feral cats or wild felids. Therefore, unmanaged feral cats and wild felids are likely exposed to *T. gondii* at an early age and then repeatedly during their lifespan through predation.

Diet likely also influences oocyst shedding, but whether or not an animal consumes a prey-based diet may not be completely sufficient for explaining shedding dynamics. The highest prevalences of *T. gondii*-like and confirmed *T. gondii* oocyst shedding were found in unmanaged feral cats and bobcats, both of which subsist predominantly on wild prey. The odds of shedding were significantly higher in these groups than in managed feral cats, whose oocyst shedding estimates were consistent with prior reports (Jones and Dubey 2010). Lower oocyst shedding in managed feral cats is not surprising, given their likely lower consumption of potential intermediate hosts. However, the low levels of oocyst shedding by mountain lions, which consume predominantly wild prey, are more challenging to interpret. Unlike bobcats and unmanaged feral cats, the odds of oocyst shedding were not significantly different in mountain lions compared to feral cats. Marchiondo and colleagues (1976) reported similar differences in oocyst shedding among wild felids, with higher levels of *T. gondii*-like and confirmed *T. gondii* oocyst shedding observed in free-ranging bobcats than mountain lions from New Mexico and Montana, USA. The confirmed *T. gondii* oocyst-shedding prevalence (2%) in sampled California mountain lions reported here was lower than the 8.3% prevalence detected in free-ranging Canadian mountain lions (Aramini et al. 1998; Dubey et al. 2008). Differences in oocyst-shedding prevalences in geographically separate populations of mountain lions, as well as among sympatric mountain lions, bobcats, and unmanaged feral cats eating prey-based diets suggest that frequency and type of prey eaten, as well as the prevalence of *T. gondii* infection in different prey species, influence felid shedding.

In experimental studies, domestic cats typically shed millions to hundreds of millions of oocysts during a single 1–3 week period following initial *T. gondii* infection (Dubey and Frenkel 1974; Fritz et al. 2012). However, repeat oocyst shedding by domestic and wild felids can occur, increasing their potential contribution to environmental oocyst loading. Chronically infected domestic cats have been experimentally shown to reshed oocysts following exposure to new strains of *T. gondii*, when treated with immunosuppressive doses of glucocorticoids, or when co-infected with another common feline parasite, *Isospora felis* (Chessum 1972; Dubey and Frenkel 1974; Dubey 1976, 1995; Jones and Dubey 2010). Natural repeat shedding of oocysts has been observed in captive wild felids, and was attributed to repeated exposures to *T. gondii* in raw meat (Lukesova and Literak 1998).

By impacting immune status, as well as exposure to *I. felis* and new strains of *T. gondii*, diet has strong potential to influence repeat shedding in free-ranging wild and domestic felids. While mountain lions feed regularly on large wild prey, primarily deer (Pierce et al. 2000), unmanaged feral cats and California bobcats tend to hunt birds and small mammals, such as lagomorphs and rodents (Liberg et al. 2000; Tewes et al. 2002; Afonso et al. 2007; Hass 2009), and likely consume higher numbers of individual animals over time. These dietary preferences may make them more likely to encounter infected intermediate hosts, which could lead to high rates of initial infection, exposure to new strains of *T. gondii*, and repeat oocyst shedding. Managed feral cats with access to prepared food and California mountain lions eating fewer, larger prey may contact new *T. gondii* strains and acquire co-infections like *I. felis* less frequently, lowering their risk of repeat shedding and their observed shedding prevalences.

To our knowledge, no longitudinal studies have been conducted to evaluate how frequently repeat oocyst shedding occurs in naturally exposed, free-ranging felids. Domestic and wild felids actively shedding oocysts can test seropositive or seronegative based on their route of exposure to *T. gondii*, which influences timing of shedding and antibody development (Dubey et al. 1970; Dubey 1976, 1995). However, Jones and Dubey (2010) propose that many seropositive cats have likely already shed oocysts in the past. In our study, felids with detectable IgG antibody titers for *T. gondii* were more likely to be actively shedding oocysts than those without detectable titers. If seropositive cats are truly likely to have previously shed oocysts, this association could indicate common repeat shedding. These data, along with likely exposure to diverse strains of *T. gondii* in wild prey and shedding by adults from all study groups, suggest a high potential for repeat shedding of *T. gondii* in free-ranging felids, contributing to terrestrial oocyst load.

Patterns of infection and oocyst shedding for wild and domestic felids in this study provide strong evidence for the potential of diet to influence *T. gondii* exposure as well as initial and repeat oocyst shedding. While it is not feasible to rule out the possibility of cross-reactivity in serologic tests for wild and domestic felids, cross-reactivity is unlikely to account for the observed differences in seroprevalence and oocyst shedding. The serologic test used in this study was previously found to be highly sensitive and specific for detecting *T. gondii* antibodies in domestic cats. In addition, high bobcat and mountain lion *T. gondii* seroprevalences detected in study felids were consistent with those reported in bobcat and mountain lion populations tested using an ELISA diagnostic test (Bevins et al. 2012). Similar seroprevalences from wild felid studies using different diagnostic tests support that the high mountain lion, bobcat, and unmanaged feral cat seroprevalences in this study reflect higher levels of parasite exposure rather than diagnostic test error.

Wild and domestic felid population patterns were inferred from an opportunistic sample of sympatric animals along the California coast. Logistical limitations precluded a more random sample of wildlife and feral cat populations. A convenience sampling approach has the potential to bias study results and population level conclusions. It is possible that animals sampled in a convenient manner (along roadways, from animal shelters, or from depredation programs) are not representative of the larger populations inhabiting the coastal landscape. For example, felids infected with *T. gondii*, a parasite known to affect behavior in other species (Berdoy et al. 2000), may be more likely to cross roads or to enter traps, which could increase group estimates of seroprevalence. Given the consistency of prevalence and shedding levels in this study with many previously reported estimates, it is reasonable to assume that this bias is not differentially affecting any single group of felids. Based on our consistent methods in collecting and analyzing specimens from sympatric wild and domestic study felids, the comparisons across felid groups provide good estimates of relative infection and shedding, even if these estimates do not reflect the population levels perfectly.

Relative Contributions to and Spatial Distribution of Terrestrial Oocyst Loading

Unmanaged feral cats, bobcats, and mountain lions in our study had higher prevalences of *T. gondii* infection and shedding than managed feral cats. However, their smaller population sizes likely limit their relative contribution to coastal California oocyst load. The total number of outdoor pet cats and managed feral cats was estimated to be over 75 times larger than the number of wild felids in the 5-km-wide coastal terrestrial environment bordering the sea otter range (Table 3). The estimated numbers of infected and shedding outdoor pet cats and managed feral cats were also drastically higher than those of unmanaged feral cats, mountain lions, and bobcats. As pet cat and managed feral cat populations are closely associated with people and developed lands, human behavior can strongly influence terrestrial parasite loading and land-to-sea transmission through domestic animal management and landscape alterations. Spaying and neutering domestic cats, keeping pet cats indoors, and reducing the number of feral cats would play a key role in limiting the number of oocysts shed into the coastal environment. In addition, preserving intact wetlands and reducing impervious surfaces like asphalt and concrete in coastal lands could reduce the transport of *T. gondii* oocysts via freshwater runoff into neighboring aquatic environments (Shapiro et al. 2010; Shapiro 2012).

As infection and oocyst shedding differ among felid groups, the distribution of these animals in the coastal landscape could influence local risk of exposure to *T. gondii*. Populations of outdoor pet and managed feral cats are likely to be concentrated in developed urban and rural areas, whereas wild felids are more likely to avoid areas with high human use (Crooks 2002; Reed and

Merenlender 2011). Less is known about population density and landscape utilization by unmanaged feral cats in California, which may inhabit undeveloped lands as well as developed areas (Liberg et al. 2000; Horn et al. 2011). Based on the estimated higher contribution of outdoor pet cats and managed feral cats to terrestrial oocyst loading, we would expect increased oocyst levels in coastal areas with human development (Fig. 1), many of which are adjacent to critical habitat for marine wildlife. A geographic cluster of *T. gondii*infection was identified in the developed landscape bordering Monterey Bay, and four of the five felids actively shedding confirmed *T. gondii* oocysts were from this area. In addition to suggesting local differences in terrestrial infection risk, this area borders a previously identified high-risk marine site for sea otter *T. gondii* infection (Conrad et al. 2005; Jessup et al. 2007). Increased infection in closely connected terrestrial and marine areas provide further evidence of *T. gondii's* transmission from terrestrial to aquatic environments. In California's changing landscapes and beyond, understanding the roles of domestic and wild hosts as well as anthropogenic and environmental influences on *T. gondii* transmission dynamics will expand our ability to protect animal and human health.

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