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ORIGINAL ARTICLE

Seasonal and spatial variation in *Toxoplasma gondii* contamination in soil in urban public spaces in California, United States

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Abstract

Background: *Toxoplasma gondii* is a zoonotic parasite that can have severe implications for human health. Acutely infected cats shed environmentally resistant *T. gondii* oocysts in their faeces that contaminate soil, and soil can serve as a reservoir of infection for humans. Free-roaming domestic cats are thought to play an important role in environmental contamination with *T. gondii*, but few studies have directly measured the direct contribution of free-roaming cats to *T. gondii* in soil.

Methods: Our goals were to determine whether *T. gondii* soil contamination occurs in public areas with free-roaming cat colonies in central California and examine spatial and temporal variation in soil contamination. We initially performed spiking experiments to compare the limit of *T. gondii* detection in soil using three conventional nested PCR assays and one real-time quantitative PCR. The nested PCR targeting the internal transcribed spacer (ITS-1) of the small subunit ribosomal RNA was the most sensitive assay, with a limit of detection between 20 and 200 oocysts per gram of soil. We applied the ITS1 PCR assay on soil from sites in city and state parks, public playgrounds and community gardens in central California, USA. Samples were collected during spring, summer and fall and in sites located along the coast and inland.

Results: We detected and sequence-confirmed *T. gondii* in 5.6% of all of our soil subsamples, but with large seasonal and spatial variation in soil contamination: we only detected *T. gondii* during fall and only in coastal sites (44.3% soil prevalence), despite similar sampling intensity across space and time.

Conclusions: Our results suggest that free-roaming cat colonies are an important source of *T. gondii* in spaces where people recreate and grow food and that soil contamination is highly seasonal and spatially variable. Management of free-roaming cats could prevent *T. gondii* infections by reducing environmental contamination with this zoonotic pathogen.

KEYWORDS
free-roaming cats, management, molecular detection, public health, sea otters

1 | INTRODUCTION

Toxoplasma gondii infection can cause miscarriage when women are first exposed during pregnancy, severe ocular and neurological lesions in newborns and systemic disease and deaths in immunocompromised individuals (Maenz et al., 2014; Sukthana, 2006; Torgerson & Mastroiacovo, 2013). Infection has also been associated with schizophrenia and neurodegenerative diseases in congenitally infected people (Brown et al., 2005; Ngô et al., 2017). Although less common, severe health outcomes in immunocompetent individuals following acquired infection with certain strains of *T. gondii* have also been reported and include ocular toxoplasmosis, pneumonia, organ failure and death (Carme, Demar, Ajzenberg, & Dardé, 2009; Cortés & Aguirre, 2018; Glasner et al., 1992; Leal et al., 2007).

Wild and domestic felids are the only known definitive hosts of *T. gondii* (Hutchison, Dunachie, Siim, & Work, 1969). Felids usually become infected after ingesting infected prey and can shed up to a billion environmentally resistant oocysts in their faeces for approximately one week following infection (Fritz, Barr, Packham, Melli, & Conrad, 2012), resulting in contaminated soil (Dubey, 1995). People can become infected with *T. gondii* by three different pathways: congenitally, after ingesting tissue cysts in undercooked meat from *T. gondii*-infected animals, or by consuming water, fruits, vegetables, soil or paratenic hosts (e.g., shellfish) contaminated with *T. gondii* oocysts (Aguirre et al., 2019; Bahia-Oliveira, Gomez-Marin, & Shapiro, 2017; Dubey & Jones, 2008; Jones & Dubey, 2010). The relative contribution of oocyst-borne infections to the overall prevalence of *T. gondii* in people is unknown for the majority of human populations. However, consumption of *T. gondii* oocysts in contaminated soil has been reported as an important risk factor (Cook et al., 2000; Jones et al., 2001; Lopez, Dietz, Wilson, Navin, & Jones, 2000; Spalding, Reis Annendoeira, Klein, & Ribeiro, 2005), and in the United States, oocyst-borne infections have been demonstrated in 78% (59/76) of acutely infected pregnant women (Hill et al., 2011).

Free-roaming domestic cats play a critical role in environmental contamination with *T. gondii* oocysts (Gotteland et al., 2014; Simon et al., 2017; VanWormer et al., 2016). Cat colonies are often found in areas with high contact with people and where access to food sources are greater (Kilgour et al., 2017; Schmidt, Lopez, & Colliter, 2007), such as parks, playgrounds and community gardens. Supplemental feeding of cats may alter *T. gondii* transmission by changing cat distribution, demographics and exposure to infected prey (Schmidt et al., 2007; VanWormer et al., 2013). Furthermore, free-roaming cats in central California, USA are known reservoirs of atypical *T. gondii* genotypes (e.g., Type X) (VanWormer et al., 2014), which are associated with more severe disease in people (Blaizot et al., 2019; Carme et al., 2009; Pomares et al., 2018). Understanding the risks of exposure to *T. gondii* from contaminated soil will help inform management of free-roaming cat colonies and assess the risk of human exposure to this zoonotic pathogen.

Impacts

- We sampled soil from under cat faeces located in parks, playgrounds and community gardens with free-roaming cat colonies and detected *Toxoplasma gondii* DNA in 5.6% of soil samples.
- We only detected *T. gondii* DNA during fall and only in coastal sites, indicating that prevalence of this zoonotic parasite in soil is highly variable seasonally and spatially.
- Free-roaming cat colonies present a risk of *T. gondii* exposure for people in contact with soil at public spaces. Managing free-roaming cat colonies can benefit human health.

In the United States and Europe, *T. gondii* infection in humans is seasonal with higher rates in late summer, fall and early winter, which has been associated with seasonal variation in activities that involve direct or indirect contact with soil (e.g., gardening and consumption of fresh produce; Contopoulos-Ioannidis, Talucod, Maldonado, & Montoya, 2015; Morin, Lobry, Peyron, & Wallon, 2012; Sagel, Mikolajczyk, & Krämer, 2010). Seasonal variation in exposure to *T. gondii* can be associated with environmental conditions that affect the viability and persistence of oocysts in soil. For example, sporulated oocysts can remain viable for at least 18 months in moist soil across a wide range of temperatures from −20°C to 35°C (reviewed by Dumètre & Darde, 2003; Lelu et al., 2012). However, very little is known about seasonal variation of *T. gondii* contamination in soil.

There are few studies of *T. gondii* oocysts in soil, in part due to limitations in current methods of sampling and detection (Dumètre & Dardé, 2004; Lelu et al., 2011; Shapiro et al., 2019; Su, Shwab, Zhou, Zhu, & Dubey, 2010). Prior investigations have been conducted in temperate or subtropical regions (Afonso, Thulliez, & Gilot-fromont, 2006; Davis et al., 2018; Du et al., 2012; Gotteland et al., 2014; Lass et al., 2009; Liu et al., 2017; Simon et al., 2017) with *T. gondii* prevalence in soil ranging from 0% (0/120, 95% CI = 0–3) in Hawaii, USA to nearly 50% (278/558, 95% CI = 45.7–54) in Northeastern France. However, few of these studies have analysed spatial or temporal variation in the patterns of *T. gondii* in soil. In China, presence of oocysts in soil varied seasonally with highest prevalence in fall and winter (Liu et al., 2017). Temporal patterns of *T. gondii* in soil may be due to seasonal variation in temperature and precipitation, which may affect *T. gondii* oocyst survival (Dumètre & Darde, 2003; Lelu et al., 2012), as well as to seasonal patterns of shedding in cats, which were higher in summer and fall in Germany (Schares et al., 2016), and higher in fall and winter in France (Simon et al., 2018). Seasonal shedding may be driven by seasonal variation in prey availability and exposure to infected prey (Simon et al., 2018) as well as to variation in cat demography. Transmission from infected prey to cats is thought to peak after kittens lose maternal antibodies against *T. gondii* following weaning and begin hunting (Gilot-Fromont et al., 2012; Nutter, Jay, & Stoskopf, 2004; Omata et al., 1994).

Our goals were to evaluate whether soil contamination with *T. gondii* occurs in recreation areas with free-roaming cat colonies and to examine spatial and temporal variation in *T. gondii* soil contamination. We focused on cat colonies occurring urban public spaces including parks, playgrounds and community gardens, where human exposure to *T. gondii* could occur. We sampled sites located in coastal and inland central California and sampled in three seasons, spring, summer and fall.

2 | MATERIALS AND METHODS

2.1 | Site selection

We searched for free-roaming cat colonies located in or near public areas including community gardens, parks and playgrounds within 50 miles of Santa Cruz, CA, with the limit set by logistical and financial considerations. We found and studied seven free-roaming cat colonies in Santa Cruz, Monterey and Santa Clara counties California, USA. Four sites were located along the coast of the Monterey Bay area, and three sites were located inland (Figure 1). We sampled each site during the first two hours after dawn, which is when cats tend to be more active (Cove, Gardner, Simons, Kays, & O'Connell, 2018). We sampled each site once in spring (April and May), summer (June and July) and fall (November) of 2017. At each site visit, we recorded the number of feeding stations and counted the total number of cats and kittens for one hour using fur colouration and eartippings to distinguish among individuals. We used size and appearance to differentiate between kittens and cats of other ages (hitherto referred as adult cats).

We sampled soil for *T. gondii* beneath scattered cat faeces and latrines. Latrines were defined as clusters of more than two faecal deposits with nearby paw prints, whereas scattered faeces were defined as no more than two clustered faeces (Simon et al., 2017). We identified each latrine and scattered faeces with an alphanumeric number and weighed and collected 20–50 g of soil from beneath the surface area of the latrines and the scattered faeces at an approximate depth of 2–5 cm as described by Simon et al. (2017). We also collected faecal samples opportunistically if they were no more than approximately two days old based on texture and smell. We kept the soil and faecal samples in plastic bags under refrigeration and processed them the following day.

2.2 | Oocyst recovery from soil

We subdivided each 20–50 g of soil sample into 4–10 replicates of 5 g (hitherto referred as sub-samples) and processed the entire sample. We processed soil sub-samples using a method modified from the sucrose flotation method described by Lelu et al. (2011) and Simon et al. (2017). Briefly, we added 2.5 ml of 2% sulphuric acid to each 5-g replicate and vortexed the solution for 1 min. The solution

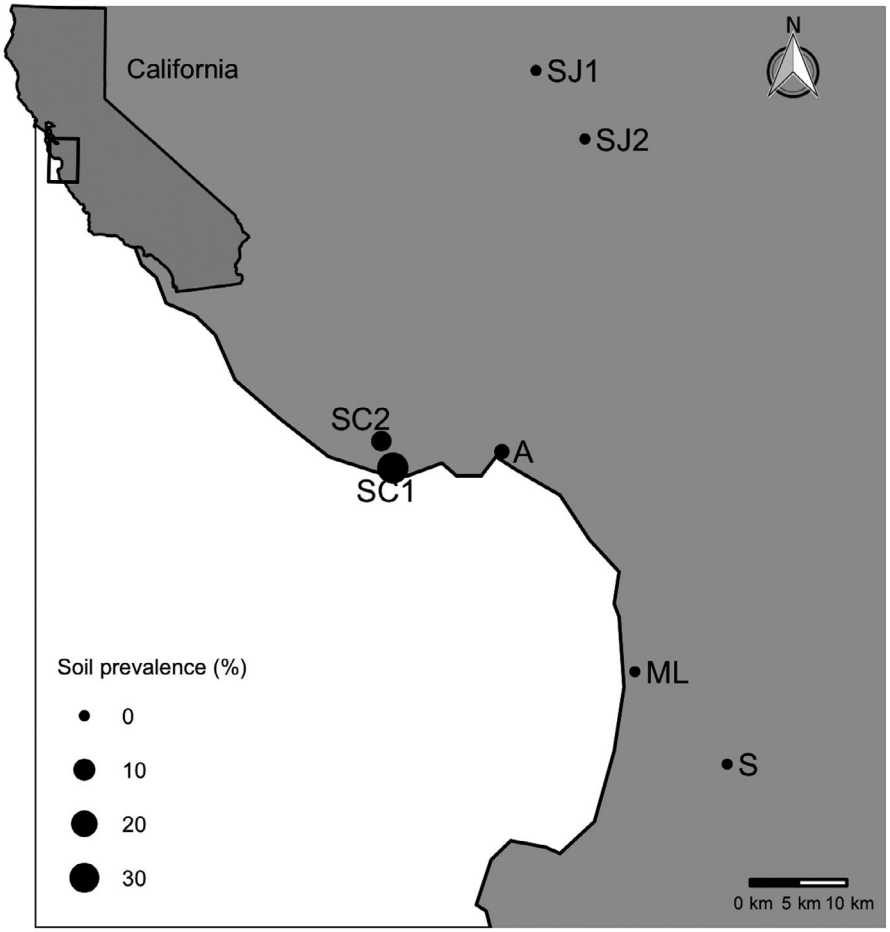

FIGURE 1 Map depicting study sites in central California where soil was collected for *Toxoplasma gondii* detection. Sites are marked as red if *T. gondii* was detected in soil and blue if *T. gondii* was not detected. Size of circles indicates prevalence of *T. gondii* in soil samples. Site abbreviations: SC1, Santa Cruz State Park; SC2, Santa Cruz Community Garden; A, Aptos State Park; ML, Moss Landing Park; S, Salinas Park; SJ1, San Jose City Park; and SJ2, San Jose City Park and playground

was left standing for 24 hr under refrigeration (4°C), after which we added 20 ml of distilled water as a dispersion solution and then vortexed for one minute. The soil mixture was then underlaid with 20 ml of cold sugar solution (1.2 specific gravity), centrifuged (1,500 × *g* for 20 min), and then the entire supernatant transferred to a sterile 50-mL conical tube. We subsequently washed the supernatant twice with 35 ml of deionized water, centrifuged (1,500 × *g* for 20 min) and retained 1 ml of pellet for analysis.

2.3 | DNA extraction and molecular analysis

We centrifuged (2,000 × *g* for 10 min) the pellets obtained following oocyst recovery from soil and processed 100 µl for nucleic acid extraction using the DNeasy Blood and Tissue Kit (QIAGEN) following manufacturer's instructions, with one modification: we performed an initial 4 min freeze (−196°C) and 4 min thaw (100°C) cycle to rupture oocyst walls prior to the addition of proteinase K (Manore, Harper, Aguilar, Weese, & Shapiro, 2019). We initially conducted systematic spiking experiments in our laboratory to select the most sensitive PCR assay and to establish a limit of detection for the approach described above (Supplemental Information). We compared four assays, including three conventional nested PCR assays that targeted three different loci: the internal transcribed spacer 1 (ITS-1) of the small subunit ribosomal RNA (SSU rRNA; Rejmanek et al., 2009), the 529 bp repeat element (RE) (Homan, Vercammen, Braekeleer, & Verschueren, 2000) and a segment of the B1 gene (Grigg & Boothroyd, 2001). In addition, we compared one real-time quantitative PCR (qPCR) assay also targeting the 529 bp RE gene (Opsteegh et al., 2010). The ITS-1 assay proved most sensitive and detected as few as 20 oocysts per g soil (Supplemental Information, Table 1).

The external PCR reaction included 36.1 µl of DNase- and RNase-free, distilled water; 5 µl of PCR buffer (10× buffer containing 15 millimolar (mM) $MgCl_2$); 1 µl of 10 mM dNTP mixture; 0.5 µL each of 50 micromolar (µM) forward and reverse primers; 1.6 µl of 10% Bovine Serum Albumin, 0.3 µl (1.5 units) of Taq Polymerase; and 5 µl of DNA template. The internal reaction was performed with 2 µl of the external amplification product and the internal primers. Each PCR batch included one positive control consisting of DNA extracted from cell culture-derived tachyzoites (RH strain) and three negative controls (one extraction reagent control with sterile water added, one PCR reagent control, and a second PCR reagent control with sterile water added). The PCR amplification products were separated through electrophoresis on a 2% agarose gel stained with Red Safe and viewed under UV light.

2.4 | Faecal analysis

We submitted faecal samples to the University of California Davis Veterinary Medical Teaching Hospital where they were processed through double centrifugal flotation, after which the cover slip was removed and rinsed into a clean 50 ml falcon tube and centrifuged to obtain a 100–500 µl pellet for nucleic acid extraction and PCR as described above.

2.5 | Sequence analysis and additional T. gondii genotyping

We purified all PCR products from soil sub-samples and faecal samples that yielded amplicons consistent with a *T. gondii* positive control with the QIAquick Gel Extraction Kit (QIAGEN) and submitted for sequencing at Sequetech (Applied Biosystems 3730xl DNA Analyzer, Mountain View, California). We analysed the forward and reverse DNA sequences using Geneious software (Biomatters, Auckland, New Zealand), and compared the consensus sequence with GenBank reference sequences for *T. gondii* using the Basic Local Alignment Search Tool, BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). To try and characterize *T. gondii* genotypes, we further processed DNA from soil sub-samples and faecal samples that were confirmed as *T. gondii* at the ITS-1 locus for PCR targeting the polymorphic B1 gene following previously published protocols (VanWormer et al., 2014). Given that there may be low quantities of *T. gondii* oocysts in soil and that the B1 locus has lower number of copies (Su et al., 2010), we attempted the B1 PCR assay twice, initially using 5 µl of template DNA and a second time using 10 µl of template DNA.

2.6 | Statistical analysis

We estimated the prevalence of *T. gondii* in soil sub-samples (i.e., 5-g replicate) as the number of positive soil sub-samples divided by the total sub-samples by location, season and sample type (i.e., latrine and

TABLE 1 Comparison of four different PCR assays for detection of *Toxoplasma gondii* in soil

		PCR Assay			
		Nested conventional PCR			Real-time quantitative PCR
Total oocysts spiked (5 g)	Oocysts/g soil	B1	ITS-1	529 bp RE	qPCR (529 bp RE)
10000	2000	3/3	3/3	3/3	3/3
1000	200	1/3	3/3	2/3	1/3
100	20	0/3	1/3	0/3	0/3
10	2	0/3	0/3	0/3	0/3
0	0	0/3	0/3	0/3	0/3

Note: PCR Assay results indicate the proportion of soil samples that yielded positive results for the detection of *T. gondii*.

scattered faeces). We found no variation in *T. gondii* in soil between sub-samples from latrines and scattered faeces (Coefficient = −2.64, 95% Credible Interval = −9.4–1.10) and thus subsequently analysed all samples together. We did not find *T. gondii* in any soil sub-sample from spring or summer nor at any of the inland sites, which resulted in complete separation of outcome data in these groups, which makes it difficult to estimate group coefficients using standard methods (Gelman, Jakulin, Pittau, & Su, 2008). Thus, we used a Bayesian generalized linear model with a binomial distribution (with the brm function in the *brms* package; Bürkner, 2017) to examine if the presence of *T. gondii* in soil was associated with season and geographic location. We fit models for the presence of *T. gondii* in soil sub-samples with season and geographic region (coastal vs. inland) as fixed effects, and site and sub-sample as random effects using a Bayesian multilevel regression model. We used weakly informative priors for the season and site coefficients: a student's *t* distribution centred at 0 with 3 degrees of freedom, and a scale parameter of 10. The effective sample sizes from the posterior distribution ranged between 145 and 1818, and all of the predictors had R-hat values <1.01 indicating model convergence (Bürkner, 2017). We used R version 3.3.3 (R Core Team, 2018) to perform all statistical analyses and created a spatial map with the *ggmap* package (Kahle & Wickham, 2013). We were unable to rigorously examine associations between *T. gondii* in soil and cat colony variables (colony size, number of kittens and number of feeding stations) because there were no *T. gondii* positive samples at four of the sites and in two of the three seasons resulting in insufficient variation in these predictors. We also did not statistically analyse prevalence patterns in faecal samples because we had too few samples for rigorous comparisons, and only one positive sample.

3 | RESULTS

Across the seven free-roaming cat colony sites, the average cat colony size was 7.5 ± 3.4 (range: 4–15), the average number of kittens was 1.2 ± 1.5 (range: 0–5), and the average number of feeding stations were 1.8 ± 0.9 (range: 0–4; Table S1). We analysed a total of 482 soil sub-samples from 80 samples (soil collected beneath 33 cat latrines and 47 scattered faeces locations) at the seven sites. All positive soil sub-samples were detected at coastal sites during the fall, and the analysis indicated that differences among sites and seasons were highly unlikely to be due to chance and that sub-samples from inland sites and spring and summer had much lower prevalence (Figure 2; Tables 2 and 3). In fall at coastal sites, we amplified *T. gondii* DNA in 44.3% (95% Confidence Interval = 31.5%–57.5%) of soil sub-samples, with within-site prevalence ranging from 0%–74% (Table 3). We also collected a total of 21 faecal samples, with *T. gondii* DNA detected in a single sample collected during summer from the inland Salinas site (Table 3). All reported PCR-positive soil sub-samples and the faecal sample were sequence confirmed as *T. gondii* (100% identity at the ITS-1 locus). We further processed all *T. gondii* -positive sub-samples for attempted genotyping via PCR at the B1 locus. However, DNA could not be amplified, both in the initial trial using 5 μl of DNA template, as well as in a second attempt using 10 μl of DNA template.

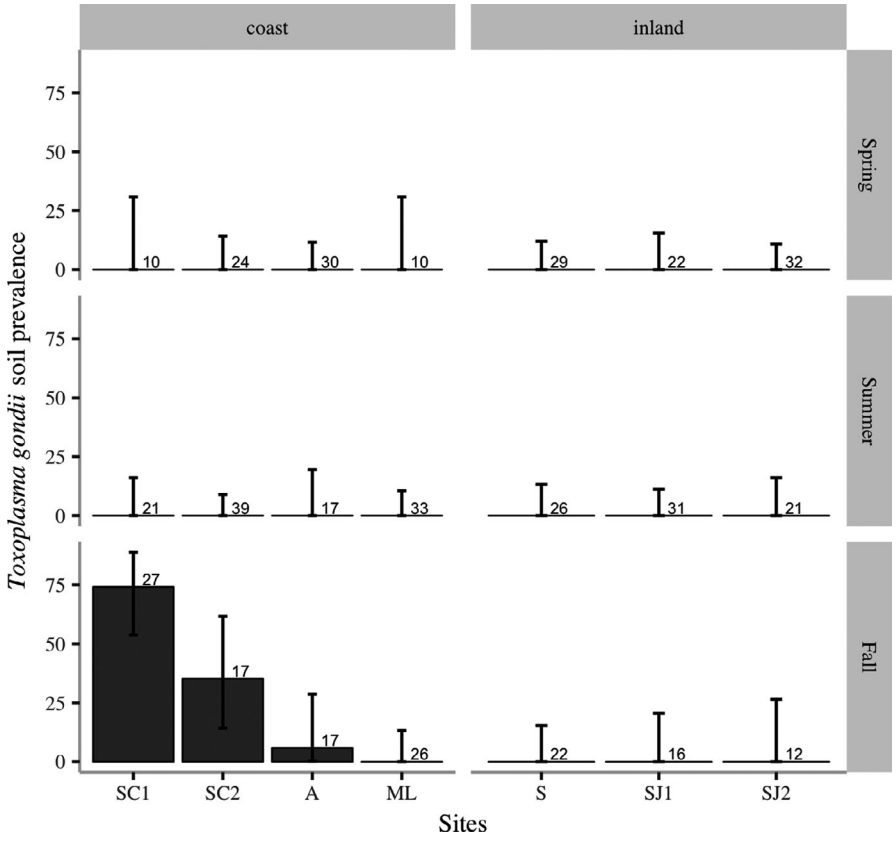

FIGURE 2 Seasonal prevalence of *Toxoplasma gondii* DNA in sub-samples of soil from latrines and scattered faeces of free-roaming cat colonies in central California by location (coastal and inland sites). Site abbreviations: SC1, Santa Cruz State Park; SC2, Santa Cruz Community Garden; A, Aptos State Park; ML, Moss Landing Park; S, Salinas Park; SJ1, San Jose City Park; and SJ2, San Jose City Park and playground. Numbers indicate number of sub-samples. Error bars represent binomial 95% confidence intervals

TABLE 2 Bayesian multilevel regression model with a binomial distribution and a logit link explaining seasonal and spatial variability of *Toxoplasma gondii* detection in all soil sub-samples (*N* = 482)

Variables	Coefficient (*SD*)	95% CI
Random effects		
Site	5.7 (4.2)	1.4–17.5
Sub-sample[a]	1.2 (1.1)	0–4.0
Fixed effects		
Season		
Fall[b]		
Spring	−97.8 (162.6)	−525.8 to −5.4
Summer	−87.8 (160.5)	−473.1 to −5.9
Geographic location		
Coastal[b]		
Inland	−77.5 (120.4)	−408.4 to −2.52

Abbreviations: CI, credible interval; *SD*, standard deviation of the posterior distribution.
[a]Sub-sample refers to each 5-g replicate belonging to samples from latrines or scattered faeces.
[b]Reference level for each predictor.

TABLE 3 Site-specific proportion of soil sub-samples and faecal samples positive for *Toxoplasma gondii* via sequence-confirmation during spring, summer and fall

	Sample type		
Site	Soil from latrines	Soil from scattered faeces	Faecal samples
Santa Cruz State Park			
Spring	0/10 (0%)	NC	NC
Summer	0/21 (0%)	NC	NC
Fall	20/27 (74%)	NC	NC
Santa Cruz Community Garden			
Spring	0/7 (0%)	0/17 (0%)	NC
Summer	0/10 (0%)	0/29 (0%)	NC
Fall	4/8 (50%)	2/9 (22.2%)	0/1 (0%)
Aptos State Park			
Spring	NC	0/30 (0%)	NC
Summer	0/10 (0%)	0/7 (0%)	NC
Fall	1/8 (12.5%)	0/9 (0%)	NC
Moss Landing Community Park			
Spring	0/11 (0%)	NC	0/1 (0%)
Summer	0/24 (0%)	0/13 (0%)	0/4 (0%)
Fall	0/29 (0%)	NC	0/3 (0%)
Salinas City Park and playground			
Spring	0/20 (0%)	0/9 (0%)	NC
Summer	0/23 (0%)	0/6 (0%)	1/4 (25%)
Fall	0/24 (0%)	NC	0/2 (0%)
San Jose City Park			
Spring	0/22 (0%)	NC	NC
Summer	0/10 (0%)	0/21 (0%)	NC
Fall	0/19 (0%)	NC	0/3 (0%)
San Jose City Park and playground			
Spring	0/10 (0%)	0/22 (0%)	0/2 (0%)
Summer	0/23 (0%)	NC	NC
Fall	0/13 (0%)	NC	0/1 (0%)

Note: Samples were collected from feral cat colonies near areas frequently used by people such as playgrounds, community gardens or parks.
Abbreviation: NC, Not collected.

4 | DISCUSSION

We found *T. gondii* to be present in soil under cat faeces at three free-roaming cat colonies in places heavily frequented by people for recreation (playgrounds and parks) or growing food (community gardens). Soil contamination with *T. gondii* was highly dependent on location and season, with *T. gondii* detection only occurring during fall and only at coastal sites, suggesting there is substantial seasonal and spatial variation in disease risk.

The marked seasonal and spatial difference in *T. gondii* soil prevalence among sites is due either to variation in persistence of oocysts in soil, or variation in shedding frequency by cats, both of which can be influenced by climatic conditions and the latter can be affected by cat demographics (Dabritz et al., 2007; Schares et al., 2016). Oocysts are known to persist and remain viable in the environment for long periods of time under mild temperatures and moist soil conditions (Dumètre & Darde, 2003; Lelu et al., 2012), whereas hotter and drier climates reduce *T. gondii* persistence in soil. Our coastal sites had more suitable conditions for persistence of oocysts in soil, and differences were even larger during fall season when a marine layer creates foggy conditions and average temperatures range from 8°C to 24°C (NOAA, 2018). In contrast, at our inland sites, temperatures frequently reach 30°C and have low humidity, especially during the summer (NOAA, 2018). Variation in *T. gondii* infection and thus shedding by cats can in turn be influenced by oocyst persistence and viability in soil. For example, availability and prevalence of infected prey may vary with seasons (Gilot-Fromont et al., 2012), as well as by seasonal reproductive patterns of cats, which will then influence the likelihood of kitten exposure to *T. gondii* (Gilot-Fromont et al., 2012; Nutter et al., 2004; Omata et al., 1994). The absence of *T. gondii* in soil at four of the seven sites and in two of the three seasons sampled poses the question of whether shedding is absent or present at very low levels during these seasons and locations. To gain a more comprehensive understanding of the effects of seasons and environmental factors on *T. gondii* shedding dynamics, future studies should try to provide more frequent soil sampling and over a longer time span (multi-year) as well as some measure of *T. gondii* shedding (e.g., by measuring seroconversion rates in cats).

The presence of *T. gondii* DNA in soil in coastal sites in fall, prior to the rainy season (winter), has important health implications for people in central California. Consuming local seafood and doing water-related recreational activities (e.g., surfing, swimming in the ocean) could lead to exposure of people to *T. gondii* oocysts because freshwater runoff can carry *T. gondii* oocysts from the terrestrial landscape to the nearshore marine environment (VanWormer et al., 2016). For example, southern sea otters (*Enhydra lutris nereis*) located in regions in California receiving freshwater runoff are frequently infected with *T. gondii* (Burgess et al., 2018; Miller et al., 2002), and our results support previous investigations suggesting free-roaming cat colonies in coastal California are an important source of *T. gondii* oocyst transmission to the marine environment (VanWormer et al., 2013, 2016). *Toxoplasma gondii* can also be dispersed through mechanical vectors such as earthworms, cockroaches, flies (Frenkel, Ruiz, & Chinchilla, 1975; Wallace, 1973) and even through dogs via fur contamination (Etheredge, Michael, Muehlenbein, & Frenkel, 2004; Frenkel, Lindsay, Parker, & Dobesh, 2003). The presence of *T. gondii* in cat latrines located in urban public spaces such as community gardens, playgrounds and parks may pose a risk for children since they might not be conscientious about washing their hands after playing with soil (Sousa, Saenz, & Frenkel, 1988). Likewise, risk of exposure may be high for people handling soil and consuming produce from organic community gardens, which have less regulations for washing and disinfecting (Williams & Hammitt, 2001).

Toxoplasma gondii infections are treatable, but not curable (Aguirre et al., 2019), which makes preventing exposure the key to preserving public health. In North America, there are efforts to reduce population sizes of free-roaming cats through trap-neuter-release programs, where neutered cats are often released into non-confined areas and occasionally provided supplemental food on an irregular basis (Guttilla & Stapp, 2010; Jessup, 2004). However, these programs are generally ineffective at controlling cat populations (Andersen, Martin, & Roemer, 2004; Foley, Foley, Levy, & Paik, 2005). Proper management of free-roaming cats could reduce the domestic cat contribution of oocysts into the environment and reduce contamination of soil, water and foods, thus preventing *T. gondii* oocyst-borne infections (Aguirre et al., 2019; Shapiro et al., 2019).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

Afonso, E., Thulliez, P., & Gilot-fromont, E. (2006). Transmission of *Toxoplasma gondii* in an urban population of domestic cats (*Felis catus*). *International Journal for Parasitology*, *36*, 1373–1382. https://doi.org/10.1016/j.ijpara.2006.07.010

Aguirre, A. A., Longcore, T., Barbieri, M., Dabritz, H., Hill, D., Klein, P. N., … Sizemore, G. C. (2019). The one health approach to toxoplasmosis: epidemiology, control, and prevention strategies. *EcoHealth*, *16*(2), 378–390.

Andersen, M., Martin, B., & Roemer, G. (2004). Use of matrix population models to estimate the efficacy of euthanasia versus trap-neuter-return for management of free-roaming cats. *Journal of the American Veterinary Medical Association*, *225*, 1871–1876. https://doi.org/10.2460/javma.2004.225.1871

Bahia-Oliveira, L., Gomez-Marin, J., & Shapiro, K. (2017). *Toxoplasma gondii*. In J. B. Rose, & B. Jimenez-Cisneros (Eds.), *Global Water Pathogen Project*. East Lansing, MI: Michigan State University.

Blaizot, R., Nabet, C., Blanchet, D., Martin, E., Mercier, A., Dardé, M.-L., … Demar, M. (2019). Pediatric Amazonian Toxoplasmosis Caused by Atypical Strains in French Guiana, 2002–2017. *The Pediatric Infectious Disease Journal*, *38*, 39–42. https://doi.org/10.1097/INF.0000000000002130

Brown, A. S., Schaefer, C. A., Quesenberry, C. P., Liu, L., Babulas, V. P., & Susser, E. S. (2005). Maternal exposure to toxoplasmosis and risk of schizophrenia in adult offspring. *American Journal of Psychiatry*, *162*, 767–773. https://doi.org/10.1176/appi.ajp.162.4.767

Burgess, T. L., Tinker, M. T., Miller, M. A., Bodkin, J. L., Murray, M. J., Saarinen, J. A., … Johnson, C. K. (2018). Defining the risk landscape in the context of pathogen pollution: *Toxoplasma gondii* in sea otters along the Pacific Rim. *Royal Society Open Science*, 5. https://doi.org/10.1098/rsos.171178

Bürkner, P.-C. (2017). brms: An R package for Bayesian multilevel models using Stan. *Journal of Statistical Software*, *80*, 1–28.

Carme, B., Demar, M., Ajzenberg, D., & Dardé, M. L. (2009). Severe acquired toxoplasmosis caused by wild cycle of *Toxoplasma gondii*, French Guiana. *Emerging Infectious Diseases*, *15*, 656–658.

Contopoulos-Ioannidis, D., Talucod, J., Maldonado, Y., & Montoya, J. G. (2015). Seasonal variation of acute toxoplasmic lymphadenopathy in the United States. *Epidemiology and Infection*, *143*, 1893–1897. https://doi.org/10.1017/S0950268814002945

Cook, A. J. C., Gilbert, R. E., Buffolano, W., Zufferey, J., Petersen, E., Jenum, P. A., … Dunn, D. T. (2000). Sources of *Toxoplasma* infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. *BMJ (Clinical Research ed.)*, *321*, 142–147.

Cortés, A. D., & Aguirre, N. (2018). Toxoplasmosis aguda diseminada fatal en paciente adulto sin inmunodeficiencia proveniente del pacífico colombiano. *Biomédica*, *38*, 2–22.

Cove, M. V., Gardner, B., Simons, T. R., Kays, R., & O'Connell, A. F. (2018). Free-ranging domestic cats (*Felis catus*) on public lands: Estimating density, activity, and diet in the Florida Keys. *Biological Invasions*, *20*, 333–344. https://doi.org/10.1007/s10530-017-1534-x

Dabritz, H. A., Miller, M. A., Atwill, E. R., Gardner, I. A., Leutenegger, C. M., Melli, A. C., & Conrad, P. A. (2007). Detection of *Toxoplasma gondii*-like oocysts in cat feces and estimates of the environmental oocyst burden. *Journal of the American Veterinary Medical Association*, *231*, 1676–1684.

Davis, A. A., Lepczyk, C. A., Haman, K. H., Morden, C. W., Crow, S. E., Jensen, N., & Lohr, M. T. (2018). *Toxoplasma gondii* detection in fecal samples from domestic cats (*Felis catus*) in Hawai'i. *Pacific Science*, *72*.

Du, F., Feng, H. L., Nie, H., Tu, P., Zhang, Q. L., Hu, M., … Zhao, J. L. (2012). Survey on the contamination of *Toxoplasma gondii* oocysts in the soil of public parks of Wuhan, China. *Veterinary Parasitology*, *184*, 141–146. https://doi.org/10.1016/j.vetpar.2011.08.025

Dubey, J. P. (1995). Duration of immunity to shedding *Toxoplasma gondii* oocysts by cats. *The Journal of Parasitology*, *81*, 410–415.

Dubey, J. P., & Jones, J. L. (2008). *Toxoplasma gondii* infection in humans and animals in the United States. *International Journal for Parasitology*, *38*, 1257–1278. https://doi.org/10.1016/j.ijpara.2008.03.007

Dumètre, A., & Darde, M.-L. (2003). How to detect *Toxoplasma gondii* oocysts in environmental samples? *FEMS Microbiology Reviews*, *27*, 651–661.

Dumètre, A., & Dardé, M. L. (2004). Purification of *Toxoplasma gondii* oocysts by cesium chloride gradient. *Journal of Microbiological Methods*, *56*, 427–430. https://doi.org/10.1016/j.mimet.2003.11.020

Etheredge, G. D., Michael, G., Muehlenbein, M. P., & Frenkel, J. K. (2004). The roles of cats and dogs in the transmission of *Toxoplasma* infection in Kuna and Embera children in eastern Panama. *Revista Panamericana De Salud Pública*, *16*, 176–186. https://doi.org/10.1590/S1020-49892004000900004

Foley, P., Foley, J. E., Levy, J. K., & Paik, T. (2005). Analysis of the impact of trap-neuter-return programs on populations of feral cats. *Journal of the American Veterinary Medical Association*, *227*, 1775–1781. https://doi.org/10.2460/javma.2005.227.1775

Frenkel, J. K., Lindsay, D. S., Parker, B. B., & Dobesh, M. (2003). Dogs as possible mechanical carriers of *Toxoplasma*, and their fur as a source of infection of young children. *International Journal of Infectious Diseases*, *7*, 292–293. https://doi.org/10.1016/S1201-9712(03)90112-3

Frenkel, J. K., Ruiz, A., & Chinchilla, M. (1975). Soil survival of *Toxoplasma* oocysts in Kansas and Costa Rica. *The American Journal of Tropical Medicine and Hygiene*, *24*, 439–443. https://doi.org/10.4269/ajtmh.1975.24.439

Fritz, H., Barr, B., Packham, A., Melli, A., & Conrad, P. A. (2012). Methods to produce and safely work with large numbers of *Toxoplasma gondii* oocysts and bradyzoite cysts. *Journal of Microbiological Methods*, *88*, 47–52. https://doi.org/10.1016/j.mimet.2011.10.010

Gelman, A., Jakulin, A., Pittau, M. G., & Su, Y. S. (2008). A weakly informative default prior distribution for logistic and other regression models. *Annals of Applied Statistics*, *2*, 1360–1383. https://doi.org/10.1214/08-AOAS191

Gilot-Fromont, E., Llu, M., Dard, M.-L., Richomme, C., Aubert, D., Afonso, E., … Villena, I. (2012). The life cycle of *Toxoplasma gondii* in the natural environment. In *Toxoplasmosis – Recent Advances* (pp. 3–36). Olgica Djurković Djaković, IntechOpen, https://doi.org/10.5772/48233

Glasner, P. D., Silveira, C., Kruszon-Moran, D., Martins, M. C., Burnier, M. Jr, Silveira, S., … Belfort, R. Jr (1992). An Unusually high prevalence of ocular toxoplasmosis in southern Brazil. *American Journal of Ophthalmology*, *114*, 136–144. https://doi.org/10.1016/S0002-9394(14)73976-5

Gotteland, C., Gilot-Fromont, E., Aubert, D., Poulle, M.-L., Dupuis, E., Darde, M.-L., … Villena, I. (2014). Spatial distribution of *Toxoplasma gondii* oocysts in soil in a rural area: Influence of cats and land use. *Veterinary Parasitology*, *205*, 629–637. https://doi.org/10.1016/j.vetpar.2014.08.003

Grigg, M. E., & Boothroyd, J. C. (2001). Rapid identification of virulent type I strains of the protozoan pathogen *Toxoplasma gondii* by PCR-restriction fragment length polymorphism analysis at the B1 gene. *Journal of Clinical Microbiology*, *39*, 398–400. https://doi.org/10.1128/JCM.39.1.398-400.2001

Guttilla, D., & Stapp, P. (2010). Effects of sterilization on movements of feral cats at a wildland – urban interface. *Journal of Mammalogy*, *91*, 482–489. https://doi.org/10.1644/09-MAMM-A-111.1

Hill, D., Coss, C., Dubey, J. P., Wroblewski, K., Sautter, M., Hosten, T., … T. S. Group. (2011). Identification of a sporozoite-specific antigen from *Toxoplasma gondii*. *Journal of Parasitology*, *97*, 328–337. https://doi.org/10.1645/GE-2782.1

Homan, W. L., Vercammen, M., De Braekeleer, J., & Verschueren, H. (2000). Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. *International Journal for Parasitology*, *30*, 69–75.

Hutchison, W. M., Dunachie, J. F., Siim, J. C., & Work, K. (1969). Life cycle of *Toxoplasma gondii*. *British Medical Journal*, *4*, 806. https://doi.org/10.1136/bmj.4.5686.806-b

Jessup, D. A. (2004). The welfare of feral cats and wildlife. *Journal of the American Veterinary Medical Association*, *225*, 1377–1383. https://doi.org/10.2460/javma.2004.225.1377

Jones, J. L., & Dubey, J. P. (2010). Waterborne toxoplasmosis–recent developments. *Experimental Parasitology*, *124*, 10–25. https://doi.org/10.1016/j.exppara.2009.03.013

Jones, J. L., Kruszon-Moran, D., Wilson, M., McQuillan, G., Navin, T., & McAuley, J. B. (2001). *Toxoplasma gondii* infection in the United States: Seroprevalence and risk factors. *American Journal of Epidemiology*, *154*, 357–365. https://doi.org/10.1093/aje/154.4.357

Kahle, D., & Wickham, H. (2013). ggmap: Spatial visualization with ggplot2. *The R Journal*, *5*, 144–161. https://doi.org/10.32614/RJ-2013-014

Kilgour, R. J., Magle, S. B., Slater, M., Christian, A., Weiss, E., & DiTullio, M. (2017). Estimating free-roaming cat populations and the effects of one year Trap-Neuter-Return management effort in a highly urban area. *Urban Ecosystems*, *20*, 207–216. https://doi.org/10.1007/s11252-016-0583-8

Lass, A., Pietkiewicz, H., Modzelewska, E., Dumetre, A., Szostakowska, B., & Myjak, P. (2009). Detection of *Toxoplasma gondii* oocysts in environmental soil samples using molecular methods. *European Journal of Clinical Microbiology and Infectious Diseases*, *28*, 599–605. https://doi.org/10.1007/s10096-008-0681-5

Leal, F. E., Cavazzana, C. L., Andrade, H. F. D., Galisteo, A. J., Mendonca, J. S. D., & Kallas, E. G. (2007). *Toxoplasma gondii* pneumonia in immunocompetent subjects: Case report and review. *Clinical Infectious Diseases*, *44*, e62–e66. https://doi.org/10.1086/511871

Lelu, M., Gilot-Fromont, E., Aubert, D., Richaume, A., Afonso, E., Dupuis, E., … Villena, I. (2011). Development of a sensitive method for *Toxoplasma gondii* oocyst extraction in soil. *Veterinary Parasitology*, *183*, 59–67. https://doi.org/10.1016/j.vetpar.2011.06.018

Lelu, M., Villena, I., Darde, M.-L., Aubert, D., Geers, R., Dupuis, E., … Gilot-Fromont, E. (2012). Quantitative estimation of the viability of *Toxoplasma gondii* oocysts in soil. *Applied and Environmental Microbiology*, *78*, 5127–5132. https://doi.org/10.1128/AEM.00246-12

Liu, X., He, Y., Han, D., Zhang, Z., Li, K., Wang, S., … Yan, R. (2017). Detection of *Toxoplasma gondii* in chicken and soil of chicken farms in Nanjing region. *Infectious Diseases and Poverty*, *6*, 1–8.

Lopez, A., Dietz, V., Wilson, M., Navin, T., & Jones, J. (2000). *Preventing congenital toxoplasmosis*. Centers for Disease Control and Prevention.

Maenz, M., Schlüter, D., Liesenfeld, O., Schares, G., Gross, U., & Pleyer, U. (2014). Ocular toxoplasmosis past, present and new aspects of an old disease. *Progress in Retinal and Eye Research*, *39*, 77–106. https://doi.org/10.1016/j.preteyeres.2013.12.005

Manore, A. J. W., Harper, S. L., Aguilar, B., Weese, J. S., & Shapiro, K. (2019). Comparison of freeze-thaw cycles for nucleic acid extraction and molecular detection of *Cryptosporidium parvum* and *Toxoplasma gondii* oocysts in environmental matrices. *Journal of Microbiological Methods*, *156*, 1–4. https://doi.org/10.1016/j.mimet.2018.11.017

Miller, M. A. A., Gardner, I. A. A., Kreuder, C., Paradies, D. M. M., Worcester, K. R. R., Jessup, D. A. A., … Conrad, P. A. A. (2002). Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). *International*

Journal for Parasitology, *32*, 997–1006. https://doi.org/10.1016/S0020-7519(02)00069-3

Morin, L., Lobry, J. R., Peyron, F., & Wallon, M. (2012). Seasonal variations in acute toxoplasmosis in pregnant women in the Rhône-Alpes region (France). *Clinical Microbiology and Infection*, 18. https://doi.org/10.1111/j.1469-0691.2012.03898.x

Ngô, H. M., Zhou, Y., Lorenzi, H., Wang, K., Kim, T. K., Zhou, Y., … McLeod, R. (2017). Toxoplasma modulates signature pathways of human epilepsy, neurodegeneration & cancer. *Scientific Reports*, *7*, 1–32.

NOAA. (2018). National Weather Service Forecast Office.

Nutter, F. B., Jay, F., & Stoskopf, M. K. (2004). Reproductive capacity of free-roaming domestic cats and kitten survival rate. *Journal of the American Veterinary Medical Association*, *225*, 9–12. https://doi.org/10.2460/javma.2004.225.1399

Omata, Y., Oikawa, H., Kanda, M., Mikazuki, K., Dilorenzo, C., Claveria, F., … Suzuki, N. (1994). Transfer of antibodies to kittens from mother cats chronically infected with *Toxoplasma gondii*. *Veterinary Parasitology*, *52*, 211–218. https://doi.org/10.1016/0304-4017(94)90113-9

Opsteegh, M., Langelaar, M., Sprong, H., den Hartog, L., De Craeye, S., Bokken, G., … van der Giessen, J. (2010). Direct detection and genotyping of *Toxoplasma gondii* in meat samples using magnetic capture and PCR. *International Journal of Food Microbiology*, *139*, 193–201. https://doi.org/10.1016/j.ijfoodmicro.2010.02.027

Pomares, C., Devillard, S., Holmes, T. H., Olariu, T. R., Press, C. J., Ramirez, R., … Montoya, J. G. (2018). Genetic characterization of *Toxoplasma gondii* DNA samples isolated from humans living in North America: An unexpected high prevalence of atypical genotypes. *The Journal of Infectious Diseases*, *218*, 1783–1791. https://doi.org/10.1093/infdis/jiy375

R Core Team. (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rejmanek, D., Vanwormer, E., Miller, M. A., Mazet, J. A. K., Nichelason, A. E., Melli, A. C., … Conrad, P. A. (2009). Prevalence and risk factors associated with *Sarcocystis neurona* infections in opossums (*Didelphis virginiana*) from central California. *Veterinary Parasitology*, *166*, 8–14. https://doi.org/10.1016/j.vetpar.2009.08.013

Sagel, U., Mikolajczyk, R. T., & Krämer, A. (2010). Seasonal trends in acute toxoplasmosis in pregnancy in the federal state of Upper Austria. *Clinical Microbiology and Infection*, *16*, 515–517. https://doi.org/10.1111/j.1469-0691.2009.02880.x

Schares, G., Ziller, M., Herrmann, D. C., Globokar, M. V., Pantchev, N., & Conraths, F. J. (2016). Seasonality in the proportions of domestic cats shedding *Toxoplasma gondii* or *Hammondia hammondi* oocysts is associated with climatic factors. *International Journal for Parasitology*, *46*, 263–273. https://doi.org/10.1016/j.ijpara.2015.12.006

Schmidt, P. M., Lopez, R. R., & Colliter, B. A. (2007). Survival, fecundity, and movements of free-roaming cats. *Journal of Wildlife Management*, *71*, 915–919. https://doi.org/10.2193/2006-066

Shapiro, K., Bahia-Oliveira, L., Dixon, B., Dumètre, A., de Wit, L. A., VanWormer, E., & Villena, I. (2019). Environmental transmission of *Toxoplasma gondii*: Oocysts in water, soil and food. *Food and Waterborne Parasitology*, *15*, e00049.

Simon, J. A., Kurdzielewicz, S., Jeanniot, E., Dupuis, E., Marnef, F., Aubert, D., … Poulle, M.-L. (2017). Spatial distribution of soil contaminated with *Toxoplasma gondii* oocysts in relation to the distribution and use of domestic cat defecation sites on dairy farms. *International Journal for Parasitology*, *47*, 357–367. https://doi.org/10.1016/j.ijpara.2017.01.004

Simon, J. A., Pradel, R., Aubert, D., Geers, R., Villena, I., & Poulle, M. (2018). A multi-event capture-recapture analysis of *Toxoplasma gondii* seroconversion dynamics in farm cats. *Parasites & Vectors*, *11*, 1–13. https://doi.org/10.1186/s13071-018-2834-4

Sousa, O. E., Saenz, R. E., & Frenkel, J. K. (1988). Toxoplasmosis in Panama: A 10- year study. *American Journal of Tropical Medicine and Hygiene*, *38*, 315–322. https://doi.org/10.4269/ajtmh.1988.38.315

Spalding, S. M., Reis Annendoeira, M. R., Klein, C. H., & Ribeiro, L. C. (2005). Serological screening and toxoplasmosis exposure factors among pregnant women in South of Brazil. *Revista Da Sociedade Brasileira De Medicina Tropical*, *38*, 173–177. https://doi.org/10.1590/S0037-86822005000200009

Su, C., Shwab, E. K., Zhou, P., Zhu, X. Q., & Dubey, J. P. (2010). Moving towards an integrated approach to molecular detection and identification of *Toxoplasma gondii*. *Parasitology*, *137*, 1–11.

Sukthana, Y. (2006). Toxoplasmosis: Beyond animals to humans. *Trends in Parasitology*, *22*, 137–142. https://doi.org/10.1016/j.pt.2006.01.007

Torgerson, P. R., & Mastroiacovo, P. (2013). The global burden of congenital toxoplasmosis: A systematic review. *Bulletin of the World Health Organization*, *91*, 501–508. https://doi.org/10.2471/BLT.12.111732

VanWormer, E., Carpenter, T. E., Singh, P., Shapiro, K., Wallender, W. W., Conrad, P. A., … Mazet, J. A. K. (2016). Coastal development and precipitation drive pathogen flow from land to sea: Evidence from a *Toxoplasma gondii* and felid host system. *Scientific Reports*, *6*, 1–9. https://doi.org/10.1038/srep29252

VanWormer, E., Conrad, P. A., Miller, M. A., Melli, A. C., Carpenter, T. E., & Mazet, J. A. K. (2013). *Toxoplasma gondii*, source to sea: Higher contribution of domestic felids to terrestrial parasite loading despite lower infection prevalence. *EcoHealth*, *10*, 277–289. https://doi.org/10.1007/s10393-013-0859-x

VanWormer, E., Miller, M. A., Conrad, P. A., Grigg, M. E., Rejmanek, D., Carpenter, T. E., & Mazet, J. A. K. (2014). Using molecular epidemiology to track *Toxoplasma gondii* from terrestrial carnivores to marine hosts: Implications for public health and conservation. *PLoS Neglected Tropical Diseases*, 8. https://doi.org/10.1371/journal.pntd.0002852

Wallace, G. D. (1973). Intermediate and transport hosts in the natural history of *Toxoplasma gondii*. *The American Journal of Tropical Medicine and Hygiene*, *22*, 456–464. https://doi.org/10.4269/ajtmh.1973.22.456

Williams, P. R. D., & Hammitt, J. K. (2001). Perceived risks of conventional and organic produce: Pesticides, pathogens, and natural toxins. *Risk Analysis*, *21*, 319–330. https://doi.org/10.1111/0272-4332.212114

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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