Toxoplasma gondii detection in fecal samples from domestic cats (*Felis catus*) in Hawai'i

By Alisa A. Davis, Christopher A. Lepczyk*, Katherine H. Haman, Clifford W. Morden, Susan E. Crow, Nicole Jensen, and Michael T. Lohr

Abstract

The presence of large numbers of free-ranging feral cats (*Felis catus*) has raised concern in terms of both native species predation and potential disease transmission in Hawai'i. A disease of particular concern is toxoplasmosis, caused by *Toxoplasma gondii,* a zoonotic protozoan parasite. We tested soil samples and cat fecal samples from cat colonies from an urban university campus and a natural, coastal ecosystem for *T. gondii* oocysts using standard molecular procedures. Soil and fecal samples were collected from cat colony sites at the University of Hawai'i at Mānoa (UHM) and additional fecal samples were collected from cats trapped within the Ka'ena Point Natural Area Reserve (KPNAR). *T. gondii* DNA was detected in 5% (3 of 60) of fecal samples from UHM, but no *T. gondii* DNA was detected from soil samples. At KPNAR, 22.2% (2 of 9) of fecal samples were positive for *T. gondii* DNA. The presence of *T. gondii* at the university study sites suggests that cat colonies may be a potential health hazard for landscaping personnel, students, staff, and visitors. Likewise, presence of *T. gondii* at KPNAR, and potentially other coastal habitat(s) for ground-nesting seabirds or marine mammals, also suggests a disease risk and should be considered when managing these areas. Toxoplasmosis is a growing concern to both people and wildlife, and further work is needed to determine the pathways of transmission both within and between terrestrial and marine ecosystems of Hawai'i.

*Corresponding Author E-mail: lepczyk@auburn.edu

Introduction

Toxoplasma gondii is a tissue-cyst forming protozoan parasite that can infect and cause disease (i.e. toxoplasmosis) in all warm-blooded animals (Dubey 2010). The lifecycle of *T. gondii* consists of both sexual and asexual stages, with members of the cat family (Felidae) being the only known definitive hosts in which the sexual life-stage occurs. Asexual, clonal reproduction can also occur in felids, presenting a unique opportunity for the parasite to undergo sexual reproduction followed by clonal amplification (Wendte et al. 2010). When this occurs, a more virulent strain of *T. gondii* may be produced and shed by the felid definitive host as highly stable, environmentally-resistant oocysts (Dubey and Jones 2008), thus increasing the risk of subsequent disease outbreaks in exposed intermediate hosts (Wendte et al. 2010; Wendte et al. 2011). Once oocysts are shed and sporulate in the environment, transmission occurs through ingestion of oocysts in soil, grass, fruits, and vegetables; inhalation; or in contaminated water (Dubey 2010; Dubey and Jones 2008; Tenter et al. 2000; Teutsch et al. 1979). Further, research has shown that oocysts shed by the definitive felid host can wash into coastal marine waters, resulting in disease of naïve marine mammals (Shapiro et al. 2012; VanWormer et al. 2013; Shapiro et al. 2014; VanWormer et al. 2016).

T. gondii in domestic cats (*Felis catus*) is of particular concern as people interact with cats both as pets and as a nuisance species. Free-ranging cats tend to have higher instances of toxoplasma infection than pet cats, due to their diet of wild prey (Dubey 2010; Dubey and Jones 2008; VanWormer et al. 2013). Cats contract *T. gondii* either by ingesting oocysts deposited in the environment or through ingestion of tissue-cysts in prey that are intermediate hosts for this protozoan parasite. Transmission of *T. gondii* can also occur congenitally. Transmission of *T. gondii* to other warm-blooded intermediate hosts, both wild and domestic, occurs through similar

2

mechanisms of direct ingestion of oocysts, tissue-cysts, or congenital infection (Dubey et al. 1986).

As with most oceanic islands, felids did not occur in Hawai'i until European explorers and settlers introduced domesticated cats (Berger 1981), which are now widespread throughout the main Hawaiian Islands as both pets and free-ranging animals both in rural and urban areas (Amarasekare 1994; Lohr et al. 2013a). The high abundance of cats in Hawai'i combined with its favorable climate for the survival of *T. gondii* oocysts has important implications for the archipelago's already decreasing native wildlife abundance (Duffy and Capece 2012; Lohr et al. 2013a) as oocysts entering the environment through felid feces are likely to become part of freshwater runoff into streams and near-shore areas (Miller et al. 2008; VanWormer et al. 2013; VanWormer et al. 2016). Though transmission pathways in Hawai'i are poorly understood, morbidity and mortality associated with *T. gondii* have been identified in a number of native Hawaiian animals, including endangered species (Hawaiian crow [*Corvus hawaiiensis*; Work et al. 2000], Hawaiian goose [*Branta sandvicensis*; Work et al. 2002, 2016], and Hawaiian monk seal [*Monachus schauinslandi*; Hawai'i Department of Health 2000; Honnold et al. 2005; Barbieri et al. 2016]), spinner dolphin (*Stenella longirostris*; Migaki, Sawa, and Dubey 1990), red-footed booby (*Sula sula*; Work et al. 2002), and in livestock (Dubey and Jones 2008). Like other island systems, the lack of native felids in Hawai'i indicates the native wildlife did not evolve with *T. gondii* and therefore may be naïve, and thus more susceptible, to *T. gondii* related pathology, morbidity, and mortality (Hollings et al. 2103). Instances of *T. gondii* infection in marine mammals in Hawaiian coastal areas suggests that *T. gondii* transmission may be occurring through freshwater runoff containing oocysts, which has further implications for other wildlife as well as ocean recreation users (Miller et al. 2008).

Despite the documented risk of *T. gondii* infection and subsequent morbidity and possible mortality in native, naïve Hawaiian wildlife, there are few studies that report environmental contamination with *T. gondii* by non-native felids. For example, on Mauna Kea, a volcano on Hawai'i Island with forests harboring many endangered bird species, 37.3% of cats were seropositive for *T. gondii* (Danner et al. 2007). On the island of O'ahu, 0.5% of feral cats tested were shedding oocysts and 20% had antibodies for *T. gondii* (Wallace 1971). Further, the transmission pathways by which native Hawaiian wildlife, both terrestrial and marine, become infected with *T. gondii* remain unknown. As such, further research is warranted to fully appreciate the transmission dynamics and full impact of this highly-infectious, potentially deadly, protozoan parasite within the fragile terrestrial and marine ecosystems of Hawai'i. Therefore, our objective was to investigate environmental contamination with *T. gondii* in soil and cat fecal samples from cat colonies located on a densely populated urban university campus and in cat fecal samples from an uninhabited coastal reserve.

Materials and Methods

Site Description

We sampled cat colonies on the island of O'ahu at the University of Hawai'i at Mānoa (UHM) main campus in Honolulu, Hawai'i and feral cats from Ka'ena Point Natural Area Reserve (KPNAR). Both cat feces and environmental (soil) samples were collected for molecular detection of *T. gondii*.

The UHM is an urban campus that supports ~14 total cat colonies, of which we randomly selected four for sampling (Figure 1). These four sites varied in both the type of vegetation and ground cover as well as the number of cats observed. Sites ranged from 13 to 36 cats, on average, with all sites having areas of vegetation that the cats used for resting and shelter. Cat

numbers are based on observations between 6:00 pm and 7:00 pm on days with favorable weather as part of a regular monitoring program (Davis and Lepczyk 2010).

Kaʻena Point NAR (KPNAR) was used as a natural area in comparison to the urban UHM campus. This natural area reserve is approximately 0.24 km^2 and is located at the north-western end of the Waiʻanae mountain range on Oʻahu Island, Hawaiʻi. The natural area is a breeding location for Laysan albatross (*Phoebastria immutabilis*) and wedge-tailed shearwaters (*Puffinus pacificus*), and is frequented by the critically endangered Hawaiian monk seal (*Monachus schauinslandi*). All non-native animals were removed from the area within a predator-proof fence in 2012 as part of the establishment of a predator free Natural Area Reserve site (Young et al. 2013), and a total of nine cats were removed and frozen for later analysis (Lohr et al. 2013b).

Sample Collection and Processing

Soil for much of the university campus, including the sample sites, is an inceptisol from the Makiki stony clay loam series with 0% to 3% slope (Natural Resource Conservation Service 2009). However, because most of the grounds have been modified through construction and landscaping, the soil at the four sites included in this study is highly mixed. A total of 30 soil samples were randomly collected from a 1 m × 1 m grid sampling scheme at each of four colonies from the main campus, yielding a grand total of 120 soil samples. We collected soil up to approximately 10 cm depth using a surface soil probe. Collected soil also included rocks (>2 mm diameter), plant material, and surface organic matter because *T. gondii* oocysts could potentially become part of any aggregate containing such material. Oocyst recovery was attempted from soil samples using a sucrose floatation method based on methodology described by Lélu et al. (2011). Specifically, ~20 g of soil was added to a 50 mL cone centrifuge tube

along with 20 ml of deionized water and then vortexed for 2 min to homogenize and disperse water-soluble aggregates. Samples were then centrifuged at $1500 \times G$ for 10 min, followed by discarding of the supernatant, and the addition of 20 ml of sucrose solution with a specific gravity of 1.15 (*T. gondii* oocysts have a density of 1.10 SG; Dubey et al. 1970). The sample was then vortexed for 2 min to ensure thorough mixing and centrifuged for 10 min ($1500 \times G$). The supernatant was poured equally into two 50 mL cone centrifuge tubes and 35 mL of deionized water was added, bringing the density of the solution to 1.05 SG. Samples were centrifuged for 10 min at $1500 \times G$ to pellet oocysts and the supernatant from each centrifuge tube was poured equally into another 50 mL cone centrifuge tube (four cone centrifuge tubes per sample) along with 20 ml of deionized water to bring the solution density to near 1.00 SG. Samples were shaken and centrifuged for 10 min at $1500 \times G$. Supernatant from each tube was carefully discarded, ensuring that the pellet remained. The pellet from each 50 mL cone centrifuge tube was pooled and 200 μL of the pellet was reserved in a 2.0 mL microcentrifuge tube and stored at -20 ˚C for DNA extraction.

Defecation sites at each colony on the university campus were targeted and 15 fecal samples were collected from each colony, for a total of 60 samples. Fecal samples were both wet and dry, though attempts were made to collect as many wet samples as possible before collecting dry samples. All fecal samples were collected on the same day and double bagged in zip-top bags. Approximately 200 mg of feces was placed into 2 mL microcentrifuge tubes for DNA extraction and stored at -20 ˚C until use.

Cat carcasses from KPNAR were thawed in October 2012 for gut content analysis (Lohr et al. 2013b) and removal of feces. Fecal samples were collected from the lower large intestine of nine cats culled as part of a multi-species eradication of introduced mammals (Young et al. 2013). We removed all feces from each cat, each of which had >200 mg, the minimum required

for analysis. Fecal samples collected from KPNAR were stored at 4 ˚C until approximately 200 mg of each sample was placed into 2 mL microcentrifuge tubes and stored at -20 ˚C until use for DNA extraction.

Molecular Analysis

DNA (from pelleted soil and feces) was extracted using the commercial kit QIAGEN QIAamp® DNA Stool Mini Kit (QIAGEN, USA) according to manufacturer's instruction (QIAGEN 2010). DNA elute of each sample was stored at -20 ˚C. *T. gondii* specific detection was conducted by polymerase chain reaction (PCR) targeting the GRA6 gene using the primers reported by Fazaeli et al. (2000). The amplification mixture contained 3.0 µL 5x GoTAQ buffer (Promega), 1.5 µL 1% bovine serum albumin, 1.2 µL $MgCl_2$ (25mM), 0.3 µM of each primer, 2.4 µL dNTP (0.2mM each dNTP), 0.2 µL (1 unit) TAQ polymerase (Promega), and 1 µL DNA template for a reaction mixture volume of 15 µL. PCR amplification was performed for 30 cycles (MJ Research DNA Engine PTC-200). Denaturation occurred at 95 ˚C for 2 min and 93 ˚C for one minute, annealing occurred at 60.8 ˚C for 30 sec, and elongation occurred at 72 ˚C for one minute. PCR products were examined by electrophoresis using 1.5% agarose gel stained with ethidium bromide and visualized under UV. Samples were compared using *T. gondii* RH strain (Advanced Biotechnologies, Inc., USA) as a positive control.

Extracted DNA from fecal samples that amplified using the GRA6 primers were further analyzed to verify the amplicon was *T. gondii* DNA. Specifically, previously published pan-apicomplexan primers that amplify across the internal transcribed space (ITS-1) anchored between the 18S and 5.8S small subunit (SSU) rDNA gene arrays were used (Gibson et al. 2011) to amplify samples that were positive using the GRA6 primers. The PCR setup and amplification (i.e. thermocycler settings) were done according to Gibson et al. (2011). *T. gondii*

7

DNA was used as a positive control and molecular grade water as the negative control. PCR

products were visualized using 1% agarose gel stained with GelRed Nucleic Acid Gel Stain

(Biotium Inc, Fremont, CA, USA). Amplicons were cleaned using ExoSAP-IT (Affymetrix,

ThermoFisher Scientific, USA) according to the manufacturer's protocol. Sequencing (Sanger)

of all positive samples that amplified at the ITS-1 locus was performed in the Genetics

Laboratory of the Washington Department of Fish and Wildlife. Sequence traces were

visualized, manually quality checked, and trimmed accordingly using FinchTV (Geospiza,

PerkinElmer, Akron, Ohio). Sequence identity was determined via a nucleotide BLAST search

in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

A total of 60 fecal samples and 120 environmental samples were collected from UHM

and nine fecal samples were collected from directly feral cats culled at KPNAR. Of these, 5

samples from UHM (8.3%, 95% CI 2.7-18.4) and 2 from KPNAR (22.2%, 95% CI 2.8-60)

amplified using the GRA6 primers (Table 1). Both locations at UHM where *T. gondii* was

isolated also had kittens present. *T. gondii* DNA was not amplified from any of the environmental

soil samples. Approximate amplicon size using the GRA6 primers was 650 base pairs. All seven

samples that were PCR positive with GRA6 primers also amplified using the ITS-1 primers.

Sequences of the ITS-1 region of five of these samples confirmed the presence of *T. gondii*

DNA. One sample from UHM was identified as *Cytauxzoon felis*, a protozoal parasite common

in domestic cats. One sample that amplified at both the GRA6 and ITS-1 regions did not provide

a clean sequence. Table 1 provides information on amplicon size and sequence results.

Discussion

8

Protozoal DNA was found in 8.3% (5/60) of fecal samples tested from cat colonies at UHM and in 22.2% (2/9) of fecal samples from feral cats culled from KPNAR. Of these, three from UHM and two from KPNAR were confirmed to be *T. gondii*. One from UHM was confirmed to be *C. felis*, a protozoal parasite that is only known to infect felids and does not pose a threat to humans or other wildlife species. The occurrence of *T. gondii* DNA in scats from UHM was thus 5% (3/60, 95% CI 1-13.9) at UHM and 22% (2/9) at KPNAR. Notably these percentages may be an underestimation of the prevalence of *T. gondii* since the initial PCR was done using primers for GRA6; there is only one copy of GRA6 in the genome of *T. gondii* (Labruyere et al. 1999). Follow-up PCR was done using ITS-1 primers, of which there is 110 copies in the *T. gondii* genome (Guay et al., 1993). Given the relative copy number of these two markers, screening all samples with ITS-1 primers may provide more positive results.

Though five samples from UHM amplified at both the GRA6 and ITS-1 regions and had amplicons of appropriate size for *T. gondii* we were unable to get clean sequences for these samples across the entire amplicon (~550bp). In general, sequence quality was poor in these samples, which is to be expected when dealing with scat samples due to inhibitors present in the sample before and after DNA extraction. Regardless, clean sequence traces for four samples from UHM and two from KPNAR were provided of approximately 150-300 base pairs and were consistent with *T. gondii* by BLAST search.

The higher occurrence of *T. gondii* DNA at the KPNAR was not expected and may be a result of the small sample size and/or because we used feces harvested directly from the intestine which . However, the presence of *T. gondii* DNA in feral cat feces indicates these cats may have been infected with *T. gondii* and likely experiencing the acute active phase of the disease in which oocysts are shed into the environment. However, recent work has shown that sensitive molecular methods (i.e. quantative PCR) may detect *T. gondii* DNA from tachyzoites (encysted

9

parasites) in prey ingested by cats and DNA detection does not necessarily imply oocyst shedding by the feline host (Poulle et al. 2016). Given we were using non-nested end-point PCR, this is unlikely, but nonetheless must be noted as a potential. Further work using quantitative PCR could be done to determine the number of oocysts being shed and provide better information on the specific concentration of *T. gondii* being shed by feral cats. Another explanation for the higher prevalence of *T. gondii* detected in the cats sampled from KPNAR is that cats at UHM obtain a large portion of their food from humans, and thus are eating canned or dry diets that have been conventionally prepared. In comparison, cats at KPNAR are a more rural population with little to no artificial diet provided by humans (Lohr et al. 2013b).

Analyses of fecal samples from free-ranging domestic cats have found prevalences between 0.11 and 9% (Pena et al. 2006; Salant et al. 2007; Schares et al. 2008). Our finding of 5% in cats in an urban setting around the university is consistent with these other studies. However, as previously mentioned, the percent positive at KPNAR (22.2%) is higher than documented at other locations, possibly due to the small sample size. Regardless, the finding of *T. gondii* at a natural area site of high conservation value highlights the risk of transmission to native, endemic, and endangered wildlife in such locations, as has recently been noted in the Hawaiian Goose on Kauai (Work et al. 2016).

Though *T. gondii* has been found in soil using molecular detection at prevalences between 5.4-17.8% in temperate environments (Afonso et al. 2008; Du et al. 2012; Lass et al. 2009), we did not detect *T. gondii* in this soil samples collected at UHM in this study. This lack of detection was in contrast to our expectations, but may be due to several factors. First, we employed a regular grid around a cat colony instead of sampling directly beneath defecation sites, which may have reduced the probability of oocyst detection as Afonso et al. (2008) demonstrated that positive *T. gondii* samples only occurred at cat defecation sites. Second, only

1% of cats are estimated to be shedding Toxoplasma oocysts at any point in time (Dubey 2010), thus, limiting the chances of detection in soil, even if under a defecation spot. Third, feces containing oocysts may simply not have been incorporated into the soil due to the rate at which degradation and potential removal by overland flow occur. Finally, the mass of our sample used in analysis was lower than has been suggested in other studies (Dumetre and Darde 2003). Thus, a larger sample size with larger amounts of soil that are sampled at defecation sites may increase the likelihood of detecting oocysts at our study sites in the future.

Our cat-sampling framework at UHM was not designed to test the cats themselves (i.e. direct sampling from euthanized/culled cats). Thus, no attempts were made to differentiate fecal samples from individual cats in each colony. However, samples from each site were collected on the same day and with the same collection criteria; attempts were made to collect fresh, non-desiccated samples in order to reduce both collecting multiple fecal samples from the same individual and also the likelihood of DNA degradation over time. Notably, both sites at UHM that were positive for *T. gondii* had kittens present.

Our results clearly demonstrate that *T. gondii* is present in cats, and possibly their prey (Poulle et al. 2016) at UHM. The occurrence of *T. gondii* DNA indicates the parasite in some form (either tachyzoite or oocyst) is present in feral cats, and thus the environment. As a zoonotic parasite, *T. gondii* in Hawaiʻi, has the potential to pose a health risk to the staff, faculty, students, and cat caregivers at UHM if they come into direct contact with infected feces or contaminated soil. *Toxoplasma gondii* also has the potential to enter the nearby aquatic and marine environments due to the proximity of the university to a stream, possibly infecting ocean recreation users, marine mammals, and/or birds (Conrad et al. 2005; Miller et al. 2002; VanWormer et al., 2016). Studies have indicated that oocysts entering the ocean through freshwater runoff may be ingested by filter-feeding invertebrates and fish (Arkush et al. 2003;

Massie et al. 2010; Miller et al. 2008), indicating there is a potential for such a relationship in Hawai'i. The most prevalent filter-feeding and detritivore invertebrates in Hawai'i include coral, and various species of molluscs, crustaceans, and polychaete worms (DeFelice et al. 1998). Although these invertebrates are not usual prey items for marine birds and mammals that occur in Hawai'i, they are prey for fish and cephalopods that marine birds and mammals prey upon. The people of Hawai'i also consume many coastal fish and invertebrate species, including *'opihi* (Family Patellidae), a filter-feeding limpet that is often eaten raw, which could pose a risk given that numerous other filter feeders around the world have been demonstrated to be infected with *T. gondii* (Aksoy et al. 2014; Esmerini et al. 2010; Putignani et al. 2011; Zhang et al. 2014). Given these potential infection pathways, research on how *T. gondii* oocysts move and are transmitted in tropical marine environments is greatly needed.

In the past, oocysts entering the environment at KPNAR could have had significant negative effects on the wildlife that occurred there. Though speculation, both Laysan albatross and wedge-tailed shearwater adults and chicks may have ingested cat feces, aerosolized particles, or soil containing oocysts when excavating burrows or while engaged in normal activities and become infected. Hawaiian monk seals could have become infected from oocysts in freshwater runoff around the near-shore area. Oocysts may also have had the potential to accumulate in the prey of the marine birds and mammals foraging in adjacent coastal waters (Conrad et al. 2005). Since the removal of all cats from within the predator-proof fence, it can be assumed that the risk of *T. gondii* exposure has decreased, though it is unknown what kind of risk might remain due to the lack of knowledge about the longevity of oocysts in coastal environments.

In conclusion, though we found *T. gondii* associated with cat colonies in both an urban and a natural area environment on Oahu, managing the presence of this potentially deadly protozoan parasite in the environment of Hawaii will prove difficult. While removal of cats may

stop *T. gondii* oocyst deposition into terrestrial environments, the oocysts can persist for years. Further, non-native, feral cat management on the Hawaiian Islands has become an extremely contentious issue (Lepczyk et al. 2011, Peterson et al. 2012). Given the high densities of cat populations in Hawaii's terrestrial ecosystems, coupled with the large number of endangered, native species, there is a critical need for improved understanding of not only the occurrence and prevalence of *T. gondii* in both the definitive felid host and also the native, naïve intermediate hosts, but also how this parasite may be transmitted across species and the subsequent impact on population health, especially of naïve Hawaiian species in this fragile island ecosystem.

Literature Cited

Afonso E, Lemoine M, Poulle ML, Ravat MC, Romand S, Thulliez P, Villena I, Aubert D, Rabilloud M, Riche B, Gilot-Fromont E. 2008. Spatial distribution of soil contamination by *Toxoplasma gondii* in relation to cat defecation behavior in an urban area. *Int J Parasitol* 38:1017-1023.

Ajzenberg D, Bañuls AL, Su C, Dumètre A, Demar M, Carma B, Dardé ML. 2004. Genetic diversity, clonality and sexuality in *Toxoplasma gondii*. *Int J Parasitol* 34:1185-1196.

Amarasekare P. 1994. Ecology of introduced small mammals on Western Mauna Kea, Hawaii. *J Mammal* 75:24-38.

Arkush KD, Miller MA, Leutenegger CM, Gardner IA, Packham AE, Heckeroth AR, Tenter AM, Barr BC, Conrad PA. 2003. Molecular and bioassay-based detection of *Toxoplasma gondii* oocyst uptake by mussels (*Mytilus galloprovincialis*). *Int J Parasitol* 33:1087-1097.

Aksoy U, Marangi M, Papini R, Ozkoc S, Bayram Delibas S, Giangaspero A. 2014. Detection of *Toxoplasma gondii* and *Cyclospora cayetanensis* in *Mytilus galloprovincialis* from Izmir Province coast (Turkey) by real time PCR/high-resolution melting analysis (HRM). *Food Microbiology* 44: 128-135.

Barbieri M, Kashinsky L, Rotstein D, Colegrove K, Haman K, Magargal S, Sweeny A, Kaufman A, Grigg M, Littnan C. 2016. Protozoal-related mortalities in endangered Hawaiian monk seals *Neomonachus schauinslandi*. *Dis Aquatic Organisms* 121: 85-95.

Berger AJ. 1981. Hawaiian Birdlife, 2nd ed. University of Hawai'i Press, Honolulu, Hawai'i, 275 pp.

Burridge MJ, Bigler WJ, Forrester DJ, Hennemann JM. 1979. Serologic survey for *Toxoplasma gondii* in wild animals of Florida. *J Am Vet Med Assoc* 175:964-967.

Conrad PA, Miller MA, Kreuder C, James ER, Mazet J, Dabritz H, Jessup DA, Gulland F, Grigg ME. 2005. Transmission of *Toxoplasma*: Clues from the study of sea otters as sentinels of *Toxoplasma gondii* flow into the marine environment. *Int J Parasitol* 35:1155-1168.

Danner RM, Goltz DM, Hess SC, Banko PC. 2007. Evidence of feline immunodeficiency virus, feline leukemia virus, and *Toxoplasma gondii* in feral cats on Mauna Kea, Hawaii. *J Wildl Dis* 43:315-318.

Davis AA, Lepczyk CA. 2010. Feral cat population surveys and management options for the University of Hawai'i at Mānoa. In: *Proceedings of the Twenty-fourth International Vertebrate Pest Conference*, 22-25 February, R.M. Timm and K.A. Fagerstone, University of California, Davis, pp. 87-71.

DeFelice RC, Coles SL, Muir D, Eldredge LG. 1998. *Investigation of the marine communities of Midway Harbor and adjacent lagoon, Midway Atoll, Northwestern Hawaiian Islands,* http:// http://hbs.bishopmuseum.org/pdf/defelice-etal98.pdf Accessed December 2013.

Du F, Feng HL, Nie H, Tu P, Zhang QL, Hu M, Zhou YQ, Zhao JL. 2012. Survey on the contamination of *Toxoplasma gondii* oocysts in the soil of public parks of Wuhan, China. *Vet Parasitol* 184:141-146.

Dubey JP. 2001. Oocyst Shedding by Cats Fed Isolated Bradyzoites and Comparison of Infectivity of Bradyzoites of the VEG Strain *Toxoplasma gondii* to Cats and Mice. *J Parasitol* 87:215-219.

Dubey JP. 2010. Toxoplasmosis of Animals and Humans, 2nd ed. CRC Press, Boca Raton, Florida, 313 pp.

Dubey JP, Miller NL, Frenkel JK. 1970. The *Toxoplasma gondii* oocyst from cat feces. *J Exp Med* 132:636-662.

Dubey JP, Murrell KD, Fayer R, Schad GA. 1986: Distribution of *Toxoplasma gondii* tissue cysts in commercial cuts of pork. *J Am Vet Med Assoc* 188:1035–37.

Dubey JP, Lindsay DS, Speer CA. 1998. Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. *Clin Microbiol Rev* 11(2):267-299.

Dubey JP, Jones JL. 2008. *Toxoplasma gondii* infection in humans and animals in the United States. *Int J Parasitol* 38:1257-1278.

Duffy DC, Capece P. 2012. Biology and Impacts of Pacific Island Invasive Species. 7. The Domestic Cat (*Felis catus*). *Pac Sci* 66:173-212.

Dumètre A, Dardé ML. 2003. How to detect *Toxoplasma gondii* oocysts in environmental samples? FEMS Microbiology Reviews 27:651-661.

Esmerini PO, Gennari SM, Pena HFJ. 2010. Analysis of marine bivalve shellfish from the fish market in Santos city, São Paulo state, Brazil, for *Toxoplasma gondii*. *Vet Parasitol* 170: 8-13.

Fazaeli A, Carter PE, Darde ML, Pennington TH. 2000. Molecular typing of *Toxoplasma gondii* strains by GRA6 gene sequence analysis. *Int J Parasitol* 30:637-642.

Flegr J. 2007. Effects of *Toxoplasma* on human behavior. *Schizophr Bull* 33:757-760.

Flegr J, Havlicek J. 1999. Changes in the personality profile of young women with latent toxoplasmosis. *Folia Parasitol* 46:22-28.

Flegr J, Havlicek J, Kodym P, Maly M, Smahel Z. 2002. Increased risk of traffic accidents in subjects with latent toxoplasmosis: a retrospective case-control study. *BMC Infect Dis* 2:11 pp.

Gibson AK, Raverty S, Lambourn DM, Huggins J, Magargal SL, Grigg ME. 2011. Polyparasitism is associated with increased disease severity in *Toxoplasma gondii*-infected marine sentinel species. *PLoS Negl Trop Dis* 5: e1142.

Guay J, Dubois D, Morency M, Gagnon S, Mercier J, Levesque R. 1993. Detection of the pathogenic parasite Toxoplasma gondii by specific amplification of ribosomal sequences using comultiplex polymerase chain reaction. J Clin Microbio 31:203-207.

Hawai‘i Department of Health. 2000. Toxoplasmosis in Hawai´i. Environmental Health Administration, 58 pp.

Hollings T, Jones M, Mooney N, McCallum H. 2013. Wildlife disease ecology in changing landscapes: Mesopredator release and toxoplasmosis. *Int J Parasitol* 2:110-118.

Honnold SP, Braun R, Scott DP, Sreekumar C, Dubey JP. 2005. Toxoplasmosis in a Hawaiian monk seal (*Monachus schauinslandi*). *J Parasitol* 91:695-697.

Jones JL, Kruszon-Moran D, Sanders-Lewis K, Wilson M. 2007. *Toxoplasma gondii* Infection in the United States, 1999-2004, Decline from the Prior Decade. *Am J Trop Med Hyg* 77:405-410.

Labruyere E, Lingnau M, Mercier C, Sibley DL. 1999. Differential membrane targeting of the secretory proteins GRA4 and GRA6 within the parasitophorous vacuole formed by *Toxoplasma gondii*. *Mol Biochem Parasitol*. 102:311-324.

Lamberton PHL, Donnelly CA, Webster JP. 2008. Specificity of the *Toxoplasma gondii*-altered behaviour to definitive versus non-definitive host predation risk. *Parasitol* 135:1143-1150.

Lass A, Pletkiewicz H, Modzelewska E, Dumètre A, Szostakowska B, Myjak P. 2009. Detection of *Toxoplasma gondii* oocysts in environmental soil samples using molecular methods. *Eur J Clin Microbiol Infect Dis* 28:599-605.

Lélu M, Gilot-Fromont E, Aubert D, Richaume A, Afonso E, Dupuis E, Gotteland C, Marnef F,

Poulle ML, Dumètre A, Thulliez P, Dardé ML, Villena I. 2011. Development of a sensitive method for *Toxoplasma gondii* oocysts extraction in soil. *Vet Parasitol* 183:59-67.

Lepczyk, C.A., Y. van Heezik, and R.J. Cooper. 2011. An issue with all-too-human dimensions. The Wildlife Professional, Spring 2011 pages 68-70.

Lindsay DS, Dubey JP. 2009. Long-Term Survival of *Toxoplasma gondii* Sporulated Oocysts in Seawater. *J Parasitol* 95:1019-1020.

Literák I, Hejlíček K, Nezval J, Folk Č. 1992. Incidence of *Toxoplasma gondii* in populations of wild birds in Czech Republic. *Avian Pathol* 21:659-665.

Lohr CA, Cox LJ, Lepczyk CA. 2013a. Costs and Benefits of Trap-Neuter-Release and Euthanasia for Removal of Urban Cats in Oahu, Hawaii. *Conserv Biol* 27:64-73.

Lohr, M., Young, L.C., VanderWerf, E.A., Miller, C.J., Leong, H. 2013b. Dietary Analysis of Free-ranging Cats at Kaʻena Point, Hawaiʻi. *ʻElepaio* 73(3):1-3.

Mason RW, Hartley WJ, Dubey JP. 1991. Lethal Toxoplasmosis in a Little Penguin (*Eudyptula minor*) from Tasmania. *J Parasitol* 77:328.

Massie GN, Ware MW, Villegas EN, Black MW. 2010. Uptake and transmission of *Toxoplasma gondii* oocysts by migratory, filter-feeding fish. *Vet Parasitol* 169:296-303.

Migaki G, Sawa TR, Dubey JP. 1990. Fatal Disseminated Toxoplasmosis in a Spinner Dolphin (*Stenella longirostris*). *Vet Pathol* 27:463-464.

Miller MA, Miller WA, Conrad PA, James ER, Melli AC, Leutenegger CM, Dabritz HA, Packham AE, Paradies D, Harris M, Ames J, Jessup DA, Worcester K, Grigg ME. 2008. Type X *Toxoplasma gondii* in a wild mussel and terrestrial carnivores from coastal California: New linkages between terrestrial mammals, runoff and toxoplasmosis of sea otters. *Int J Parasitol* 38:1319-1328.

Miller MA, Gardner IA, Kreuder C, Paradies DM, Worcester KR, Jessup DA, Dodd E, Harris, MD, Ames JA, Packham AE, Conrad PA. 2002. Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). *Intl. J. Parasitol.* 32: 997-1006.

Natural Resource Conservation Service. 2009. Web Soil Survey. United States Department of Agriculture (USDA); [updated 2009 Nov 11; accessed 2011 Apr 5]. Available from: http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm.

Novotna M, Hanusova J, Klose J, Preiss M, Havlicek J, Roubalva K, Flegr J. 2005. Probable neuroimmunological link between *Toxoplasma* and cytomegalovirus infections and personality changes in the human host. *BMC Infect Dis* 5:54 pp.

Pena HFJ, Soares RM, Amaku M, Dubey JP, Gennari SM. 2006. *Toxoplasma gondii* infection in cats from São Paulo state, Brazil: Seroprevelence, oocyst shedding, isolation in mice, and biologic and molecular characterization. *Res Vet Sci* 81:58-67.

Pederson MG, Mortensen PB, Norgaard-Pederson B, Postolache TT. 2012. *Toxoplasma gondii* Infection and Self-directed Violence in Mothers. *Arch Gen Psychiatry* 69(11):1123-1130.

Peterson, MN, Hartis B, Rodriguez S, Green M, Lepczyk CA. 2012. Opinions from the front lines of cat colony management conflict. *PLoS ONE* 7(9):e44616.

Poulle ML, Forin-Wiart MA, Josse-Dupuis É, Villena I, Aubert D. 2016. Detection of *Toxoplasma gondii* DNA by qPCR in the feces of a cat that recently ingested infected prey does not necessarily imply oocyst shedding. *Parasite* 23:29.

Prandovszky E, Gaskell E, Martin H, Dubey JP, Webster JP, McConkey GA. 2011. The neurotrophic parasite *Toxoplasma gondii* increases dopamine metabolism. *PLoS ONE* 6:9 pp (e23866).

Putignani L, Mancinelli L, Del Chierico F, Menichella D, Adlerstein D, Angelici MC, Marangi

M, Berrilli F, Caffara M, Frangipane di Regalbono DA, Giangaspero A. 2011. Investigation of *Toxoplasma gondii* presence in farmed shellfish by nested-PCR and real-time PCR fluorescent amplicon generation assay (FLAG). *Exp Parasitol* 127: 409-417.

QIAGEN. 2010. QIAamp DNA Stool Handbook. 2nd ed. Hilden, Germany, 44 pp.

Ratcliffe HL, Worth CB. 1951. Toxoplasmosis of captive wild birds and mammals. *Am J Pathol* 27:655-667.

Salant H, Markovics A, Spira DT, Hamburger J. 2007. The development of a molecular approach for coprodiagnosis of *Toxoplasma gondii*. *Vet Parasitol* 146:214-220.

Schares G, Globakar Vrhovec M, Pantchev N, Herrmann DC, Conraths FJ. 2008. Occurrence of *Toxoplasma gondii* and *Hammondia hammondi* oocysts in the faeces of cats from Germany and other European countries. *Vet Parasitol* 152:34-45.

Shapiro K, Miller M, Mazet J. 2012. Temporal association between land-based runoff events and California Sea Otters (*Enhydra lutris nereis*) protozoal mortalities. *J Wildl Dis* 48: 392-404.

Shapiro K, VanWormer E, Aguilar B, Conrad P. 2014. Surveillance for *Toxoplasma gondii* in California mussels (*Mytilus californianus*) reveals transmission of atypical genotypes from land to sea. *Environ Microbiol* 17:4177–4188.

Tanyüksel M, Uzun Ö, Araz E, Koru Ö, Babür C. 2010. Possible role of toxoplasmosis in patients with first-episode schizophrenia. *Turk J Med Sci* 40(3):399-404.

Tenter AM, Heckeroth AR, Weiss LM. 2000. *Toxoplasma gondii*: from animals to humans. *Int J Parasitol* 30:1217-1258.

Teutsch SM, Juranek DD, Sulzer A, Dubey JP, Sikes KS. 1979. Epidemic toxoplasmosis associated with infected cats. *N Engl J Med* 300:695-699.

VanWormer E, Carpenter T, Singh P, Shapiro K, Wallender W, Conrad P, Largier J, Maneta M, Mazet J. 2016. Coastal development and precipitation drive pathogen flow from land to sea: evidence from a *Toxoplasma gondii* and felid host system. *Scientific Reports* 6.

VanWormer E, Fritz H, Shapiro K, Mazet J, Conrad P. 2013. Molecules to modeling: *Toxoplasma gondii* oocysts at the human-animal-environment interface. *Comp Imm Micro Infect Dis* 36: 217-231.

VanWormer, E, Conrad PA, Miller MA, Melli AC, Carpenter TE, Mazet JA. 2013. *Toxoplasma gondii*, source to sea: higher contribution of domestic felids to terrestrial parasite loading despite lower infection prevalence. *EcoHealth* 10: 277-289.

Wallace GD. 1971. Isolation of *Toxoplasma gondii* from the Feces of Naturally Infected Cats. *J Infect Disease* 124:227-228.

Webster JP, McConkey GA. 2010. *Toxoplasma gondii*-altered host behavior: clues as to mechanism of action. *Folia Parasitol* 57:95-104.

Wendte J, Miller M, Lambourn D, Magargal S, Jessup D, Grigg M. 2010. Self-mating in the definitive host potentiates clonal outbreaks of the Apicomplexan parasites *Sarcocystis neurona* and *Toxoplasma gondii*. *PLoS Genetics* 6: e1001261.

Wendte JM, Gibson AK, Grigg ME. 2011. Population genetics of *Toxoplasma gondii*: New perspectives from parasite genotypes in wildlife. *Vet Parasit* 182: 96-111.

Work TM, Massey JG, Rideout BA, Gardiner CH, Ledig DB, Kwok OCH, Dubey JP. 2000. Fatal toxoplasmosis in free-ranging endangered 'alalā from Hawai´i. *J Wildl Dis* 36:205-212.

Work TM, Massey JG, Lindsay DS, Dubey JP. 2002. Toxoplasmosis in three species of native and introduced Hawaiian birds. *J Parasitol* 88:1040-1042.

Work TM, Verma SK, Su C, Medeiros J, Kaiakapu T, Kwok OC, Dubey JP. 2016. *Toxoplasma gondii* antibody prevalence and two new genotypes of the parasite in endangered

Hawaiian gees (nene: *Branta Sandvicensis*). *J Wildl Dis* 52:253-257.

Yagmur F, Yazar S, Temel HO, Cavusoglu M. 2010. May *Toxoplasma gondii* increase suicide attempt-preliminary results in Turkish subjects? *Forensic Sci Int* 199:15-17.

Young LC, VanderWerf EA, Lohr MT, Miller CJ, Titmus AJ, Peters D, Wilson L. 2013. Multi-species predator eradication within a pest-proof fence at Ka'ena Point, Hawai`i. *Biol Inv* 15: 2627–2638.

Zhang M, Yang Z, Wang S, Tao L, Xu L, Yan R, Song X, Li X. 2014. Detection of *Toxoplasma gondii* in shellfish and fish in parts of China. *Vet Parasitol* 200: 85-89.

Table 1. Amplicon size from GRA6 and ITS-1 PCRs and sequence results of ITS-1 amplicons

from fecal samples collected from cats (*Felis catus*) at two locations in Hawai'i.

Sample ID	Location[1]	GRA6[2]	ITS-1[3]	ITS-1 Sequence	Total Sequence length[4]
UHGH-F5	UHM	650	400	*Cytauxzoon felis*	413
UHKS-F7	UHM	650	550	*Toxoplasma gondii*	308
UHKS-F15	UHM	650	550	NA[5]	
UHGH-3	UHM	650	550	*Toxoplasma gondii*	304
UHKS-F1	UHM	650	550	*Toxoplasma gondii*	293
KPGH-F4	KPNAR	650	550	*Toxoplasma gondii*	233
KP-5	KPNAR	650	550	*Toxoplasma gondii*	307

[1] UHM = University of Hawai'i at Mānoa; KPNAR = Ka'ena Point Natural Area Reserve

[2, 3] Estimated amplicon size based on running amplified DNA on 1.5% agarose gel stained with

ethidium bromide (GRA6) and 1% agarose gel stained with gel red (ITS-1). Both were

visualized under UV light.

[4] Total sequence length that was BLASTed to determine the identity of the amplicon. Sequences

were visualized and manually cleaned using FinchTV.

[5] NA = not applicable. Though this sample provided amplicons at both the GRA6 and ITS-1 we

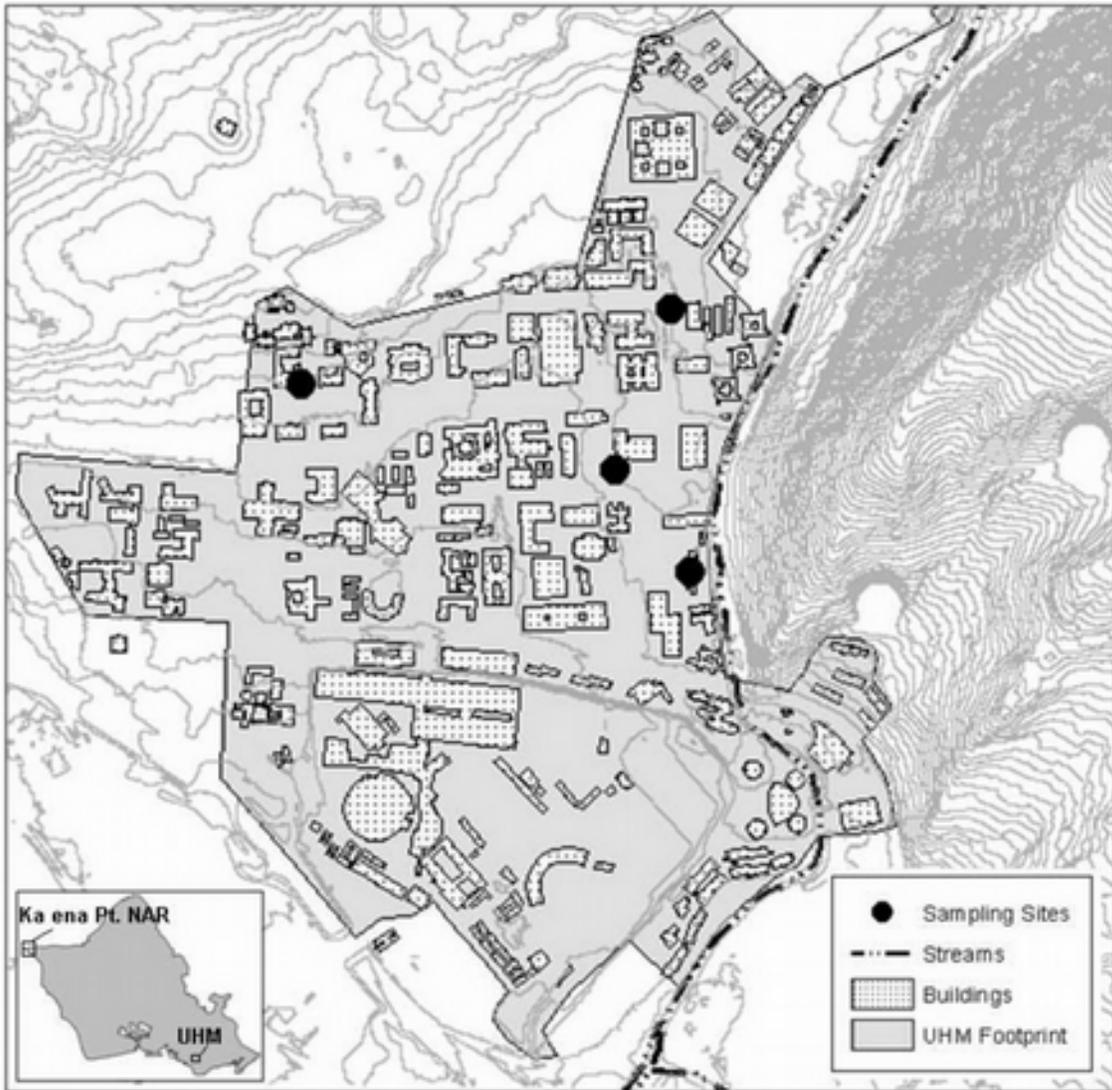could not obtain a clean sequence and thus cannot identify the amplified DNA.

Figure 1. Map of the University of Hawai'i at Mānoa with sampling locations and KPNAR