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INVASIVE CATS, TOXOPLASMOSIS AND BIODIVERSITY: USING PUBLIC HEALTH AS A LEVER FOR CONSERVATION

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By

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ABSTRACT

Invasive cats, toxoplasmosis and biodiversity: using public health as a lever for conservation

Luz A. de Wit

Human health and ecosystem health are intricately linked through ecosystem services and through common threats. For example, deforestation, resource overexploitation, pollution, and introduction of invasive species are the four main threats to biodiversity, and these have also been linked to the emergence of pathogens of public health concern and overall human health issues. Non-native species, which often become invasive in ecosystems that have been anthropogenically disturbed and that have been depleted of or lack native competitors or predators, are also reservoirs of several pathogens that affect humans. Because they can reach large population sizes and displace other host species, invasive species can modify and potentially amplify disease transmission dynamics through the introduction of novel pathogens or through changes in community structure. Invasive species are often found in close association to humans, usually as human commensals (e.g. peri-domestic rodents, cats, mosquitos), which can increase the risk of human exposure to pathogens transmitted or carried by these species. Thus, management of invasive species through population control, eradication (in the case of islands) or ecosystem management, which are frequently implemented as biodiversity conservation tools, have the potential to also benefit human health. Although the relationship between ecosystem and human

health has just recently emerged as an important area of study, there are few concrete examples of the potential for synergistic interventions benefiting both (Wolfe et al. 2005, Ostfeld et al. 2006, Herrera et al. 2017). Here I focus on the public health impacts of invasive cats (*Felis catus*) and through three case studies, I provide evidence of the potential for combined public health and conservation benefits of eradicating invasive cat populations from islands and controlling free-roaming colonies on continental areas.

Cats have been introduced to approximately 179,000 islands, and co-occur with people in approximately 560 of them (Medina et al. 2011, Threatened Island Biodiversity Database Partners 2014). Cats are the second most widespread invasive predator on islands and are responsible at least in part for 14% of all bird, mammal and reptile extinctions on islands (Doherty et al. 2016, Jones et al. 2016b). Free-roaming cats on the mainland are also opportunistic predators and in many cases are additionally subsidized directly with supplemental food or indirectly with peri-domestic rodents (Lepczyk et al. 2004, Baker et al. 2005). Management schemes for free-roaming populations range from complete elimination of the population (i.e. eradication) to varying degrees of population control (e.g. trap-neuter-release (TNR), trap-neuter-adopt or trap-euthanize). Most countries and many states in the USA have few, if any, regulations concerning cat ownership and cat owner responsibilities, making free-roaming cat population control inconsistent and difficult to enforce (LaCroix 2006).

Programs such as TNR adhere more closely to the demands of cat advocates, resulting in negligible benefits to wildlife, high long-term management costs, and usually uncontrolled free-roaming cat colonies (Andersen et al. 2004, Foley et al. 2005). In theory, these forms of management can significantly reduce cat population sizes if they cover a large proportion of the population and are done consistently (Andersen et al. 2004). However, supplementary feeding, pet abandonment, and persistence of fertile females may attract cats from nearby colonies and increase local cat densities (Bengsen et al. 2015), eventually making trapping efforts a waste of resources. On the other hand, eradications are only implemented when the goal is to permanently eliminate cat populations and are thus only conducted in closed systems such as islands, requiring considerable financial investment, capacity building, and ongoing biosecurity measures to prevent reintroduction (Donlan and Wilcox 2007). Eradications on human-inhabited islands represent additional logistical, social and economic constrains because some invasive species have a social and/or economic value for island inhabitants (Oppel et al. 2011). As a result, successful eradication of domestic cat populations on human-inhabited islands have been limited to islands with no more than 1,000 people (Jones et al. 2016b).

Cats and other wild felids are the only known definitive hosts of *Toxoplasma gondii*, and infected cats can shed up to one billion or hundreds of million oocysts in their feces (Hill and Dubey 2002). Infection with *T. gondii* can

cause miscarriage or severe ocular and neurological lesions in newborns, systemic disease in immunocompromised individuals, and has been associated with neurological disorders such as Alzheimer's and schizophrenia (Brown et al. 2005, Mortensen et al. 2007, Torrey et al. 2007, Maenz et al. 2014, Ngô et al. 2017). Exposure to *T. gondii* oocysts (the environmental stage of the parasite) through contact with contaminated soil is one of the most common routes of *T. gondii* infection (Cook et al. 2000a, Spalding et al. 2005, Jones et al. 2009, Egorov et al. 2018). Soil-related activities such as gardening or having an occupation involving soil exposure are associated with high risk of *T. gondii* infection (Jones et al. 2001). There is currently no vaccine against *T. gondii*, and treatment is often limited to reducing the risk of congenital transmission in women who become infected during pregnancy, or severe illness in people with a compromised immune system (SYROCOT (Systematic Review of Congenital Toxoplasmosis) study group 2007, Robert-Gangneux and Dardé 2012). Following proper food handling and hygiene practices can reduce exposure to *T. gondii* (Bahia-Oliveira et al. 2017). However, because free-roaming cats contribute significantly to the environmental load of *T. gondii* (VanWormer et al. 2013a), implementing management strategies that effectively control cat populations can be a more effective approach for reducing environmental contamination at its source. Likewise, on the majority of islands that do not harbor native felids, the local environmental source of *T. gondii* can be eliminated through

cat eradications.

To examine whether cat eradication or population control on islands could benefit human health by reducing the burden of *T. gondii* infection, I compared the seroprevalence and risk factors associated with *T. gondii* exposure in people on seven islands with variation in cat density, including one island in which cats were eradicated in the year 2000, and another island in which cats had never been present. I found that eradication of introduced cats on islands could significantly reduce human risk of exposure to *T. gondii*. Exposure to *T. gondii* was completely absent on the island that never had cats and near zero on the island where cats were eradicated. Furthermore, all island resident children born after cats were eradicated showed no evidence of exposure to the parasite. The odds of *T. gondii* infection were nearly five-fold higher in people that had cats near their homes. On islands with cats, we found no association between local cat density and *T. gondii* seroprevalence, suggesting that complete eradication rather than control of cat population densities is necessary to reduce public health impacts of *T. gondii* infection.

Understanding the burden of *T. gondii* infection is required to assess whether cat eradication can benefit human health while also reducing one of the main threats to native species. The burden of *T. gondii* infection can be further integrated into cost-benefit analyses of cat eradications to determine if the costs of *T. gondii* infection and biodiversity threats outweigh the socioeconomic costs of

eradication. However, epidemiological information on *T. gondii* infection in people is only known for a small number of islands (N=18) where invasive cats co-occur with people and threatened island native species. This paucity of epidemiological information may be due to lack of reporting and/or limitations in public health services. To estimate the burden of *T. gondii* infection on islands where this epidemiological information was missing, I used *T. gondii* prevalence as a proxy for disease burden, and readily available ecological and socioeconomic variables as predictors. I found prevalence of *T. gondii* to be significantly and negatively correlated with human population size and with the per capita gross domestic product of islands. I used the results of the predictive model to estimate seroprevalence of *T. gondii* infection for the remaining islands where epidemiological information was missing. This predictive model can be used as a tool for identifying islands where *T. gondii* prevalence is high and where cat eradication has potential for benefiting human health. These models should be used with caution as further on-the-ground measurement is required before cost-benefit analyses are implemented.

Shedding and persistence of *T. gondii* oocysts in soil can change through time and can also be influenced by environmental factors, such as temperature and humidity as well as by cat demographic factors, such as cat density, juvenile abundance and supplemental feeding. To examine the spatial and temporal patterns of *T. gondii* prevalence, I

sampled soil at free-roaming cat colonies present in community gardens, playgrounds and parks associated during three seasons (Spring, Summer and Fall) and in areas located along the coast and inland Central California, USA. I used seasons as proxies for temporal variation in both environmental and cat demographic variables, and geographic location of sites to examine spatial differences in prevalence of *T. gondii* in soil. I detected *T. gondii* only during Fall and in coastal sites. Results from this study suggest that environmental conditions play an important role in *T. gondii* presence, or at least persistence in soil, and cat abundance (or juvenile abundance) may be influencing *T. gondii* transmission dynamics. These results indicate that free-roaming cat colonies in spaces where people (including children) recreate is an important source of *T. gondii*.

DEDICATION

A mis padres, Diego y a Paulo

Por su apoyo, paciencia, cariño e inspiración.

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CHAPTER 1. POTENTIAL PUBLIC HEALTH BENEFITS FROM CAT ERADICATIONS ON ISLANDS

Abstract

Cats (*Felis catus*) are reservoirs of several pathogens that affect humans, including *Toxoplasma gondii*. Infection of pregnant women with *T. gondii* can cause ocular and neurological lesions in newborns, and congenital toxoplasmosis has been associated with schizophrenia, epilepsy, movement disorders, and Alzheimer's disease. We compared seroprevalence of *T. gondii* and risk factors in people on seven islands in Mexico with and without introduced cats to determine the effect of cat eradication and cat density on exposure to *T. gondii*. Seroprevalence was zero on an island that never had cats and 1.8% on an island where cats were eradicated in 2000. Seroprevalence was significantly higher (12-26%) on the five islands with cats, yet it did not increase across a five-fold range of cat density. Having cats near households, being male and spending time on the mainland were significant risk factors for *T. gondii* seroprevalence among individuals, whereas eating shellfish was protective. My results suggest that cats are an important source of *T. gondii* on islands, and eradicating, but not controlling, introduced cats from islands could benefit human health.

Introduction

Cats (*Felis catus*) are reservoirs of many pathogens that affect humans, including the parasite *Toxoplasma gondii* (Gerhold and Jessup 2013). Cats are also the second most widespread introduced predator found on islands (Doherty et al. 2016, Jones et al. 2016a), and have contributed to 14% of global bird, reptile and mammal extinctions on islands (Doherty et al. 2016). The dual impact of introduced cats on wildlife and human health increases the potential benefits of eradicating cats from islands (Crowley et al. 2017, de Wit et al. 2017). Introduced cats have been eradicated for conservation reasons from 80 islands globally (DIISE 2015), resulting in rapid recoveries of native species on many of those islands (Jones et al. 2016a). Fifteen of these cat eradications were on islands with permanent human settlements (DIISE 2015). A key gap in our knowledge is whether eradication or control of introduced species also result in public health benefits.

Toxoplasmosis is one of the most widespread zoonotic diseases with a significantly greater burden in low-income countries, and cats are a key reservoir host (Torgerson and Mastroiacovo 2013). Domestic cats and wild felids are the only known definitive hosts for *T. gondii* (Dubey 1998). Cats can become infected after ingesting *T. gondii* bradyzoites found in tissue cysts of infected intermediate hosts (i.e. prey such as rodents or birds)(Gilot-Fromont et al. 2012). Acutely infected cats host the sexual cycle of the parasite and subsequently shed millions of *T. gondii* oocysts in their feces (Hill and Dubey 2002, Fritz et al. 2012, Gilot-

Fromont et al. 2012), thereby contaminating the soil or bodies of water (Dubey 2004). Oocysts sporulate in the environment and become infectious to intermediate hosts and people (Dubey 1998). The burden of toxoplasmosis tends to be highest in low-income countries from tropical regions, with prevalence rates ranging between 35.8% and 85.4% (Sánchez-Gutiérrez et al. 2003, Adou-Bryn et al. 2004, Hung et al. 2007, Ramsewak et al. 2008, Rosso et al. 2008, Lago et al. 2009, Caballero-Ortega et al. 2012b, Gelaye et al. 2015). Women exposed to *T. gondii* during pregnancy can transmit the parasite to their fetus, which can lead to miscarriage or congenital toxoplasmosis (Torgerson and Mastroiacovo 2013). Congenital toxoplasmosis can result in severe ocular and neurological lesions in newborns (Foulon et al. 1999, SYROCOT (Systematic Review of Congenital Toxoplasmosis) study group 2007, Maenz et al. 2014) and has been linked to schizophrenia, epilepsy, movement disorders and Alzheimer's disease (Brown et al. 2005, Ngô et al. 2017). Furthermore, *T. gondii* infection can be acquired postnatally leading to vision loss (Maenz et al. 2014) and systemic disease in immunocompromised individuals (Dabritz and Conrad 2010). There is currently no vaccine against *T. gondii* and treatment is commonly restricted to acute infections, particularly for women infected during pregnancy or immunosuppressed patients (Foulon et al. 1999, Hill and Dubey 2002).

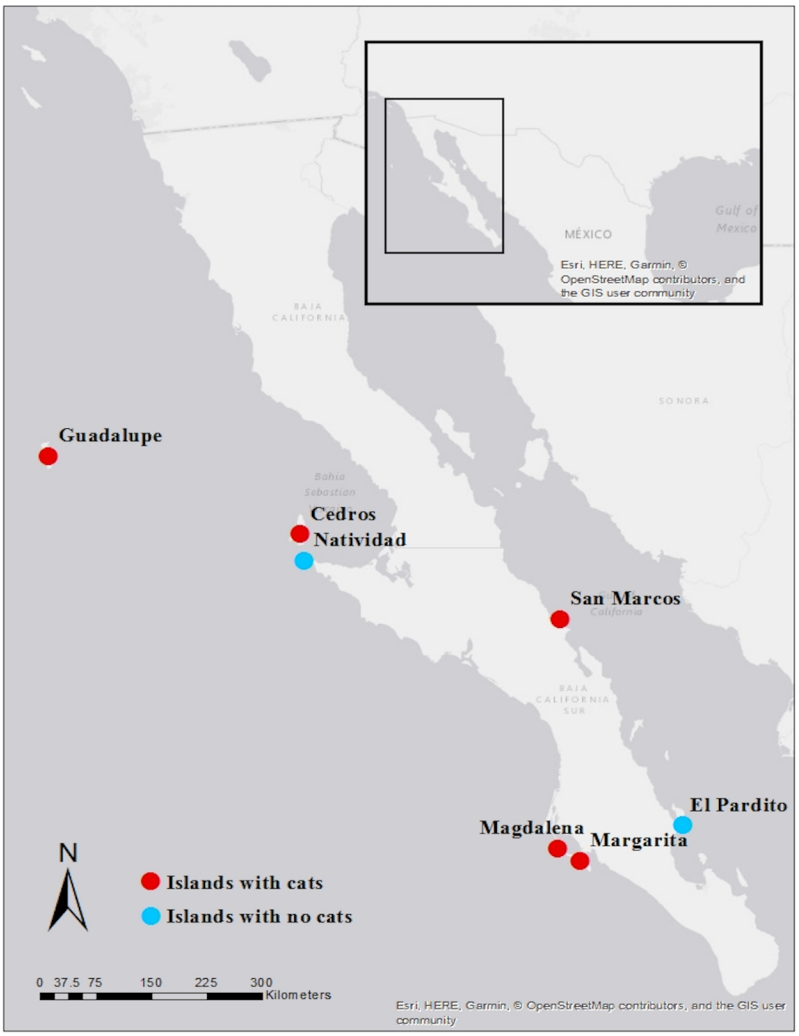
Most islands do not have native felid species (Inskip and Zimmermann 2009), creating the potential to reduce the

burden of *T. gondii* infection in people living on islands by reducing or eliminating introduced cat populations. Local sources of *T. gondii* on islands may include contact with shedding cats, oocyst-contaminated soil or consumption of local shellfish that have been contaminated by runoff that carries *T. gondii* oocysts from land to sea (Miller et al. 2002, VanWormer et al. 2013a). External sources of infection include consumption of contaminated meat and vegetable products that are imported from the mainland, and exposure during travel to a region where *T. gondii* is endemic.

Although there have been many studies that attempt to correlate prevalence of *T. gondii* in soil, or *T. gondii* seroprevalence in pigs, humans, rodents, or cats with some measure of cat abundance or exposure (Wallace et al. 1972, Meerburg et al. 1998, Afonso et al. 2008a, Ortega-Pacheco et al. 2011, Thomasson et al. 2011, Du et al. 2012b, Gotteland et al. 2014a, 2014b, Boughattas et al. 2017, Retmanasari et al. 2017), only one of these studies (Afonso et al. 2008a) estimated cat density using a standardized approach, and none attempted to determine the quantitative relationship between cat density and *T. gondii* exposure in humans. This relationship is needed to determine how low cat density must be reduced to achieve a reduction in *T. gondii* exposure in humans. Thus, my goal was to determine if reducing or eliminating populations of introduced cats could reduce *T. gondii* exposure in human populations on islands. I examined risk factors and the seroprevalence of *T. gondii* exposure in people on seven human inhabited islands

located off the coast of the Baja California Peninsula, Mexico (Figure 1.1). These islands do not harbor native felids and have a range of introduced cat densities, including one where cats have never been present and another where cats were present but eradicated in 2000.

Figure 1.1 Geographic distribution of the seven human inhabited islands of Baja California, Mexico.



Methods

I conducted research on seven islands in Baja California, Mexico (Figure 1.1), between February 2016 and July 2017. The human communities on these islands rely on fishing as their main source of protein and income, except for San Marcos, which relies mostly on gypsum mining. The climate is tropical and subtropical desert with high annual mean temperatures (19.5 – 23.7 °C), and low precipitation (47.6 – 281.19 mm/year) ("Servicio Meteorologico Nacional" 2017). All islands except Natividad and El Pardito had populations of introduced cats at the time of the study. El Pardito is a small island with a fishing community of 13 people and has never had cats. Cats were introduced to Natividad in the 1920s, and fluctuated over time until they were eradicated in 2000 (Keitt et al. 2002, Keitt and Tershy 2003). Cats on the rest of the islands live mostly as free-roaming (57% of the participants report occasionally feeding food scraps to cats) or feral (38% of participants do not report feeding cats), and a small fraction of cats are owned (6% of participants report buying cat food and regularly feed cats) but all are allowed to go outdoors. Cat populations may have decreased on Guadalupe and Magdalena Islands over the past decade due to control efforts (Luna Mendoza 2014), but no data on cat abundance over time in the human inhabited regions of the islands is available.

Sample collection

I systematically visited households and visited community aggregation centers (i.e. schools, convenience

stores, administrative offices of the fish cooperatives and a gypsum mine), where we distributed information pamphlets about the study. To determine *T. gondii* seropositivity and examine possible risk factors I collected blood samples and applied a questionnaire to people who had given informed consent, and in the case of underage children, if they were accompanied by their parent or tutor and had given informed consent.

Seroprevalence estimates

I used the fingerprick method to collect approximately 10 μL of blood on Guthrie cards (filter paper) and refrigerated cards at 4°C until we analyzed them at the *Laboratorio de Inmunología Experimental* of the *Instituto Nacional de Pediatría, México*. I tested all samples for presence of IgG antibodies against *T. gondii* using an indirect ELISA (Cañedo-Solares et al. 2009). I determined the cutoff value for seropositivity in each ELISA run as the average optical absorbance of the negative controls plus three standard deviations of the absorbance from negative samples (Caballero-Ortega et al. 2014). I ran each sample in duplicate and considered it as positive if the average optical absorbance was greater than the cutoff value.

I estimated crude *T. gondii* antibody prevalence and created age-adjusted estimates using the direct method (Roalfe et al. 2008) with the age-structure of the 2015 population of the States of Baja California and Baja California Sur, Mexico (INEGI 2015).

Questionnaire

To examine associations between risk factors and *T. gondii* exposure, I interviewed participants using a standardized questionnaire (Cook et al. 2000b, Jones et al. 2009) adapted to the social context of communities in the islands of Baja California. For each individual we recorded gender; age; educational level; source of drinking water; whether they had contact with soil through outdoor activities; consumption of raw or undercooked meat or poultry; annual frequency of meat (including poultry and pork), and shellfish consumption; annual frequency of travel outside the island; and fraction of time spent outside the island. I asked people if they had cats; whether cats were allowed indoor, outdoor, or both; if they were in contact with cat feces when cleaning their household; and I asked them to estimate the number of cats observed near their house. To better understand cat ownership and the relationship between people and cats in the islands, I asked people if they fed the cats that roamed near their household and whether they fed them food scraps or cat food. I also asked people whether they owned a dog, and if it was allowed indoor, outdoor, or both, as dogs may act as carriers of *T. gondii* oocysts in their fur (Frenkel et al. 2003). I excluded water as a potential source of *T. gondii* exposure because all islands obtain water from local desalination plants or from fishing boats with desalination equipment (El Pardito), from which water is delivered to each household through water pipes or barrels.

I interviewed and collected blood samples from all 13 residents on El Pardito, and 59-325 participants on each of the six remaining islands (representing 25-75% of each population), for a total of 724 participants of ages 9 to 70 (Table 1.1).

Cat density

I used distance sampling along transects (178-433 meters in length) on each island to measure cat density (Thomas et al. 2012). I placed transects in human-occupied areas, including main roads in towns. I walked transects between 30 and 90 minutes after dawn on each of two consecutive days, counted all cats, and estimated the distance to each cat with the aid of a rangefinder (Bushnell Yardage Pro Sport 450). I used the ds function in the *Distance* package in R (Miller et al. 2016, R-Core-Team 2018) to estimate cat density on each island. I selected the best fitting detection function on each island using Akaike's Information Criterion with correction for small sample sizes (AICc) and used the Cràmer-von Mises test to assess the goodness of fit of the best fitting function (Miller et al. 2016). To examine the relationship between *T. gondii* seroprevalence and cat density among islands, we fit a nonlinear saturating function (Seroprevalence = $Y_{int}+c_0*(1-e^{(-c1*\text{Cat Density})})$ to the data with a binomial distribution using the mle2 function in the *bbmle* package in R. In this model, Y_{int} is the seroprevalence when cat density is zero, (c_0+Yint) is the asymptote (the seroprevalence at high cat densities), and c_1

is the slope parameter describing the increase in seroprevalence with cat density.

Statistical analysis

I used R version 3.3.3 to run all statistical analysis (R-Core-Team 2018). I used logistic regression models to compare age-adjusted seroprevalence among islands. I used Fisher's Exact Tests to compare seroprevalence among islands for children born after 2000, when cat eradication took place in Natividad. For the risk factor analysis, the predictor variables associated to cats were correlated ($r>0.3$). To avoid including many collinear variables, I ran two generalized linear mixed effects models (with a binomial distribution and a logit link) including data from all the islands, with island as a random effect. In the first model, I included the presence/absence of cats reported near households, and all non-cat related predictor variables, but removed all other variables related to cats. I then fit a second model for the subset of people that reported having cats near their households that included all non-cat related variables, exposure to cat feces and the number of cats a person reported having near their house.

Ethics Statement

All research was performed under the human subjects protocols CONBIOETICA02CEI00520131206 and CONBIOETICA03CEI00120131203 approved by the Human Subjects Research review committees of the State of Baja

California and Baja California Sur, Mexico, and protocol HS2385 approved the Office of Research Compliance Administration of the University of California Santa Cruz. All adult subjects provided written informed consent, and in the case of underage children who participated in the study, a parent or guardian provided written informed consent on the child's behalf.

Results

T. gondii seroprevalence

I sampled a total of 724 participants of ages 9 to 70 (Table 1.1). All 13 inhabitants from El Pardito, where cats have never been present, were seronegative for *T. gondii* IgG antibodies. The age-adjusted seroprevalence was 1.8% on Natividad, where cats were eradicated in the year 2000. Age-adjusted seroprevalence on the remaining five islands varied from 11.6% to 25.7%, which was significantly higher than on the two cat-free islands (Table 1.1; Figure 1.2).

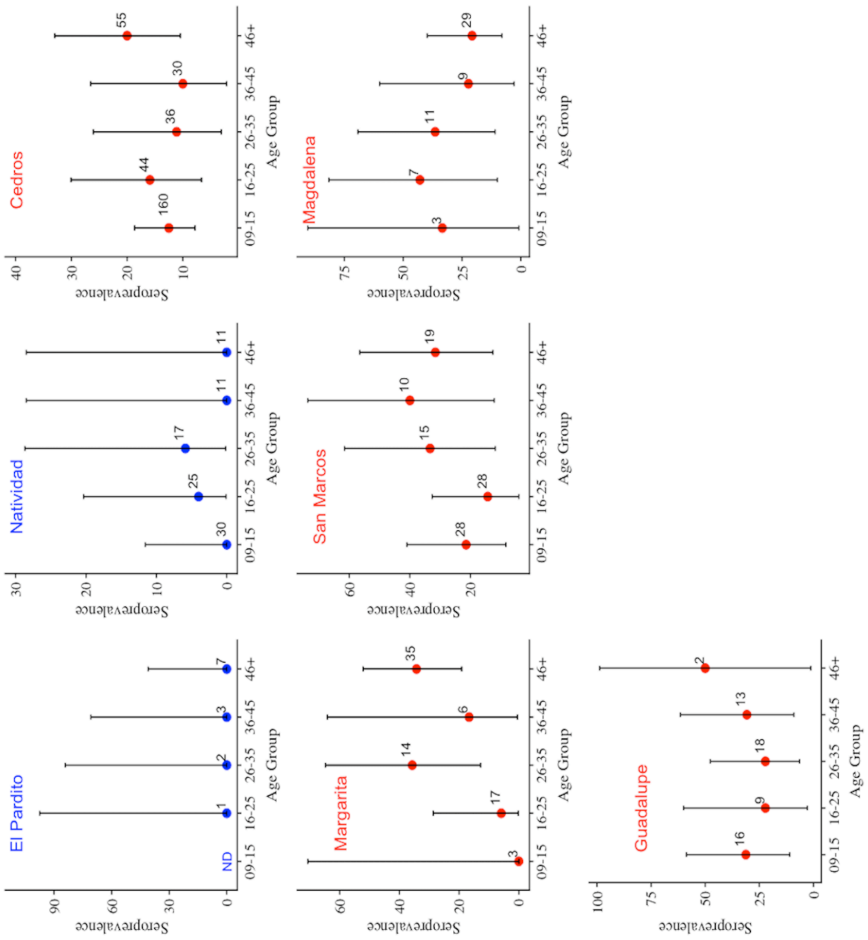
Table 1.1 Estimated crude and age-adjusted *Toxoplasma gondii* seroprevalence and odds ratio in the islands of Baja California, Mexico. CI= Confidence Interval; ND= not defined; * $P < 0.01$; ** $P < 0.001$

Island	% Crude seroprevalence (no. positive / no. sampled)	% Age-adjusted seroprevalence (95%CI)	odds-ratio relative to Natividad (95% CI)
El Pardito	0 (0/13)	0 (0 - 0)	ND
Natividad	2.13 (2/94)	1.76 (1.75 - 1.77)	1
Cedros	13.84 (45/325)	11.62 (11.59 - 11.65)	7.4 (2.2 - 45.8) *
San Marcos	25 (25/100)	22.89 (22.85 - 22.94)	15.3 (4.4 - 97) **
Margarita	25.3 (19/75)	16.61 (16.58 - 16.66)	15.6 (4.3 - 100.3) **
Magdalena	27.11 (16/59)	25.03 (24.98 - 25.1)	17.1 (4.6 - 111.4) **
Guadalupe	27.6 (16/58)	25.68 (25.64 - 25.73)	17.5 (4.7 - 114.1) **

Seroprevalence varied with age across the islands, with seroprevalence being significantly greater in age groups 26-35, and 46 and older, than 9-16 year olds (Figure 1.2; Table 2.2). The island where cats were eradicated (Natividad) had significantly lower seroprevalence in all age groups compared to most islands with cats. Seroprevalence in the 30 children 9-15 years of age (who were born after cats were eradicated) on Natividad was 0%, which was significantly lower than in children of the same age from

three other islands (Fisher's exact tests: Cedros: 12.5% (20/160), P = 0.04; Guadalupe: 31.2% (5/16), P = 0.01; San Marcos: 21.4% (6/28), P = 0.03) but not in the two others where sample sizes were very small (Margarita: 0% (0/3), P = 1; Magdalena: 33.3% (1/3), P = 0.13). In addition, seroprevalence in people born when cats were still present on Natividad (16 years and older), was significantly lower (3.1%, 2/64, 95% CI = 0.3 - 10.8%) than that of people of the same age from islands with cats (21.87%, 89/407, 95% CI = 17.9 - 26.2 %).

Figure 1.2. Age-specific *Toxoplasma gondii* seroprevalence. Error bars indicate 95% confidence intervals and numbers indicate sample size. Islands where cats are absent are color-coded blue; islands where cats are present are color-coded red. Children from Natividad in the 09-15 age group were born after cats were eradicated.



Risk factors

I found that interactions with cats, gender, visitation to the mainland, and diet were important factors influencing *T. gondii* seroprevalence. The odds of seropositivity to *T. gondii* were 4.8-fold higher in people that had cats near their homes. For the subset of people that reported having cats near their homes, *T. gondii* seroprevalence decreased with the number of cats reported. The odds of seropositivity were 1.6-fold higher in men (Table 2.1), 1.03-fold higher for every percent increase of time spent on the mainland, and 0.97-fold lower for every percent increase of shellfish

consumption (Table 2.1). The fitted model indicated that seroprevalence of *T. gondii* was 4.3% (1.7% - 6.4%) higher in men than women for the most common age classes of each island.

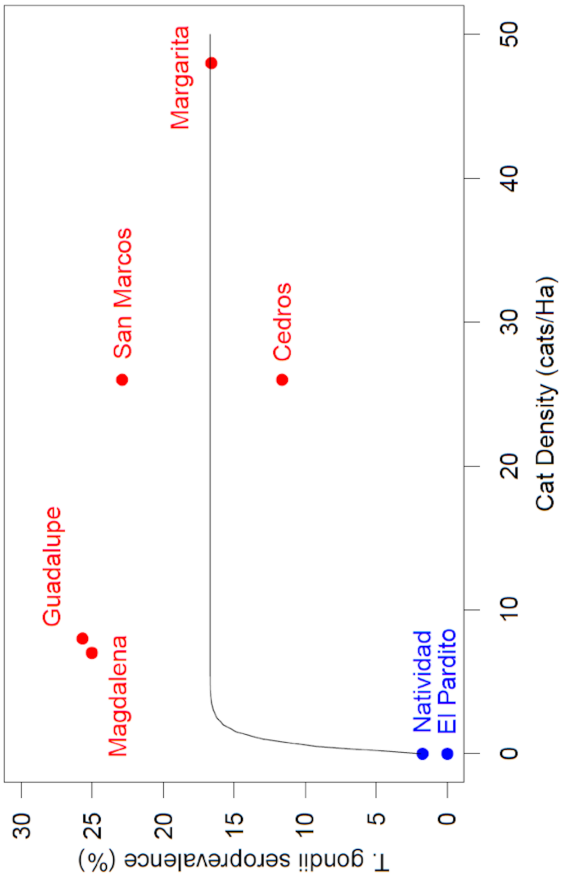
Table 1.2. Risk factors for *Toxoplasma gondii* seropositivity using a generalized linear mixed effects model with a binomial distribution, a logit link and with island as a random effect. Asterisks indicate the reference level for each predictor.

Risk factor	OR (95% CI)	P value
Age class		
9-15*	1	
16-25	1.20 (0.58 - 2.50)	0.61
26-35	2.03 (1.01 - 4.10)	0.04
36-45	1.49 (0.73 - 3.06)	0.26
> 46	1.88 (1.10 - 3.36)	0.03
Educational level	0.88 (0.70 - 1.11)	0.27
Gender		
Female*	1	
Male	1.57 (1.03 - 2.40)	0.03
Presence of cats near household		
No*	1	
Yes	4.79 (2.30 - 9.97)	< 0.001
Dog range		
No dog*	1	
Indoor-outdoor	0.88 (0.53 - 1.46)	0.61
Outdoor only	1.13 (0.63 - 2.01)	0.67
Frequency of shellfish consumption	0.97 (0.95 - 0.99)	0.005
Frequency of meat consumption		
Raw meat consumption	1.01 (0.99 - 1.02)	0.36
No*	1	
Yes	0.85 (0.42 - 1.71)	0.64
Proportion of time spent outside the island	1.03 (1.01 - 1.06)	0.02
Outdoor activities		
No*	1	
Yes	1.13 (0.67 - 1.90)	0.64

Cat density

T. gondii seroprevalence increased with cat density, but the best fitting model showed a sharp rise in seroprevalence between zero cats and the lowest non-zero cat density island, and no change thereafter (Figure 1.3).

Figure 1.3. Age-adjusted *Toxoplasma gondii* seroprevalence plotted against the density of cats on seven islands in Baja California. Islands where cats are absent are color-coded blue; islands where cats are present are color-coded red. The best fitting model was Seroprevalence = 0.019+0.15*(1-$e^{(-1.37*\text{Cat Density})}$); slope coefficient P = 0.05; 95% CI: 0.001 - ∞).



Discussion

I found evidence that introduced cats are a key source of human exposure to *T. gondii* on islands and that eradication,

but not control, of cats can reduce the burden of this zoonotic disease. Seroprevalence of *T. gondii* was near or equal to zero and significantly lower on the two islands where cats were absent. Moreover, children born after cat eradication were all seronegative. Further, I found that the odds of being seropositive for *T. gondii* were nearly fivefold greater for people that reported having cats near their households. However, I found seroprevalence did not increase with overall cat density on islands with cats, and I was surprised to find that for the subset of people that reported having cats near their homes, risk of seropositivity to *T. gondii* actually decreased with number of cats reported. Although my findings are based on a relatively small number of study populations from a single geographical region, my focus on island populations (including an island where cats were eradicated) allowed me to examine the effect of cat presence and density, exposure routes, and age more easily than in mainland populations.

My results suggest that exposure to *T. gondii* occurs both in children and in young adults, but may have occurred through different exposure routes. Seroprevalence was lowest in 9-15 year-olds (the youngest group I sampled) with a predicted seroprevalence across all islands of 14%, which increased to 17.0%, 25.8%, 20.3%, and 24.3% in the next four age classes. Studies have suggested that exposure to *T. gondii* in children occurs via exposure to oocysts when children play with soil that is contaminated with *T. gondii* (Jones et al. 2001, Caballero-Ortega et al. 2012b). Exposure of young

adults may occur through ingestion of bradyzoite cysts in raw or undercooked meat and contaminated produce, likely imported from the mainland (Jones et al. 2001, Caballero-Ortega et al. 2012b). To better understand the main sources of *T. gondii* infection would require the use of serological tests designed specifically for detecting antibodies against *T. gondii* oocysts (e.g. (Hill et al. 2011)). Finally, I also found older individuals from the island where cats were eradicated (Natividad) to be seronegative to *T. gondii*, which may suggest that T. gondii antibodies wane without antigenic stimulation, as has been suggested elsewhere (Rougier et al. 2017).

Gender, diet, and travel also influenced the risk of *T. gondii* exposure. Men had higher risk of T. gondii seropositivity than women, suggesting that men may engage in activities that increase risk of exposure to *T. gondii*. I also found that spending time on the mainland was an important risk factor for being exposed to *T. gondii*. People may be exposed to contaminated soil, meat or vegetables when traveling to the mainland. The majority of people (83.3%) reported travelling to the northwestern states of Mexico, where the average prevalence of *T. gondii* is 39.9% $\pm$ 12.9 (Range 20 – 59.9%) (Caballero-Ortega et al. 2012a). In contrast, eating shellfish was associated with reduced *T. gondii* seroprevalence. How shellfish consumption reduces *T. gondii* seroprevalence is unknown, because consumption of shellfish was positively ($r = 0.23$; $N = 724$; $P < 0.001$), not negatively correlated with

meat, poultry and pork consumption (potential sources of *T. gondii* (10,38)).

My finding that *T. gondii* seroprevalence decreased with the number of cats reported for people with cats near their homes suggests that greater cat abundance reduces *T. gondii* transmission. While this may seem counterintuitive, higher numbers of cats near a home could actually decrease rodent populations through predation as well as impose sub-lethal effects on rodents through fear and lower fecundity (Beckerman et al. 2007). Reduced rodent density could reduce exposure of T. gondii in cats by interrupting the predator-prey transmission route of the parasite (Gilot-Fromont et al. 2012). Determining whether increased cat abundance reduces transmission of *T. gondii* by reducing rodent abundance would require measuring *T. gondii* shedding (or at least seroprevalence) in cats as well as rodent abundance across a range of cat densities. Regardless, management or eradication of cat populations should also incorporate management of rodent populations to avoid an increase in rodents as cat predators are removed (Rayner et al. 2007, Doherty and Ritchie 2016). Increases in rodent population could lead to increasing outbreaks of rodent-borne diseases as well as increased rodent predation on native species (Rayner et al. 2007, Doherty and Ritchie 2016).

Interestingly, I found no increase in seroprevalence with cat density among islands where there were cats. Initially, this appears to contrast with several studies that have found

higher *T. gondii* seroprevalence in pigs, or humans or *T. gondii* in soil in areas with “high” cat density than “low” cat density, or nearer to farms with cat populations than farther away (Meerburg et al. 1998, Afonso et al. 2008a, Ortega-Pacheco et al. 2011, Du et al. 2012b, Gotteland et al. 2014a, 2014b, Boughattas et al. 2017, Retmanasari et al. 2017). However, none of these studies examined seroprevalence across a continuous range of cat densities (all treated cat abundance as a categorical variable), and the combination of multiple *T. gondii* exposure routes (e.g. soil, food, direct contact with cats) makes studies of human exposure critical. As a result, the actual relationship between cat density and *T. gondii* transmission to humans is very poorly understood. The lack of a relationship between cat density and *T. gondii* seroprevalence on islands with cats could result from focal aggregation of *T. gondii*-contamination in common latrine areas (Afonso et al. 2008a, Gotteland et al. 2014b, Simon et al. 2017) where cats defecate, but only limited *T. gondii*-contamination outside these areas. Spatial sampling of *T. gondii* in soil at sites across a range of cat densities would provide data to test this hypothesis. In addition, my estimates of cat density came from a single point in time, and cat densities have likely varied over time. Temporal variation in cat density would make it more difficult to detect a relationship between cat density and seroprevalence. Likewise, the demographic structure of cat populations, which can also vary temporally and as a result of cat population control (Doherty and Ritchie 2016), may also

influence transmission dynamics among cats and subsequently exposure to people. This is because kittens lose maternal antibodies against *T. gondii* after being weaned and as they begin to consume potentially infected intermediate hosts they are more likely to become infected and shed *T. gondii* oocysts (Omata et al. 1994, Gilot-Fromont et al. 2012).

Overall, my results suggest that there are opportunities to achieve measurable public health benefits from cat eradications on islands. In contrast, I found little evidence to indicate that controlling cat abundance on islands is an effective tool to reduce human *T. gondii* exposure. It remains to be determined how *T. gondii* transmission to humans varies with cat density in continental populations, and whether control of feral cat colonies will result in public health benefits without complete or near eradication. Regardless, eradicating zoonotic diseases such as *T. gondii* by eliminating their introduced reservoir hosts is much more feasible on islands than in continental populations, and eradicating introduced cats from islands contributes to a "One Health" approach in that this intervention simultaneously benefits human health and native biodiversity (Keitt and Tershy 2003, Buxton et al. 2014, Newton et al. 2016).

CHAPTER 2. ESTIMATING BURDENS OF NEGLECTED TROPICAL ZOONOTIC DISEASES ON ISLANDS WITH INTRODUCED MAMMALS

Abstract

Many neglected tropical zoonotic diseases are maintained by introduced mammals, and on islands the most common introduced species are rodents, cats, and dogs. Management of introduced mammals, including control or eradication of feral populations, which is frequently done for ecological restoration, could also reduce or eliminate the diseases these animals carry. Understanding the burden of these zoonotic diseases is crucial for quantifying the potential public health benefits of introduced mammal management. However, epidemiological data is only available from a small subset of islands where these introduced mammals co-occur with people. I examined socioeconomic and climatic variables as predictors for disease burdens of angiostrongyliasis, leptospirosis, toxoplasmosis, toxocariasis and rabies from 57 islands or island countries. I found strong correlates of disease burden for leptospirosis, *Toxoplasma gondii* infection, angiostrongyliasis and toxocariasis with more than 50% of the variance explained, and an average of 57% (range 32-95%) predictive accuracy on out of sample data. I used these relationships to provide estimates of leptospirosis incidence and *T. gondii* seroprevalence infection on islands where non-native rodents and cats are present. These predicted estimates of disease burden could be used in an

initial assessment of whether the costs of managing introduced mammal reservoirs might be less than the costs of perpetual treatment of these diseases on islands.

Introduction

Introduced mammals are reservoirs of zoonotic pathogens and are also well known drivers of decline and extinction of native species and changes to ecosystems on islands.(Chanteau et al. 1998, Reaser et al. 2007, Levy et al. 2008, Aguirre-Muñoz et al. 2008) Rodents (*Mus* spp., *Rattus* spp.), cats (*Felis catus*), and dogs (*Canis familiaris*) are the most common introduced mammals. (Atkinson 1985, Oppel et al. 2011) These introduced mammals co-occur with over 470 million people on at least 560 islands that also harbor Critically Endangered and Endangered vertebrates as defined by the International Union for Conservation of Nature (IUCN).(Threatened Island Biodiversity Database Partners 2014, IUCN 2015) Peri-domestic rodents and domestic, stray and feral cats and dogs are reservoirs for many neglected zoonotic diseases, including angiostrongyliasis, some strains of leptospirosis and rabies, as well as toxoplasmosis, toxocariasis and echinococcosis. (Centers for Disease Control and Prevention 2015, World Health Organization 2016) Although these diseases have a worldwide distribution, they have disproportionate impacts on marginalized human populations with limited access to healthcare services. (King 2011, Molyneux et al. 2011, Torgerson and Mastroiacovo 2013) Treatment of these

diseases includes antibiotics, anti-protozoals, and vaccinations, but treatment is challenging to administer to remote populations. In mainland areas, controlling the reservoir host populations can reduce the source of infection(Victoriano et al. 2009, Hampson et al. 2009, Dabritz and Conrad 2010), but the ongoing costs for control can be prohibitively expensive.(Patronek et al. 1997, Flores-Ibarra and Estrella-Valenzuela 2004, Easterbrook et al. 2005) In contrast, on islands where immigration of introduced mammals is low and potentially preventable, management actions to control (reduce populations) or eradicate (completely remove) introduced mammals represent significant public health opportunities to reduce or potentially eliminate these diseases.

Management of introduced mammals on islands is a common practice to protect native biodiversity. For many islands, complete eradication of introduced rodents, and feral cats and dogs is possible, with island size and human population size being key limiting factors on where that can occur (Howald et al. 2007, Phillips 2010, Oppel et al. 2011). Where this is not currently feasible, controlling rodents, and feral dogs and cats on islands utilizes techniques to reduce populations of these introduced mammals to a desired state, ideally an outcome state for a native species (e.g. (Reardon et al. 2012, Pender et al. 2013)).

Islands represent a potential opportunity for reducing zoonotic disease burden because control or eradication of introduced mammal populations could result in disease

alleviation or disease elimination, particularly if reintroductions of reservoirs can be prevented. Determining whether control or eradications are a cost-effective approach to controlling zoonotic diseases requires weighing the potential ecological, economic, and social costs of management against the human health and ecological benefits. The costs include the economic cost of control or eradication, economic and cultural benefits of the introduced mammal, and non-target ecological impacts of removal (Howald et al. 2007, Oppel et al. 2011). The benefits potentially include reduction of zoonotic disease in human populations, enhanced economic growth due to alleviation of economic costs of zoonotic disease on human livelihoods and local economies (Bonds et al. 2012), reduced costs of disease control, and recovery of impacted native diversity (Beltran et al. 2014). A first step in quantifying the potential benefits of management of introduced mammals is an assessment of the burden of zoonotic diseases on island communities.

Although there has been a considerable effort to estimate the global burden of neglected zoonotic diseases such as rabies (Hampson et al. 2015), congenital toxoplasmosis (Torgerson and Mastroiacovo 2013), and leptospirosis (Costa et al. 2015), very little is known about the burden of zoonotic diseases transmitted by introduced mammals on islands. Only a fraction of the 560 islands where people co-occur with introduced mammal host populations (Threatened Island Biodiversity Database

Partners 2014) has information on disease burdens for any zoonotic disease. However, if disease burdens could be estimated using other readily available ecological and socioeconomic data, these estimates could then be used to inform preliminary cost-effectiveness analyses for management of introduced mammal populations on islands, and identify where local disease burden assessments could then be conducted.

The goals of my study were to examine whether a suite of climatic and socioeconomic factors can be predictive of burden of zoonotic diseases transmitted by introduced mammals on islands, and thereby to provide a tool that can be used in cost-effectiveness analyses to identify where control of introduced mammals could be applied as a public health mitigation tool. Several readily available socioeconomic and climatic variables could serve as useful predictors of disease burdens through direct, indirect, or correlated influences on pathogen transmission.

Socioeconomic factors such as Gross Domestic Product (GDP) per capita and population size are often correlated with zoonotic disease burden possibly due to their inherent connection to public health care services and infrastructure. Infrastructure in public services is predicted to scale exponentially with increasing population size through economies of scale (Bettencourt et al. 2007). In particular, efficiency in public services such as solid waste management and access to waste disposal centers lead to decreased rodent population sizes, and decreased contact

with commensal rodents and rodent-borne diseases.(Barcellos and Sabroza 2000, Traweger et al. 2006, Reis et al. 2008) This in turn may have a cascading effect on cat abundance through bottom-up processes (Ringler et al. 2015) and in consequence affect the environmental load of parasites like *Toxoplasma gondii*.(Dabritz et al. 2007, Gotteland et al. 2014b) However, this pattern has only been studied in large metropolitan areas, and whether it persists at smaller population sizes is currently unknown. Climate can influence disease dynamics by affecting host and pathogen population fluctuations via availability of resources in the form of vegetation or prey, or by creating suitable environmental conditions for free-living stages of pathogens.(Altizer et al. 2006)

I searched the literature and other sources for data on population size, economic development, and climatic variables. I correlated these variables with disease burden data for five important zoonotic diseases: angiostrongyliasis, leptospirosis, toxoplasmosis, toxocariasis, and rabies. I focused on these diseases because of their public health importance and neglected status (Torgerson and Budke 2003, World Health Organization 2006, Wang et al. 2008, King 2011, Torgerson and Mastroiacovo 2013), as well as on the availability of epidemiological information.

Methods

I collected disease data for angiostrongyliasis, leptospirosis, toxoplasmosis, toxocariasis, rabies, and

echinococcosis from the Global Infectious Disease and Epidemiology Network (GIDEON) online database (GIDEON 2019) and by searching Google Scholar and PubMed for published epidemiological studies from islands, island countries, or countries within islands. I found incidence data for cystic echinococcosis and alveolar echinococcosis for only eight and one islands, respectively. Given the limited availability of incidence data for these diseases, I did not pursue further analysis.

For islands that do not harbor native felids, *T. gondii* can be exclusively maintained by introduced cats (Dubey 1998) and both introduced cats and dogs can maintain *Toxocara* spp. (Overgaauw and van Knapen 2013) However, some species and serotypes of *Leptospira* and rabies can be maintained by native mammal species in islands. (Cleaveland et al. 2006, Desvars et al. 2011) For these two diseases I only included cases of species or serotypes associated with rodents or dogs, respectively.

I obtained the majority of disease information for leptospirosis and rabies from the GIDEON online database, which reports number of cases per year. I calculated incidence using the average number of cases reported between 2005 and 2015 to account for variability between years. I calculated the average yearly incidence of these diseases by dividing the number of cases by the island's population. I estimated countrywide incidence for archipelagos when cases were not reported for a specific island. I obtained the majority of information for

angiostrongyliasis from epidemiological studies. To meet assumptions of normality, I log transformed leptospirosis and rabies incidence data after adding one to account for islands that reported zero cases, and log transformed angiostrongyliasis incidence data.

Reporting of cases is thought to be heterogeneous, with underreporting being higher in rural populations. As a result, I performed analyses on estimates of incidence, one using total population size, and another using urban population size in order to account for underreporting in rural areas. I primarily used The World Bank Group (World Bank) (World Bank Group 2016) and CIA World Factbook (The CIA World Factbook 2016) to obtain information on total and urban population size. However, when data was not available in these databases, I searched the country profiles of the UNdata (UNdata 2015), UNICEF Migration Profiles(UNICEF 2014), and the Insee (Institut national de la statistique et des etudes économiques). (INSEE 2015)

I obtained data for prevalence of *T. gondii* infection and toxocariasis from epidemiological studies. These diseases were reported as seroprevalence and for both diseases the age classes for which data were collected for each island differed. To compare seroprevalence between islands, I only included data from islands with disease information stratified by age classes. For seroprevalence of *T. gondii* infection, I imputed missing values using pairwise multivariate correlations.(Sterne et al. 2009) I analyzed adjusted estimates for the 31-40 year age-class for

seroprevalence of *T. gondii* infection, and the 5-15 year age class for toxocariasis, which were the most commonly reported age classes in the epidemiological studies. Seroprevalence was arcsine square-root transformed to normalize the data.

For islands with disease information, I obtained data on climate, population size and per capita GDP. I obtained climatic data from Bioclim World Climate (Hijmans et al. n.d.) and focused on mean annual temperature, temperature variability, annual precipitation and precipitation variability. This database reports temperature variability as the standard deviation of the monthly values of temperature, and precipitation variability as the coefficient of variation in monthly precipitation. I used ESRI ArcMap version 10.2 (ESRI (Environmental Systems Resource Institute) n.d.) to obtain the climate values for each island by extracting the corresponding BioClim layers using the 30 arc-seconds spatial resolution. I primarily used the World Bank and CIA World Factbook to obtain information on per capita GDP and population size that corresponded to the year the epidemiological study was done. I log-transformed population size as well as per capita GDP to equalize leverage in the analyses.

I used R version 3.2.1 (R CoreTeam 2018) to perform regression analyses. I performed stepwise backwards regression for leptospirosis and *T. gondii* infection, starting with 6 predictor variables (annual temperature, temperature variability, annual precipitation,

precipitation variability, population size, and per capita GDP), until only significant ($p < 0.05$) predictors remained in the model. Models obtained through stepwise regression have been shown to have higher predictive power than regression trees and similar predictive power to models obtained through exhaustive subset search and stepwise elimination using AIC (Murtaugh 2009). Although stepwise regression can lead to bias in coefficients when predictor variables are correlated, the alternative, including non-significant and possibly spurious variables can lead to overfitting. In my analyses, coefficients of predictors in reduced models were very similar to those estimated for the full models, suggesting that bias in coefficients due to variable selection was minor. Sample sizes of islands for rabies, angiostrongyliasis and toxocariasis were too small to examine in multiple regression analyses. Thus, I performed univariate regression analyses for these diseases. I included population size as a predictor variable in my analyses, and although very low incidence can result in spurious negative relationships with population, analyses in which I excluded islands that reported zero and one cases were qualitatively identical to the results reported below. The residuals of all analyses did not significantly deviate from normality (Shapiro-Wilks tests, $p > 0.05$) and inspection of residual plots did not reveal any clear non-linearities.

For seroprevalence of *T. gondii* infection and toxocariasis, I also fit the non-transformed seroprevalence data with a generalized linear model with a binomial

distribution and logit link. This analysis weights each seroprevalence estimate by the sample size in the study, rather than giving each data point equal weight, as in the Gaussian regression.

I tested the prediction accuracy of the Gaussian models by using leave-one-out cross-validation (LOOCV). I used prediction accuracy to determine whether total population or urban population was more appropriate for estimating incidence. In addition, I calculated the correlation coefficients of the socioeconomic predictors with the residuals of the final model of each disease to test for potential reverse causation between disease data and the socioeconomic variables, which would indicate a need to add instrumental variables to my models (Martens et al. 2006). For all models, I did not find any correlation between residuals and the socioeconomic predictor variables, suggesting that there was no need to use instrumental variables.

I used the Gaussian regression models for leptospirosis and *T. gondii* infection, which were cross validated, and were based on the largest sample size of islands, to predict disease burdens on a subset of islands lacking local disease data to illustrate the potential use of the fitted models. I used the Threatened Island Biodiversity database, which is a global dataset of islands based on the presence of breeding populations of IUCN Critically Endangered and Endangered vertebrates to identify islands with human population, and introduced rodents and/or cats.

(Threatened Island Biodiversity Database Partners 2014, IUCN 2015) I used the Threatened Island Biodiversity database to obtain population size, and the sources described above to obtain climate data and per capita GDP. I primarily focused on islands with known per capita GDP, or that form part of an island country or state with known per capita GDP. However, when GDP per capita was not available for the island or island country, I assigned the GDP per capita value of the sovereign country of the island.

To predict estimates of leptospirosis and *T. gondii* infection I used the "sp" package in R(R CoreTeam 2018) to create bivariate polygon regions to identify islands with values that fell within the range of the predictor values that were used to fit the models. I expect the average prediction accuracy for these estimates to be close to that reported in my analyses. I did not predict disease for islands with values that fell outside the range of these predictors, except for population size. I extrapolated disease estimates for islands with small population sizes (and clearly identify them as extrapolations) because population size is a limiting factor for management of introduced mammals. Specifically eradications are currently feasible on islands with population sizes less than approximately 1,000 people(Jones et al. 2016a). A substantial fraction of the islands that are therefore suitable for management were less populous than the range of islands in the dataset I used to fit the models. As a result, these extrapolated predictions should be used in planning with extreme caution and, as with all my predicted

estimates, should be verified by on-the-ground measurement before any rigorous cost-benefit analyses are undertaken.

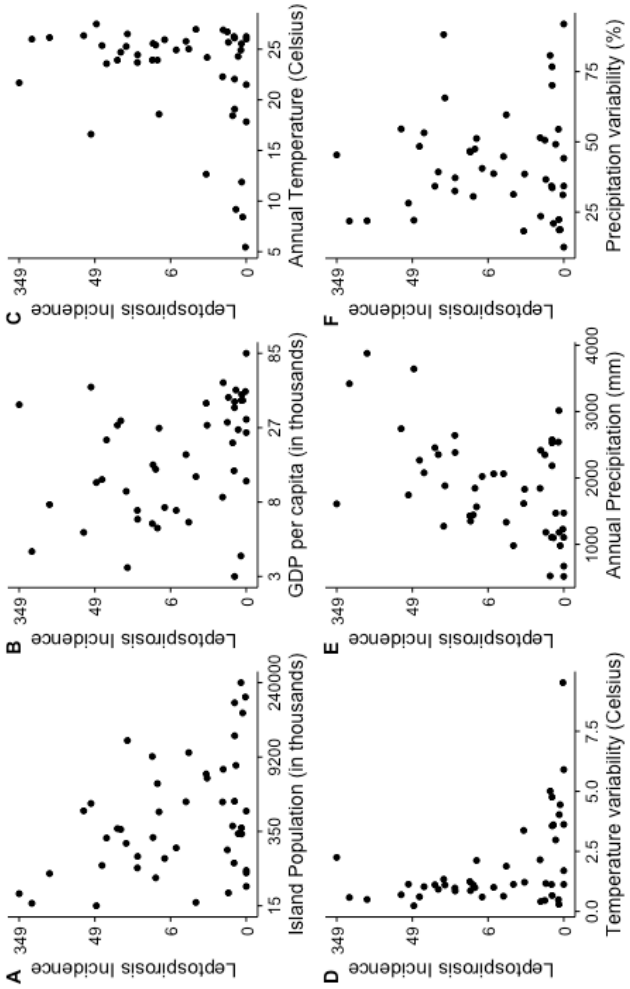
Results

Data existed for at least one disease on 57 populated islands (including island countries or countries within islands) that were also inhabited by introduced rodents, cats, and/or dogs.(Threatened Island Biodiversity Database Partners 2014) I found significant associations for four of the five diseases with 53%-95% of the variance explained. Based on the average predictive accuracy values of the regression models, incidence of leptospirosis and angiostrongyliasis were better described when estimated using total population size than urban population size.

The best fitting model for incidence of leptospirosis was the full model. Leptospirosis decreased with increasing values of population size, GDP per capita, temperature variability and annual temperature, and decreasing values of annual precipitation and precipitation variability, explaining 57% of the variability with 35% out of sample average predictive accuracy (Figure 2.1). When incidence was estimated using the most recent (2013-2015) reported number of cases, the same model showed an even better fit with higher predictive accuracy ($R^2 = 0.64$, $p < 0.0001$, LOOCV = 0.44). Island population size and per capita GDP were not correlated with the residuals in the models.

Figure 2.1. Univariate plots of leptospirosis incidence versus six predictor variables: (A) Total population; (B) Gross

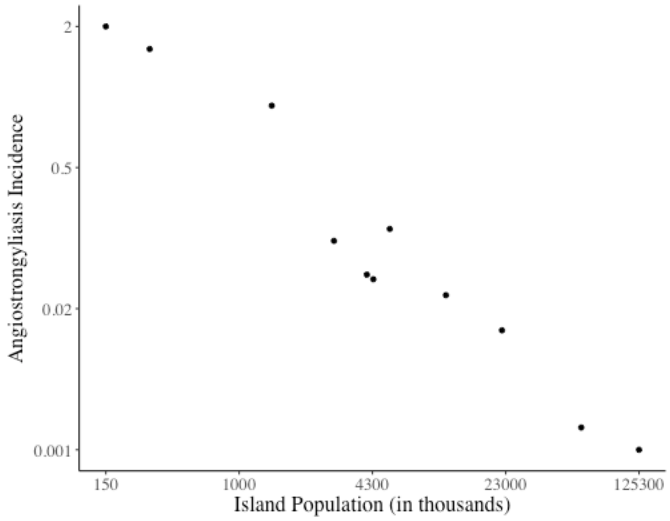
Domestic Product (GDP) per capita; (C) Annual temperature; (D) Temperature variability; (E) Annual precipitation; and (F) Precipitation variability. Leptospirosis is in per 100,000 individuals and is on a log (x+1) scale in all plots, population and per capita GDP are shown on a log scale, temperature variability is the standard deviation of the monthly values of temperature, and precipitation variability is the coefficient of variation in monthly precipitation. Although not all of these univariate plots reveal strong relationships, all six predictors were significant in a multiple regression (leptospirosis incidence = 6.96 - 0.38 (log population size) – 0.57 (log GDP per capita) – 0.11 (annual temperature) – 0.23 (temperature variability) + 0.0004 (annual precipitation) + 0.01 (precipitation variability); R2 = 0.57, n = 46, p < 0.0001, LOOCV = 0.35).



Incidence of angiostrongyliasis decreased significantly with population size, explaining 95% of the variability with 94% out of sample average predictive

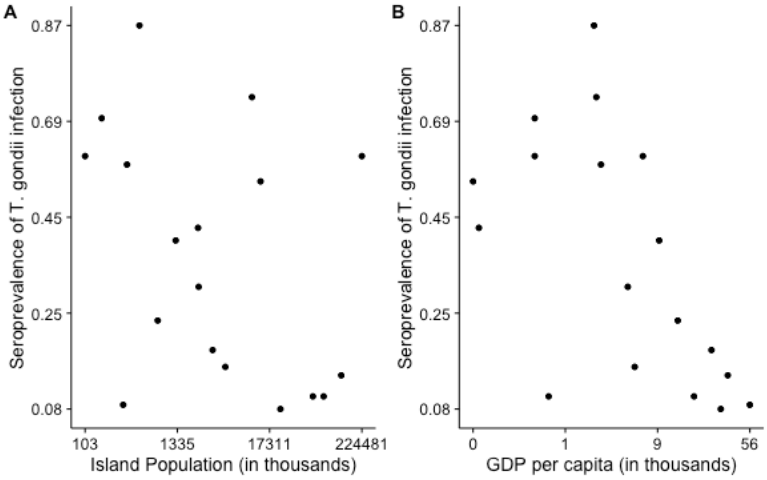
accuracy (Figure 2.2). Island population was not correlated with the residuals in the models.

Figure 2.2. Univariate plot of angiostrongyliasis incidence versus population. Angiostrongyliasis is in per 100,000 individuals and on a log scale and population size is log transformed. The model was highly significant (angiostrongyliasis incidence = 6.36 – 1.16 (log population size); R2 = 0.96, n =11, p < 0.0001, LOOCV = 0.94).



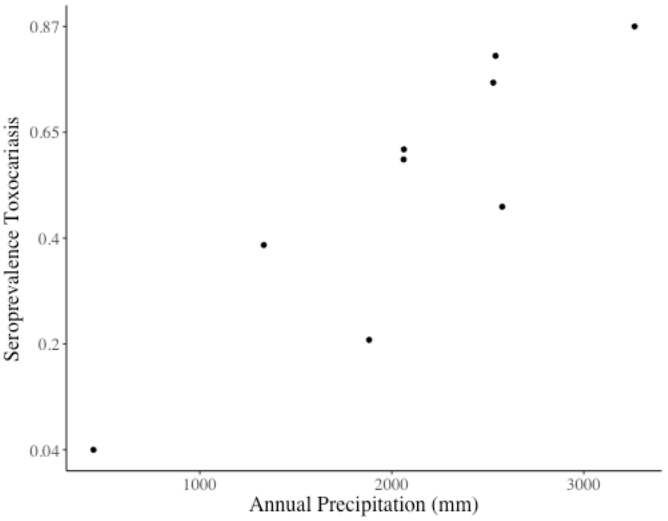
Seroprevalence of *T. gondii* infection decreased with increasing per capita GDP and increasing population size in both Gaussian and binomial regression models. The predictors in the Gaussian model explained 53% of the variability with 32% average out of sample predictive accuracy (Figure 2.3). Island population and per capita GDP were not correlated with the residuals in the Gaussian model.

Figure 2.3. Univariate plots of seroprevalence of *T. gondii* infection versus (A) Total population and (B) Gross Domestic Product (GDP) per capita. Seroprevalence is shown on an arcsine square root scale, and GDP per capita and total population are shown on a log scale. Both predictors were significant in a multiple regression model (seroprevalence of *T. gondii* infection = 2.32 - 0.25 (log per capita GDP) - 0.11 (log population size); R2 = 0.53, n =18, p = 0.003, LOOCV = 0.32).



Seroprevalence of toxocariasis was significantly and positively correlated with annual precipitation in both the Gaussian and binomial regression models. Annual precipitation explained 77% of the variability with 67% out of sample average predictive accuracy (Figure 2.4).

Figure 2.4. Univariate plot of seroprevalence of toxocariasis and annual precipitation. Toxocariasis is arcsine square root transformed. The model was highly significant (toxocariasis seroprevalence = 0.09 + 0.0003 (annual precipitation); R2 = 0.77, n = 9, p = 0.002, LOOCV = 0.67).

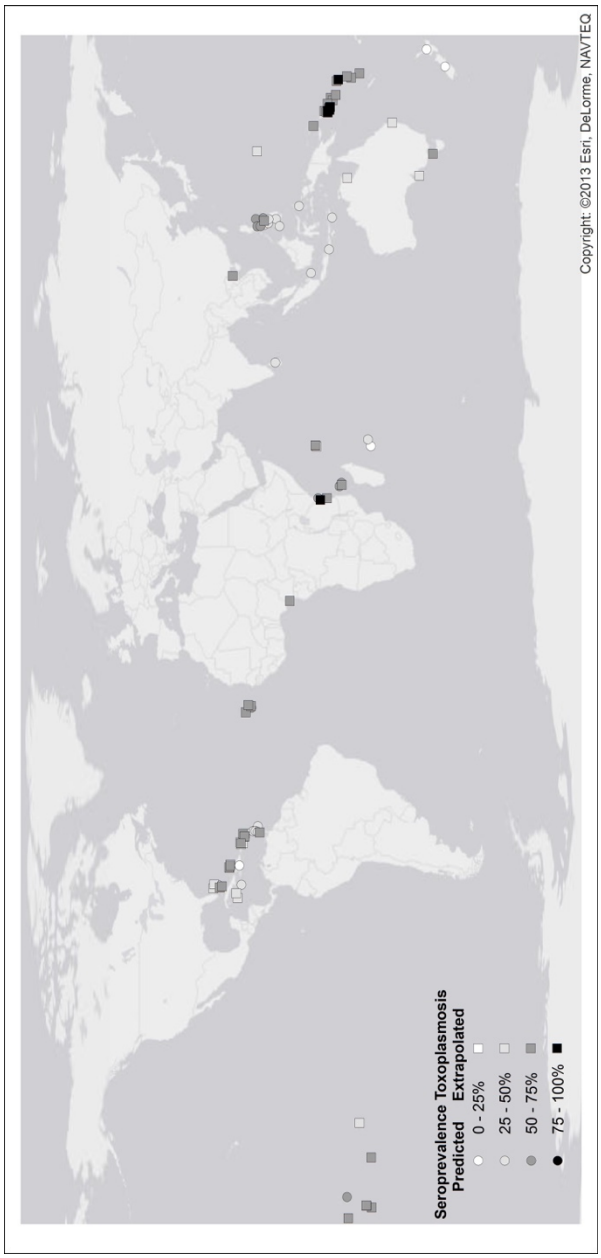


Rabies incidence was not significantly correlated with any of the predictor variables.

I used the fitted models for leptospirosis and *T. gondii* infection to generate preliminary estimates of disease burdens on islands lacking local information. Preliminary estimates included seven and 32 islands with values within the bivariate polygon regions of predictors of my fitted models for leptospirosis (Figure 2.5; S10 Table) and *T. gondii* infection (Figure 2.6; S11 Table), respectively, and 30 and 61 additional islands for which I extrapolated outside the range of population size.

Figure 2.5. Predicted incidence of leptospirosis. Circles show predicted values and squares show extrapolated values. Leptospirosis is reported as prevalence per 100,000 people.

Figure 2.6. Predicted seroprevalence of *Toxoplasma gondii* infection. Circles show predicted values and squares show extrapolated values. Seroprevalence of *T. gondii* infection is reported as a percentage.



Discussion

Introductions of non-native mammals have facilitated the establishment and persistence of several important zoonotic diseases. (Torchin et al. 2003) Efforts to reduce or eradicate introduced mammal hosts from islands could result in elimination or reduction of the zoonotic pathogens they transmit, particularly if they are the sole pathogen reservoirs

on the island (i.e. cats for *T. gondii*, dogs and cats for *Toxocara* spp.). However, the costs of controlling or eradicating introduced mammals from islands is non-trivial and cost-effectiveness analyses are needed to assess whether management is worthwhile to attempt as a public health mitigation tool (Martins et al. 2006, Donlan and Wilcox 2007, Howald et al. 2007). Cost-effectiveness analyses require estimates of disease incidence or prevalence, which are lacking for most islands globally.

I developed models to provide initial estimates of incidence or seroprevalence on islands where diseases transmitted by rodents, cats or dogs are present. I found that widely available island attributes explained most of the variation in incidence and seroprevalence for four zoonotic diseases. Although the socioeconomic and ecologic variables rarely directly influence the incidence of zoonotic disease, they appear to be useful correlates in providing an estimate of incidence when local data is lacking. Due to the correlative nature of my analyses, my estimates should be used in the initial identification stage. After prospective islands for management of introduced mammals are identified, local incidence estimates should be obtained before formal cost-effectiveness analyses are undertaken.

Determining whether management of introduced mammal populations offers a conclusive benefit to public health requires an assessment of the disease burden and a cost-effectiveness analysis of the proposed management action at an island scale. (Fox-Rushby and Hanson 2001)

Disability-adjusted life-years (DALYs) are commonly integrated into cost-effectiveness analyses as a value for healthy years of life lost through premature mortality or disability (Murray et al. 2012). The monetary value of DALYs can be estimated based on the region's income, the funds allocated towards disease alleviation, and the DALY value of the disease (Brent 2011). The fitted models above could be used to provide an initial estimate of disease incidence or seroprevalence on islands, island countries, or countries within islands where this information is missing to identify prospective islands in which local data should be collected. Ultimately, these results could be converted into DALYs (Murray et al. 2012). The results can be used to estimate the number of DALYs potentially saved through management of introduced mammal populations on a particular island, and then be assigned a monetary value to be compared to the monetary costs of the management action (Brent 2011).

Management of introduced mammals on inhabited islands implies social, cultural, and economic challenges that should be considered when assessing the feasibility of management actions (Nogales et al. 2004, Donlan and Wilcox 2007, Townsend et al. 2013). For example, costs of eradication vary greatly, mostly as a function of population size, island area (e.g. US$3 to $20,000 per hectare), the type of eradication method (target species dependent), and other variable costs such as capacity building, environmental compliance, and mitigation of non-target species. (Martins et al. 2006, Donlan and Wilcox 2007, Howald et al. 2007)

Incorporating disease burden estimates into feasibility assessments for introduced mammal eradications can potentially expand the rationale and utility of these actions.

I validated my Gaussian models using the leave-one-out cross-validation technique. For leptospirosis and *T. gondii* infection, prediction accuracy was 35% and 32%, respectively, and thus, for islands whose traits fall within the bivariate polygon regions of predictors, the predicted values should offer a useful initial estimate of disease burdens. However, disease data was particularly limited for islands with small populations and low GDP per capita. The paucity of epidemiological information from islands with these traits limits the extent of my predictions, yet highlights islands where disease surveillance could be relevant based on my model results. Furthermore, given that population size is currently a limiting factor for management of introduced mammals on islands, I extrapolated burden estimates of leptospirosis and *T. gondii* infection on islands in which eradication might be feasible, yet based on information from islands with larger population sizes. This may lead to less accurate predicted estimates of disease burden. Obtaining local data from the prospective islands for formal cost-effectiveness analyses is thus especially important for islands with population values outside those used to build the models described above.

Three recent studies have developed estimates of countrywide incidence for rabies (Hampson et al. 2015), congenital toxoplasmosis (Torgerson and Mastroiacovo

2013), and leptospirosis(Costa et al. 2015), to guide surveillance, vaccination and reservoir control programs. These studies include incidence estimates for some islands, the majority of which have large populations. My estimates for predicted and extrapolated incidence of leptospirosis were similar to some of those developed by Costa *et al.* (Costa et al. 2015). Unfortunately, I could not compare my estimates of *T. gondii* burden because I calculated seroprevalence estimates for the 31-40 year old age-group, whereas Torgerson and Mastroiacovo (Torgerson and Mastroiacovo 2013) examined incidence of congenital toxoplasmosis. I did not develop estimates for rabies incidence that could be compared to Hampson et al. (Hampson et al. 2015), because none of my predictors reached statistical significance. However, as more data become available, all my models should be updated and estimated values should be compared with other estimates, and if possible, merged into a single dataset to identify islands for potential eradications of introduced mammals.

Improving public health and reducing threats from introduced species are recognized in global targets for sustainable development and biodiversity conservation. (Convention on Biological Diversity 2011, United Nations 2015). There has been substantial recent attention investigating the relationship between biodiversity and human health [26,65], but there are few concrete examples of the potential for synergistic interventions that benefit both (Ostfeld and Keesing 2000, Wolfe et al. 2005). However, the

biodiversity benefits of eradicating introduced mammals from islands has been well documented (Lavers et al. 2010, Jones et al. 2016a) and my study enables a way to connect these biodiversity benefits to improve public health.

CHAPTER 3. *TOXOPLASMA GONDII* CONTAMINATION OF SOIL IN URBAN PUBLIC SPACES OF CENTRAL CALIFORNIA, UNITED STATES

Abstract

Toxoplasma gondii is a zoonotic parasite that can have severe implications to human health. Acutely infected cats shed environmentally resistant *T. gondii* oocysts in their feces that contaminate soil and soil serves as a reservoir of infection for humans. Free- roaming domestic cats are thought to play an important role in environmental contamination with *T. gondii*. Persistence of *T. gondii* oocysts in soil is largely affected by environmental conditions, which can in turn affect risk of human exposure to contaminated soil. Few studies have directly measured the direct contribution of free-roaming cats to *T. gondii* in soil, and understanding the impacts of cats on public health is relevant to policy and management of free roaming cat colonies. My goal was to evaluate if soil contamination with *T. gondii* occurs in public areas with free-roaming cat colonies and examine variation in soil contamination associated to environmental factors. I collected soil samples from sites in city and state parks, public playgrounds and community gardens in central California, USA. To test for differences in environmental factors I sampled during spring, summer and fall and in sites located along the coast and inland. I tested soil samples using conventional nested PCR targeting the internal transcribed spacer 1 (ITS-1) of the small subunit ribosomal RNA. I detected and sequence-

confirmed *T. gondii* in 5.6% of my soil samples. Despite similar sampling intensity in different seasons, I only detected *T. gondii* in coastal sites during fall and not during spring or summer months. My 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 affected by local climatic conditions and season. Management of free-roaming cats could prevent *T. gondii* oocyst-borne infections by reducing environmental contamination with this zoonotic pathogen.

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 (Sukthana 2006, Torgerson and Mastroiacovo 2013, Maenz et al. 2014). 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 (Glasner et al. 1992, Leal et al. 2007, Carme et al. 2009, Cortés and Aguirre 2018). Wild and domestic felids are the only known definitive hosts of *T. gondii* (Hutchison et al. 1969). Felids usually become infected after ingesting infected prey and can shed up to a billion environmentally resistant oocysts in their feces for approximately one week (Fritz et al. 2012), thus contaminating soil (Dubey 1995).

People can become infected 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 (Dubey and Jones 2008, Jones and Dubey 2010, Bahia-Oliveira et al. 2017, Aguirre et al. 2019). 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. 2000b, Lopez et al. 2000, Jones et al. 2001, Spalding et al. 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. 2014b, VanWormer et al. 2016, Simon et al. 2017), with colonies often found in areas of overlap with people where access to food sources are greater (Schmidt et al. 2007, Kilgour et al. 2017), such as parks, playgrounds and community gardens. 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 (Jessup 2004, Guttilla and Stapp 2010). However, these programs are generally ineffective at controlling cat populations (Andersen et al. 2004, Foley et al. 2005, Nutter 2005), and supplemental feeding may inadvertently alter *T. gondii* transmission cycles by changing cat distribution, demographics, and exposure to infected prey (Schmidt et al. 2007, VanWormer et al. 2013a). 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 (Carme et al. 2009, Pomares et al. 2018, Blaizot et al. 2019). Understanding the risks of exposure to *T. gondii* from contaminated soil may inform management of free-roaming cat colonies and reduce human exposure to this zoonotic pathogen.

There is reported seasonal variation in the incidence of *T. gondii* infection in humans in the United States and Europe*,* with higher rates in late summer, fall and early winter, which has been associated with either seasonal variation in activities that involve direct or indirect contact with soil (e.g. gardening and consumption of fresh produce) or climatic factors (e.g. rain and temperature) (Bobic et al. 2010, Sagel et al. 2010, Morin et al. 2012, Contopoulos-Ioannidis et al. 2015). The viability and persistence of oocysts in soil is largely influenced by environmental conditions, as 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 and Darde 2003, Lelu et al. 2012)). However, very little is known about the potential role of seasonal variation in *T. gondii* prevalence in soil.

Studies of *T. gondii* oocysts in soil are scarce mostly due to limitations in current methods of sampling and detection (Dumètre and Dardé 2004, Su et al. 2010, Lelu et al. 2011, Shapiro et al. 2019a). Prior investigations have been conducted in temperate or subtropical regions 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 (Afonso et al. 2006, Lass et al. 2009, Du et al. 2012a, Gotteland et al. 2014b, Liu et al. 2017, Simon et al. 2017, Davis et al. 2018). However, few of these studies have analyzed variation in the patterns of *T. gondii* in soil. In China, presence of oocysts in soil varies 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 and 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 cat demographics, since transmission from infected prey to cats is thought to peak after kittens lose maternal antibodies against *T. gondii* following weaning (Nutter et al. 2004) and begin hunting (Omata et al. 1994, Gilot-Fromont et al. 2012).

My goals were to evaluate whether soil contamination with *T. gondii* occurs in recreation areas with free-roaming cat colonies in central California and examine spatial and temporal variation in *T. gondii* soil contamination. I focused on urban public spaces such as parks, playgrounds and community gardens where human exposure to *T. gondii* could occur. I sampled sites located in coastal and inland central California to examine how local climate affects the geographic distribution of *T. gondii* in soil

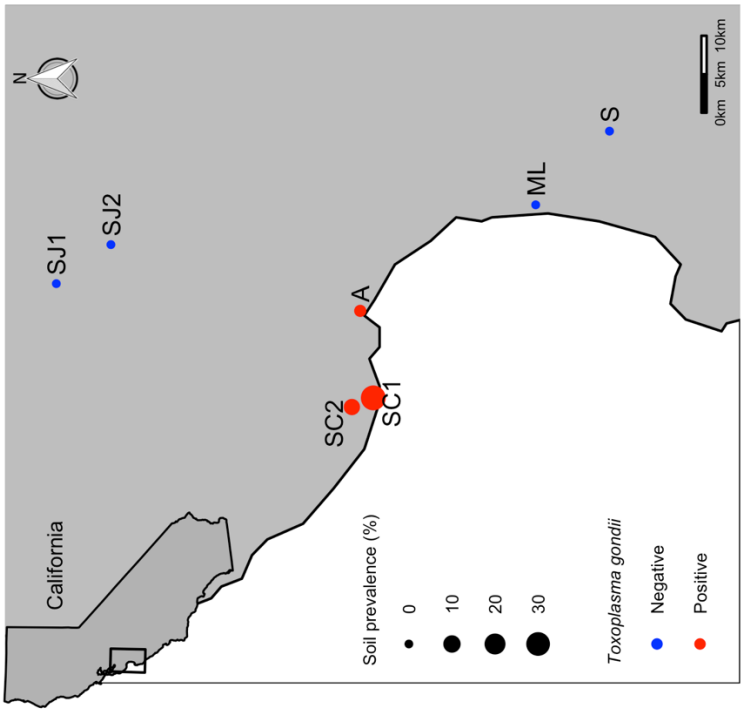
(NOAA 2018). I specifically examined if presence of *T. gondii* was associated with season, geographic location (coastal vs inland), cat demographics, and provision of supplemental food.

Materials and methods

Site selection

I studied seven free-roaming cat colonies located in or near public areas including community gardens, parks and playgrounds 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 3.1). I sampled each site during the first two hours after dawn, which is when cats tend to be more active (Cove et al. 2018). I sampled each site once in spring (April and May), summer (June and July) and fall (November) of 2017. At each site visit, I recorded the number of feeding stations and counted total cats and kittens for one hour using fur coloration and ear clippings to distinguish among individuals. I used size and appearance to differentiate between kittens and cats of other ages (hitherto referred as adult cats).

Figure 3.1. 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 indicate 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; SJ2 = San Jose City Park and playground.



I sampled soil beneath scattered cat feces and latrines. Latrines were defined as clusters of more than two fecal deposits that contained paw prints and scattered feces were defined as no more than two clustered feces (Simon et al. 2017). I identified each latrine and scattered-feces with an alphanumeric number and weighed and collected 20-50 grams of soil from beneath the surface area of the latrines and the scattered feces at an approximate depth of 2 to 5 cm as described by (Afonso et al. 2008b). I also collected fecal samples opportunistically if they were no more than approximately two days old based on texture and smell. I kept the soil and fecal samples in plastic bags under refrigeration and processed them the following day.

Oocyst recovery from soil

I subdivided each 20 to 50-gram of soil sample into 4 to 10 replicates of 5 grams (hitherto referred as sub-samples). I processed soil sub-samples using a modified sucrose flotation method (Lelu et al. 2011, Simon et al. 2017). Briefly, I added 2.5 mL of 2% sulphuric acid to each 5-gram replicate and vortexed the solution for 1 min. The solution was left standing for 24 hours under refrigeration (4 °C), after which I 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 (1500 X g for 20 min), and then the entire supernatant transferred to a sterile 50-mL conical tube (Simon et al., 2017). I subsequently washed the supernatant twice with 35 mL of deionized water, centrifuged (1500 X g for 20 min), and retained 1 mL of pellet for analysis.

DNA extraction and molecular analysis

I centrifuged (2000 X 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: I 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 et al. 2019). I initially conducted systematic spiking

experiments in my laboratory to select the most sensitive PCR assay and to establish a limit of detection for the approach described above (Supplemental Information). I 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 529bp repeat element (RE) (Homan et al. 2000), and a segment of the B1 gene (Burg et al. 1989). In addition, I compared one real-time quantitative PCR (qPCR) assay also targeting the 529bp 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 S1).

The external PCR reaction included 36.1 μL of DNase- and RNase-free, distilled water; 5 μL of PCR buffer (10X 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.

Fecal analysis

I submitted fecal 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.

Sequence analysis and additional T. gondii *genotyping*

I purified all PCR products from soil sub-samples and fecal 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). I analyzed 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 I further processed DNA from soil sub-samples and fecal 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), I attempted the B1 PCR assay twice, initially using 5 μL of template DNA and a second time using 10 μL of template DNA.

Statistical analysis

I estimated the prevalence of *T. gondii* in soil as the number of positive soil sub-samples divided by the total sub-samples by location, season and sample type (i.e. latrine and scattered feces). I 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. Thus, I used a Bayesian approach to examine if the presence of *T. gondii* in soil was associated with season and geographic location (coastal vs inland). I fit models for the presence of *T. gondii* in soil sub-samples with season and geographic location as fixed effects, and site and sub-sample as random effects using Bayesian multilevel regression models with a Bernoulli distribution and a logit link, a student's t prior distribution centered at 0 with 3 degrees of freedom, and a scale parameter of 10. The effective sample sizes ranged between 145 and 1818, and none of the predictors had R-hat values greater than 1.01. Given the relatively small sample size, I could not rigorously examine associations between *T. gondii* in soil and cat colony variables (colony size, number of kittens and number

of feeding stations) because the outcome data were limited to one sampling period (fall season). I used R version 3.3.3 (R CoreTeam 2018) to perform all statistical analyses and create a spatial map with the *brms* (Bürkner 2017) and *ggmap* (Kahle and Wickham 2013) packages, respectively. I did not statistically analyze fecal samples because I had too few samples for rigorous comparisons.

Results

I analyzed a total of 482 soil sub-samples from 80 samples (soil collected beneath 33 cat latrines and 47 scattered feces locations) at the seven sites. I amplified *T. gondii* DNA at the ITS-1 locus in 27 of the 482 soil sub-samples (5.6%, 95% CI = 3.7 – 8%), which belonged to six of the 80 samples (7.5%, 95% CI = 2.8 – 15.6%). All positive samples were detected at coastal sites during the fall, resulting in site and season being strong predictors in the Bayesian regression for *T. gondii* contamination of soil (Figure 3.2; Tables 3.1 and 3.2). In fall samples, prevalence of *T. gondii* in coastal sites was 31% (95% CI= 21.5 – 41.9%) and prevalence ranged from 0 – 74% across seasons (Table 3.2). I collected a total of 21 fecal samples and *T. gondii* DNA was amplified in a single sample collected during summer from the inland Salinas site (Table 3.2). All reported PCR-positive sub-samples and the fecal sample were sequence confirmed as *T. gondii* (100% identity at the ITS-1 locus on BLAST). I further processed all *T. gondii* -positive sub-samples for attempted genotyping via

PCR at the B1 locus. However, DNA could not be amplified, both at the initial trial using 5 μL of DNA template, as well as in a second attempt using 10 μL of DNA template.

Figure 3.2. Seasonal prevalence of *Toxoplasma gondii* DNA in soil from latrines and scattered feces 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; SJ2 = San Jose City Park and playground. Numbers indicate number of sub-samples. Error bars represent 95% Confidence Intervals estimated for binomial proportions.

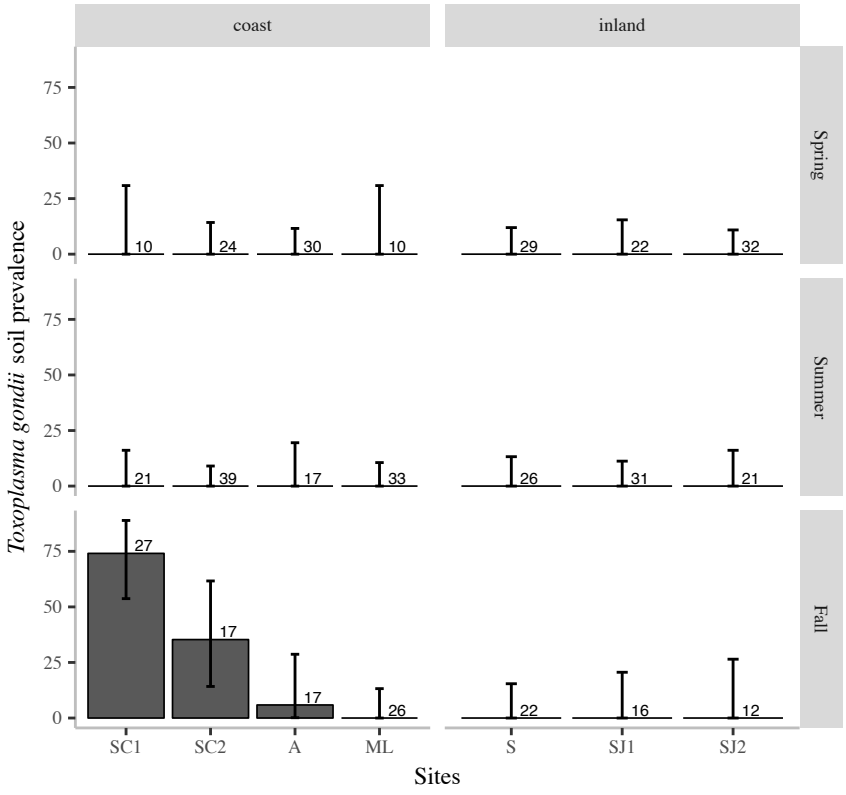


Table 3.1. Bayesian multilevel regression model with a Bernoulli distribution and a logit link explaining seasonal and spatial variability of *Toxoplasma gondii* detection in all soil sub-samples (N=482).

Variables	Coefficient (SD)	95% CI
Random effects		
Site	5.67 (4.09)	1.04 – 16.35
Sub-sample¶	1.26 (1.23)	0 – 4.6
Fixed effects		
Season		
Spring§	1	
Summer	6.57 (359.23)	-542.4 – 613.2
Fall	154.97 (337.86)	5.6 – 1020.6
Geographic location		
Inland§	1	
Coastal	108.19 (187.25)	2.9 – 556.3

¶ Sub-sample refers to each 5-gram replicate belonging to samples from latrines or scattered feces; CI = credible interval; SD = standard deviation of the posterior distribution; § = reference level for each predictor.

Table 3.2. Site-specific proportion of soil sub-samples and fecal samples positive for *Toxoplasma gondii* via sequence-confirmation during spring, summer and fall. Samples were collected from feral cat colonies near areas frequently used by people such as playgrounds, community gardens or parks. NC = Not collected

Site	Soil sub-samples from latrines	Soil sub-samples from scattered feces	Total positive soil sub-samples	Positive fecal samples
Santa Cruz State Park				
Spring				
Summer	0/10 (0%)	NC	0/10 (0%)	NC
Fall	0/21 (0%)	NC	0/21 (0%)	NC
	20/27 (74%)	NC	20/27 (74%)	NC
Santa Cruz Community Garden				
Spring				
Summer	0/7 (0%)	0/17 (0%)	0/24 (0%)	NC
Fall	0/10 (0%)	0/29 (0%)	0/39 (0%)	NC
	4/8 (50%)	2/9 (22.2%)	6/17 (35.3%)	0/1 (0%)
Aptos State Park				
Spring	NC	0/30 (0%)	0/30 (0%)	NC
Summer	0/10 (0%)	0/7 (0%)	0/17 (0%)	NC
Fall	1/8 (12.5%)	0/9 (0%)	1/17 (5.9%)	NC
Moss Landing Community Park				
Spring	0/11 (0%)	NC	0/11 (0%)	0/1 (0%)
Summer	0/24 (0%)	0/13 (0%)	0/37 (0%)	0/4 (0%)
Fall	0/29 (0%)	NC	0/29 (0%)	0/3 (0%)
Salinas City Park and playground				
Spring	0/20 (0%)	0/9 (0%)	0/29 (0%)	NC
Summer	0/23 (0%)	0/6 (0%)	0/29 (0%)	1/4 (25%)
Fall	0/24 (0%)	NC	0/24 (0%)	0/2 (0%)
San Jose City Park				
Spring				
Summer	0/22 (0%)	NC	0/22 (0%)	NC
Fall	0/10 (0%)	0/21 (0%)	0/31 (0%)	NC
	0/19 (0%)	NC	0/19 (0%)	0/3 (0%)
San Jose City Park and playground				
Spring				
Summer	0/10 (0%)	0/22 (0%)	0/32 (0%)	0/2 (0%)
Fall	0/23 (0%)	NC	0/23 (0%)	NC
	0/13 (0%)	NC	0/13 (0%)	0/1 (0%)

Discussion

I found *T. gondii* to be prevalent in soil under cat feces at free-roaming cat colonies that were located in close proximity to places heavily frequented by people for recreation (playgrounds and parks) or growing food (community gardens). Contamination in soil was highly

dependent on location and season, with *T. gondii* detection only occurring during fall and only at coastal sites.

The marked seasonal and spatial difference in *T. gondii* soil prevalence among sites may be due to persistence of oocysts in soil influenced by season and local climatic conditions. Oocysts are known to persist and remain viable in the environment for long periods of time under mild temperatures and moist soil conditions (Dumètre and Darde 2003, Lelu et al. 2012). The Mediterranean climate of coastal California may provide suitable conditions for persistence of oocysts in soil, particularly during late summer and fall season when a marine layer creates foggy conditions and average temperatures range from 8°C to 24°C (NOAA 2018). In contrast, the hotter and drier climate in inland California, where temperatures frequently reach 30°C with low humidity may reduce *T. gondii* persistence in soil, especially during the summer (NOAA 2018). The relatively low soil prevalence of *T. gondii* in my study may be explained by my small sample size, by low shedding frequency of cats (Dabritz et al. 2007, Schares et al. 2016) and by other factors limiting the accurate detection of *T. gondii* in environmental matrices such as oocyst age and naturally occurring components in soil that can inhibit reagents used in PCR and reduce the sensitivity of PCR assays (Dumètre and Dardé 2004, Su et al. 2010, Lelu et al. 2011). My protocol had a limit of detection of 20 oocysts per gram of soil (Table S1), proving less sensitive compared to

other studies (Lelu et al. 2011), which can also explain my low detection rate.

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 from central California consuming local seafood and involved in water-related recreational activities, because freshwater runoff can carry *T. gondii* oocysts from the terrestrial landscape to the nearshore marine environment (VanWormer et al. 2016). For example, dead southern sea otters (Enhydra lutris nereis) located in regions in California receiving freshwater runoff are frequently infected with *T. gondii* (Miller et al. 2002, Burgess et al. 2018), of which 72% are infected with the atypical genotype X (Miller et al. 2004, Conrad et al. 2005). While I was unable to genotype the *T. gondii* I detected in soil, the two most common *T. gondii* genotypes present in infected free-roaming domestic cats from the Monterey Bay area are Type X (20.3% prevalence) and Type II (42.9% prevalence) (VanWormer et al. 2014). The discrepancy between the genotype prevalence in sea otters and free roaming cats may be due to differences in strain virulence, as reported genotypes in sea otters have been largely reported in sea otters that died due to protozoal encephalitis (Miller et al. 2004). Overall, my results support previous investigations suggesting free-roaming cat colonies in coastal California as an important source of *T. gondii* oocyst transmission to the marine environment (VanWormer et al. 2013a, 2016).

My results suggest that soil from cat feces and latrines are a significant foci of *T. gondii* and that free-roaming cat colonies located in public spaces represent a risk of exposure to this zoonotic pathogen. Although direct contact of people with soil from latrines is probably low, oocysts can further disperse to adjoining areas and surfaces through soil transport from wind and rain (Dumètre and Darde 2003, Gotteland et al. 2014b) or through mechanical vectors such as earthworms, cockroaches, flies (Wallace 1973, Frenkel et al. 1975) and even through dogs via fur contamination (Frenkel et al. 2003, Etheredge et al. 2004). The presence of *T. gondii* in latrines located in urban public spaces such as community gardens, playgrounds and parks may pose a greater risk for children since they might be less conscientious about washing their hands after playing with soil (Sousa et al. 1988). Likewise, risk of exposure may be high for people handling soil and consuming produce from community gardens, which is normally considered organic and thus has less regulations for washing and disinfecting (Williams and Hammitt 2001). *Toxoplasma gondii* infections are treatable, yet not curable (Aguirre et al. 2019), thus preventing exposure is a key public health measure. 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. 2019a).

CONCLUSIONS

Understanding the links between human and ecosystem health can serve to integrate solutions and inform policy and management decisions that can maximize benefits to public health and conservation of biodiversity. The effects of introduced and free-roaming cats on native species, and in particular on island native species are well known, yet mitigation strategies are spatially and/or logistically limited in many regions of conservation importance (Lepczyk et al. 2004, Donlan and Wilcox 2007, Loss et al. 2013). The role of cats as reservoirs of zoonotic pathogens such as *T. gondii* have also been well documented (Robertson et al. 2000, Robertson and Thompson 2002, Dabritz and Conrad 2010, Gerhold and Jessup 2013), yet better understanding the direct threats of free-roaming cats on human health, while accounting for the effects of cat management strategies on pathogen transmission dynamics, could inform policy makers as well as public and private land owners on best practices to control introduced free-roaming and invasive cat populations.

I examined the effect of cat population control and cat eradication on prevalence of *T. gondii* infection in island inhabitants. I found evidence that introduced cats are a key source of human exposure to *T. gondii* on islands and that eradication, but not control, of cats can reduce the burden of this zoonotic disease. Although the findings of this study are based on a relatively small number of study populations from

a single geographical region (Baja California Peninsula, Mexico), by focusing on island populations (including an island where cats were eradicated) allowed me to examine the effect of cat presence and density, exposure routes, and age more easily than in mainland populations.

I also found *T. gondii* to be prevalent in soil at free-roaming cat colonies located in close proximity to places heavily frequented by people for recreation (playgrounds and parks) or growing food (community gardens). The distribution of *T. gondii* in soil varied temporally and spatially, with prevalence concentrated during the fall season (19.7% of fall samples tested positive for *T. gondii*) and at sites located on the coast (10% of total coastal samples and 31% of coastal samples collected in the fall season tested positive for *T. gondii*). The presence of *T. gondii* in urban public spaces such as community gardens, playgrounds and parks poses an especially important risk for children since they might be less conscientious about washing their hands after playing with soil (Sousa et al. 1988). Likewise, risk of exposure may be high for people handling soil and consuming produce from community gardens, which is normally considered organic and thus has less regulations for washing and disinfecting (Williams and Hammitt 2001).

Although *T. gondii* infection through exposure to oocysts has been documented (Hill et al. 2011) and exposure to soil has been reported to be an important risk factor in several studies (Cook et al. 2000b, Lopez et al. 2000, Jones et al. 2001, Spalding et al. 2005), published

studies investigating presence of *T. gondii* oocysts in soil are limited. In the few studies that have analyzed the patterns of *T. gondii* in soil, a seasonal patterns of *T. gondii* distribution in soil was reported. For example, in China, *T. gondii* has been predominantly found in soil samples collected during fall and to a lesser extent during Winter (Liu et al. 2017); and in Germany, shedding in domestic cats is significantly associated with high temperatures and precipitation during winter (Schares et al. 2016). The marked seasonal and spatial difference in prevalence of *T. gondii* in soil among the sites in my study suggests that presence and possibly persistence of oocysts in soil may be influenced by environmental conditions. These seasonal patterns in *T. gondii* soil prevalence and shedding coincide with seasonal peaks in incidence of *T. gondii* infection in humans (Logar et al. 2005, Bobic et al. 2010, Sagel et al. 2010, Morin et al. 2012, Contopoulos-Ioannidis et al. 2015).

The effect of cat density on prevalence of *T. gondii* (in people and in soil) requires further investigation. There was either no association or a negative association between some measurement of cat abundance and *T. gondii* prevalence in people and soil, respectively. Initially, this appears to contrast with several studies that have found higher *T. gondii* seroprevalence in pigs and humans, or higher load of *T. gondii* in soil, in areas with “high” cat density than “low” cat density, or closer to farms with cat populations than farther away (Meerburg et al. 1998, Afonso et al. 2008a, Ortega-Pacheco et al. 2011, Du et al. 2012b,

Gotteland et al. 2014a, 2014b, Boughattas et al. 2017, Retmanasari et al. 2017). However, none of these studies examined seroprevalence across a continuous range of cat densities (all treated cat abundance as a categorical variable), and the combination of multiple *T. gondii* exposure routes (e.g. soil, food, direct contact with cats) makes studies of human exposure critical. As a result, the actual relationship between cat density and *T. gondii* transmission to humans is very poorly understood. The lack of a relationship between cat density and *T. gondii* seroprevalence on islands with cats could result from focal aggregation of *T. gondii*-contamination in common latrine areas (Afonso et al. 2008a, Gotteland et al. 2014b, Simon et al. 2017) where cats defecate, but only limited *T. gondii*-contamination outside these areas. Likewise, prevalence of *T. gondii* in soil was negatively associated with cat colony size. A possible explanation for this is that *T. gondii* transmission dynamics can potentially be affected in colonies with high cat numbers as these can impose lethal (via predation) and sub-lethal (via fear) effects on prey when they are found at high densities (Beckerman et al. 2007) and thus interrupt the predator-prey transmission stage of *T. gondii.*

Studies have suggested that the predator-prey transmission phase of *T. gondii* can also be interrupted when free-roaming cats are fed supplemental food, where cats (which may still be hunting) may not be consuming potentially infected intermediate hosts (VanWormer et al.

2013a). While providing supplemental food could inadvertently interrupt the life cycle of *T. gondii* by reducing the likelihood of cats consuming infected prey (VanWormer et al. 2013a), supplemental feeding could also increase survival and fecundity of cats and promote migration of cats from nearby colonies (Foley et al. 2005, Schmidt et al. 2007), thereby increasing the number of susceptible individuals that can become infected and shed *T. gondii*. Furthermore, increasing the carrying capacity of feral cat colonies through supplemental feeding may also have negative implications for native wildlife through increased hunting or predation. (VanWormer et al. 2013b). I found no association between supplemental food and *T. gondii* prevalence in soil. However, a better approach at determining whether supplemental feeding influences cat exposure to *T. gondii* would require tracking seroconversion rates of *T. gondii* in cats with and without supplemental feeding and interpreting these results with respect to the seroprevalence of *T. gondii* in local prey populations.

Finally, I developed a model using readily available socioeconomic variables that can be used to predict the burden on *T. gondii* infection on islands where this information is unavailable. Ideally, as more data become available, this predictive model should be updated and estimated values should, if possible, be merged into a single dataset to identify islands for potential eradications of introduced cats. Management of introduced cats on inhabited islands implies social, cultural, and economic

challenges that should be considered when assessing the feasibility of management actions (Nogales et al. 2004, Donlan and Wilcox 2007, Townsend et al. 2013). For example, costs of eradication vary greatly, mostly as a function of population size, island area (e.g. US$3 to $20,000 per hectare), the type of eradication method (target species dependent), and other variable costs such as capacity building, environmental compliance, and mitigation of non-target species (Martins et al. 2006, Donlan and Wilcox 2007, Howald et al. 2007). Incorporating disease burden estimates into feasibility assessments for introduced cat eradications can potentially expand the rationale and utility of these actions.

The existence of free-roaming and invasive cats in their current global distribution is a result of the accumulation through time of legally and ethically undefined cat ownership responsibilities. Furthermore, despite the known impacts of invasive species on ecosystem function and biodiversity, their management is often hampered by opposing ethical, socioeconomic and environmental views (Donlan and Wilcox 2007, Crowley et al. 2017). However, better understanding the direct effects of invasive cats on human health could be equally, if not more, relevant to policy on their management. Overall, my dissertation research suggests that there are opportunities to achieve measurable public health benefits from management of introduced and free-roaming cat populations. Eradicating or controlling zoonotic diseases such as *T. gondii* by eliminating or controlling their main

reservoir host contributes to a “One Health” approach in that this intervention simultaneously benefits human health and native biodiversity.

APPENDICES

Supplemental Information: Validation and comparison of different PCR assays for detection of *Toxoplasma gondii* in soil

Spiking experiments were performed to compare the limit of detection of conventional nested PCR (3 assays) and real-time quantitative PCR (one assay) for detection of known numbers of spiked *T. gondii* oocysts added to soil samples. The three conventional nested PCR assays targeted three different loci: the internal transcribed spacer 1 (ITS-1) of the small subunit ribosomal DNA (SSU rDNA) (Rejmanek et al. 2009), the 529bp repeat element (RE) (Homan et al. 2000), and a segment of the B1 gene (Burg et al. 1989). In addition, one real-time quantitative PCR assay also targeting the 529bp RE gene was also included (Opsteegh et al. 2010).

Type II *T. gondii* (M4 strain (Gutierrez et al. 2010)) oocysts were produced in experimentally-infected cats as previously described (Fritz et al. 2012). Ninety percent of produced oocysts were sporulated and oocyst stock solutions were enumerated using a hemocytometer chamber under light microscopy at 400 X magnification and serial dilutions of 10, 100, 1000, and 10,000 oocysts/mL were prepared in sterile deionized (DI) water.

Three replicates of 5-gram autoclaved and homogenized soil samples were seeded with oocysts at a concentration of 0 to 10^4. For initial oocyst recovery, a procedure was followed using a modified protocol initially validated by (Lelu et al. 2011). In brief, 2.5 mL 2% sulphuric

acid was added to each 5-gram replicate of autoclaved soil. The spiking solution was homogenized by thoroughly vortexing and repeated pipetting. The solution was left standing in refrigeration (4° C) for 24 hrs, after which 20 mL of distilled water were added as a dispersion solution and then vortexed for one minute. The soil mixture was underlaid with 20 mL cold sugar solution (1.2 specific gravity). The mixture was then centrifuged at 1200 g for 20 minutes and then the entire supernatant was transferred to a sterile 50 mL conical tube. The supernatant was washed twice with 35 mL of deionized water, centrifuged at 1200 g for 20 min and 1 mL of centrifuged sediment was preserved.

Soil pellets (100 μL) were processed for nucleic acid extraction using the DNeasy Blood and Tissue Kit (QIAGEN) following manufacturer's instructions, with one modification: An initial freeze (-196°C)/thaw (100°C) cycle was performed to rupture oocyst walls prior to the addition of proteinase K. Nested PCR assays were performed on extracted DNA using primers targeting the ITS-1, B1 and the 529 bp RE loci as previously described (Shapiro et al. 2015). The external PCR reaction included 36.1 μL of DNase- and RNase-free, distilled water; 5 microliters of PCR buffer (10X buffer containing 15 millimolar (mM) MgCl2); 1 μL of 10 mM dNTP mixture; 0.5 microliters each of 50 micromolar (mM) forward and reverse primers; 1.6 microliters of 10% Bovine Serum Albumin, 0.3 microliters (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 primer. Each PCR batch included one positive control consisting of DNA extracted from cell culture-derived tachyzoites (RH strain) and three negative controls (extraction reagents with sterile water added; PCR reagents only; and PCR reagents with sterile water). The PCR amplification products were separated through electrophoresis on a 2% agarose gel stained with Red Safe and viewed under UV light.

In addition to the three conventional PCR assays described above, one quantitative polymerase chain reaction (qPCR) targeting the 529 bp RE for *T. gondii* was selected from a previously reported publication (Opsteegh et al. 2010). Each 25-µl qPCR reaction mixture contained 10 µl of template DNAand final concentrations of 1x TaqMan® Environmental Master Mix 2.0 (Applied Biosystems™) as well as a forward and reverse primers (700nM final concentration) and probe (100nM final concentration). The thermal cycling conditions included a pre-incubation step of 50ºC for 2 min and denaturation at 95ºC for 10 min, followed by 40 cycles of a denaturation step at 95ºC for 15 seconds and an annealing step at 60ºC for 1 min. The standard curve was generated from 10-fold dilutions (1-1,000 oocysts per reaction) of *T. gondii* oocyst genomic DNA in six replicates. The lowest limit of quantification (lowest concentration where all replicates amplified in the standard curve) for the qPCR assay was determined as 1 oocyst per reaction. The assay limit of quantification was determined on the standard curve using the lowest concentration where at least 4 of 8

replicates were amplified (Shapiro et al. 2019b). For spiking sample analysis, three serial dilutions (3- to 27-fold) of template DNA in nuclease-free water were used to assess inhibition in qPCR analysis. Plasmid DNA containing qPCR target sequences and nuclease-free water were used as positive and negative control, respectively, in qPCR analyses.

Results depicting the performance of each assay in detection of spiked oocysts in soil are depicted in Table S1. The assay with the highest sensitivity was the conventional PCR targeting the ITS-1 locus with a limit of detection between 20 and 200 oocysts per gram of soil. Conventional nested PCR assays were previously shown to be more sensitive than qPCR when applied to environmental matrices (Shapiro et al. 2010, 2019b).

Table S1. Comparison of four different PCR assays for detection of *Toxoplasma gondii* in soil

		PCR Assay			
Total oocysts spiked (5g)	Oocysts / g soil	B1	ITS-1	529bp RE	qPCR (529bp 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

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