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***Toxoplasma gondii* Detection in Urban Hawai'i**

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ABSTRACT: Feral cats have flourished in urban areas of Hawai'i due to the state's favorable climate and people's positive perception of cats. However, the presence of large numbers of feral cats has raised concern both in terms of predation of native species and as vectors of disease. One disease, in particular, that has aroused a great deal of attention is toxoplasmosis, caused by the *Toxoplasma gondii* parasite. Cats are the definitive host of *T. gondii* and concerns arise regarding transmission to humans due to the relationships people have with cats. Another concern is the fact that the parasite has infected endemic and endangered species found in the state. *Toxoplasma gondii* oocysts are shed in cat excrement and can persist in soil between 1 and 4 years. The presence of *T. gondii* at cat colony sites could be an important factor when making decisions for the management of feral cats in the state. We intend to test soil samples taken from cat colony sites at the University of Hawai'i at Mānoa for *T. gondii* oocysts using molecular identification methods. Cat colony sites are defined by feeding stations maintained by cat colony caretakers. Given that cats are definitive hosts of *T. gondii*, we hypothesize that the presence of toxoplasmosis in soil is correlated to cat colony locations. Because most cats within a colony remain in close proximity to their feeding location, we predict that toxoplasmosis is spatially contained within tight proximity to cat colonies. If *T. gondii* oocysts are present in soil at the University of Hawai'i at Mānoa, then cat colonies may cause potential health hazards for landscaping personnel, students, staff, and visitors.

KEY WORDS: cats, colonies, disease, *Felis catus*, feral cat, Hawaii, parasite, polymerase chain reaction (PCR), soil aggregate, *Toxoplasma gondii*, toxoplasmosis

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INTRODUCTION

Toxoplasma gondii is a tissue-cyst forming coccidian protozoan that causes the toxoplasmosis infection in humans and other mammals and in birds (Beazley and Egerman 1998, Tenter et al. 2000). The life cycle of *T. gondii* consists of both sexual and asexual stages, with the first occurring in definitive hosts only, and the latter being the stage within intermediate hosts. The definitive hosts of *T. gondii* are members of the cat family Felidae and are the only hosts to accommodate both the asexual and sexual stages of the protozoan and shed the environmentally-resistant oocysts in their excrement (Figure 1) (Dubey and Jones 2008). The infectious stages in the parasite's life cycle are the tachyzoites (rapidly multiplying stage), bradyzoites (encysted stage within tissue cysts), and oocysts (the stage that is shed into the environment) (Dubey 2010). Tachyzoites penetrate a wide range of host cells and rapidly multiply asexually, while bradyzoites are found primarily in nervous and muscle tissues and less frequently in visceral tissues. *Toxoplasma gondii* oocysts are extremely resistant to most environmental conditions. The amount of time that oocysts can survive is dependent on favorable environmental conditions such as low light intensity, warm temperatures, high humidity, and medium (soil, water, or cat feces) (Dumetre and Darde 2003). For instance, oocysts are generally less likely to survive for long periods of time at colder temperatures, in dry climates, water, and/or direct sunlight. Though certain conditions are favorable for oocyst survival, studies have shown that oocysts have survived in fecal deposits in soil

Figure 1. Definitive (sexual) *Toxoplasma gondii* life cycle in cats.

for 18 months outdoors in temperatures ranging from -20 to 35°C and for 2 to 12 months outdoors in temperatures ranging from 15 to 30°C (Frenkel et al. 1975). Notably, one study found oocyst survival to be up to ~54 months in water at 4°C (Dubey 1998). Thus, *T. gondii* oocysts have been found to have a wide range of environmental conditions under which they can survive. Transmission usually occurs through ingesting cysts in raw or rare meat, ingesting feed or grass contaminated with felid feces, ingesting fruits and vegetables that have grown in soil with oocysts, inhaling oocysts that have been aerosolized when soil is highly disturbed, or drinking water contaminated with oocysts (Dubey and Jones 2008, Tenter et al. 2000).

Toxoplasmosis is of special concern because of the close relationship people have with domestic cats (*Felis catus*). Feral and stray cats living free outdoors tend to have a higher incidence of toxoplasmosis infection than pet cats, since they primarily hunt for their food (Dubey 2010, Dubey and Jones 2008). Cats contract toxoplasmosis by ingesting prey items that have been infected by *T. gondii* and generally develop immunity to the protozoan after initial infection, though transmission occurs congenitally as well. Transmission from cats to other species has been documented in a number of instances, including pets (other cats, rabbits, etc.), livestock, wildlife, marine species, and endangered species (Dubey 2010, Dubey and Jones 2008, Tenter et al. 2000). While symptoms vary by species, lethargy and delayed reaction time are common, while abortion and death sometimes occur.

In humans, toxoplasmosis can be contracted either congenitally or clinically, and most cases remain asymptomatic (latent). However, symptoms are severe in the fetus of pregnant women (transplacentally by tachyzoites) or in individuals with compromised immune systems either due to an immunodeficiency disease (such as AIDS) or undergoing immunotherapy (transplant patients, etc.) (Dubey and Jones 2008, Tenter et al. 2000). Infections in healthy individuals have been considered asymptomatic, though recent studies have found correlations between toxoplasmosis infection rates and behavioral differences, schizophrenia, higher risk for traffic accidents, and higher suicidal attempt rates (Flegr 2007, Flegr and Havlicek 1999, Flegr et al. 2002, Novotna et al. 2005, Webster and McConkey 2010, Yagmur et al. 2010). A study by Prandovszky et al. (2011) has also found that *T. gondii*-infected mice had a significant increase in dopamine metabolism, supporting evidence of behavioral changes related to toxoplasmosis infection. Although the likelihood of contracting toxoplasmosis has been decreasing in the U.S., there is concern among certain at-risk groups. In general, these groups include people with immunity disorders, pregnant women, landscaping personnel, and waste handling personnel who are in close proximity to cat colonies.

The state of Hawai'i is famous for many things, including its year-round warm, humid climate and an overabundance of invasive and endangered species. Feral and stray cats occur in Hawai'i's forests (Amarasekare 1994) and in multiple colonies found throughout urban areas in the islands. Because of the high number of feral cats, there is concern over toxoplasmosis transmission to Hawai'i's people, and native and endangered species. For example, a study on Mauna Kea, Hawai'i, a volcano with forests

consisting of many endangered bird species, 37.3% of cats were seropositive for toxoplasmosis (Danner et al. 2007). Toxoplasmosis infections have been identified in a number of Hawaiian animals, including three endangered species (the endemic Hawaiian crow or 'alala, *Corvus hawaiiensis*; the endemic nēnē goose, *Branta sandvicensis*; the endemic Hawaiian monk seal, *Monachus schauinslandi*; and one other native bird species, the red-footed booby, *Sula sula*) (Dubey and Jones 2008, Honnold et al. 2005, Ikeda 2000, Work et al. 2000, 2002). Within O'ahu's livestock, 48.5% of pigs tested positive for toxoplasmosis (Dubey and Jones 2008). Because Hawaiian monk seals are marine mammals, the infection infers *T. gondii* transmission through soil runoff, which may have further implications for ocean recreation users in Hawai'i and other wildlife. However, no studies have been conducted on environmental contamination of *T. gondii* oocysts in Hawai'i. Given the high cat densities in urban areas of Hawai'i, coupled with the large number of endangered animals, there is a critical need for understanding the prevalence of *T. gondii*.

Goals and Objectives

The University of Hawai'i at Mānoa in Honolulu, Hawai'i supports feral cat colonies that contain >200 cats (Davis and Lepczyk 2010). Due to the high number of cats present, there is a concern that the landscaping personnel may be coming in contact with *T. gondii* oocysts in the soil. Another concern is that oocysts in the soil may be spreading to endemic and endangered species on the island. Given these concerns, the over-arching purpose of the study is to detect *T. gondii* oocysts in soil samples at cat colony sites and aid management decisions for cat colonies on the University of Hawai'i at Mānoa. The goals of this study are to: 1) test if aggregations in the topsoil hold onto *T. gondii* oocysts, and 2) measure *T. gondii* presence in soil in urban areas on the island of O'ahu. Furthermore, we hypothesized that strong macroaggregates in Hawai'i's soils will protect oocysts and will require disruption, and low concentrations of the soil dispersant sodium hexametaphosphate will increase oocyst detection rate. Given that cats are definitive hosts of *T. gondii*, we also hypothesized that toxoplasmosis will be spatially contained within tight proximity to cat colonies, since most cats within a colony seem to remain in close proximity to their feeding location. Detection rates at each colony are also expected to be higher at large cat colonies and will be affected by management practices. To test these hypotheses, soil samples were collected from cat colonies using a target sampling scheme. Future work will include using a grid sampling scheme with randomly selected points to test for spatial distribution of oocysts at cat colony sites.

MATERIALS AND METHODS

To date, there is no single method to detect *T. gondii*, rather, there are a variety that differ based on sample mediums, including microscopy, antibody evaluation, bioassays, and molecular analysis. Molecular analyses have been found to be the most sensitive, least time consuming, and most economically feasible approach for detecting *T. gondii* oocysts (Garcia et al. 2008, Salant et al. 2010). Several previous studies have used different methods for DNA

extraction, depending upon such factors as the medium (water, soil, and food) in which *T. gondii* is found (Dumetre and Darde 2003). For soil, the procedures include homogenizing the samples using agitation and washing with dispersion solution, pre-filtration, sucrose floatation and, finally, destroying oocysts with freeze-thaw events (Afonso et al. 2008) or grinding with glass beads and proteinase K digestion (Salant et al. 2007). Several studies have also used commercial DNA extraction kits (Homan et al. 2000, Lass et al. 2009, Mancianti et al. 2010, Salant et al. 2007, 2010). Viable polymerase chain reaction (PCR) markers were identified by Su et al. (2006) for the different strains of *T. gondii*.

Soil sample collection took place within known cat colonies and may have disturbed cats, but we did not directly contact any cats. Samples were collected during the afternoon, when most cats were hiding and sleeping, to minimize disturbance.

Molecular analysis methods were used to detect oocysts from samples. For this study, methods were pooled from 4 studies on soil detection of *T. gondii* oocysts. Specifically, oocyst recovery from soil and DNA extraction was modified from Dumetre and Darde (2003), Lass et al. (2009), Mancianti et al. (2010), and Salant et al. (2010).

Site Description

The University of Hawai'i at Mānoa main campus is located in the Mānoa-Ala Wai watershed in Honolulu, Hawai'i. The campus supports ~20,000 students and is approximately ~129 ha, consisting of both permanent and demountable structures (UHM 2011). Soil at the University is an Inceptisol from the Makiki stony clay loam series with 0% to 3% slope (NRCS 2009) modified through construction and landscaping. Makiki series soils are typically well drained, very sticky and plastic, and consist mainly of gibbsite and goethite clay minerals (Fan et al. 1995). The soil management practices and clay soils present at the University favor aggregate formation, since the soil present is primarily utilized for ornamental plants and do not receive regular mixing.

The campus currently supports a population of feral cats that are managed by cat colony caregivers. Past surveys have estimated the campus to have 14 colonies and approximately 200-250 cats within an area of approximately 42 ha, though previous surveys have indicated higher densities (Davis and Lepczyk 2010, A. Davis unpubl. data). The results presented here are for one cat colony with ~20 cats; however, more will be sampled in the future. The site is located next to the Mānoa stream at a parking lot with both managed and unmanaged planters along the borders and an unmanaged riparian zone between the parking lot and stream.

The current cat management method being utilized is a variation on trap-neuter-return (TNR) methods, in which authorized cat caregivers are responsible for ensuring that cats in their respective colony/colonies are sterilized, vaccinated, have identification, are ear notched to indicate sterilization, and are properly fed and maintained in good health. Though some cat caregivers remain authorized under the University's Office of Facilities and Grounds, there are several that remain unauthorized and may not be properly managing their colony/colonies).

Sample Collection

Five soil samples of ~12.1 cm³ were target-sampled, at a cat colony on the University of Hawai'i at Mānoa main campus, from defecation sites based on the presence of feces and dig marks. Soil was collected up to ~10 cm depth using a soil probe. Samples, double-bagged in zip-top bags, were refrigerated for a short period of time (approx. 2-3 days). Cat colonies were determined based on caregivers' feeding locations.

Aggregate Dispersion and Oocyst Recovery

Though water and mechanical dispersion methods may disperse most macroaggregates, dispersion solution may be required to break down smaller water-stable aggregates. Lelu et al. (2011) demonstrated that the use of a dispersion solution had no significant effect on *T. gondii* oocyst recovery; however, it is important to note that soils tested in the study differ from the soil tested for this study and may have different results. Soil macroaggregates are 0.25 - 2 mm in diameter and bonded together by plant roots, fungal hyphae, and microbial and plant polysaccharides. *T. gondii* oocysts have the potential to be trapped within soil macroaggregates, and several studies have used dispersion solutions to release the oocysts (Afonso et al. 2008, Lelu et al. 2011). The purpose of this experiment is to determine whether different concentrations of a dispersion solution will release oocysts from soil aggregates.

The dispersion solution used for this experiment was sodium hexametaphosphate (NaPO₃), which is used as a dispersant in soil particle size analysis and soil carbon analysis (Tyner 1939). Soil samples received 3 different treatments: deionized water, 1% sodium hexametaphosphate, and 5% sodium hexametaphosphate, with 25 mL of the treatment added to ~10 mL soil. Samples were vortexed 5 min to ensure that macro-aggregates were dispersed, and centrifuged 10 min at 1500 × G.

To recover *T. gondii* oocysts, the supernatant was discarded and 25 mL of 1.15 density sucrose solution was added to the pellet to float *T. gondii* oocysts. Samples were vortexed to homogenize and centrifuged for 10 min at 1500 × G. The supernatant was separated and the pellet discarded. Deionized water was added to the supernatant, then it was manually shaken to dilute the sucrose solution, and centrifuged to form a pellet containing *T. gondii* oocysts. For molecular analysis, 200 µL of the pellet was reserved in triplicates.

Molecular Analysis

DNA extraction was conducted according to manufacturer's instructions for the QIAamp DNA Stool Mini Kit for DNA analysis (QIAGEN QIAamp DNA Stool Mini Kit, Valencia, CA). The QIAamp DNA Stool Mini Kit can accommodate soil samples and account for contaminants and inhibitors in feces and soil (QIAGEN 2012). The stool mini kit procedure includes lysis of samples using Buffer ASL, removal of impurities by absorbing the impurities into a matrix, and DNA purification (QIAGEN 2010). *Toxoplasma gondii* RH strain was used as a control and the primers TOX4 (5'-CGC TGC AGG GAG GAA GAC GAA AGT TG-3') from the 5' end and TOX5 (5'-CGC TGC AGA CAC AGT GCA TCT GGA TT-3') from the 3' end were used for PCR analysis (Homan et al. 2000,

Salant et al. 2007). The TOX4 and TOX5 primers were chosen based on studies conducted by Homan et al. (2000) and Salant et al. (2007), which showed that those primers resulted in amplification of *T. gondii* DNA and not other protozoan species. Homan et al. (2000) also found that the sequence was present in all 60 *T. gondii* strains tested. Other markers have been identified (Su et al. 2006) and may be tested if the TOX4 and TOX5 markers prove unsuccessful. The PCR amplification mix consisted of 3.0 µL 5X buffer, 1.5 µL 1% Bovine Serum Albumin, 1.2 µL MgCl₂, 2.4 µL dNTP, and 1 µL of DNA template to a final volume of 15 µL. The PCR amplification was conducted for 30 cycles; denaturation occurred at 95°C for 2 min and 93°C for one min, annealing at 52°C for 1 min, and elongation at 72°C for 1.5 min.

PRELIMINARY RESULTS

Preliminary results were inconclusive. None of the 5 targeted samples with differing dispersion concentrations amplified *T. gondii* DNA. It is important to note that these preliminary results were from only one site and only 5 targeted samples. Soil sampling rates were higher in previous studies (Afonso et al. 2008, Du et al. 2012, Lass et al. 2009) and greater sampling effort is planned for this study in the future. Though no oocysts were detected, this preliminary study has proven useful for preparing for higher sampling intensity in the future. As stated previously, there is no one standard method for detecting oocysts in the environment. This study will attempt to demonstrate a more simplified method for recovering *T. gondii* oocysts from soil samples in Hawai'i.

Work in Progress

Along with higher sampling intensity for targeted samples from defecation sites, detection of *T. gondii* oocysts to identify spatial extent is in progress. Soil will be sampled randomly using a 1×1-m grid sampling scheme. The grid for each site will be mapped with ArcGIS software (impervious surfaces and buildings will be excluded) and the extent of each grid will be based on observational data and barriers for each site, such as the location of a road or building. Until oocysts can be detected, soil macroaggregate dispersion will utilize deionized water, with experimentation on different disruption concentrations to follow, once oocysts are successfully recovered. PCR analysis will also follow the procedure described above. Furthermore, depending on whether oocysts are detected, the number of cats for each location and management strategies utilized may be included in a mixed model regression to test for a relationship.

DISCUSSION

The results of this preliminary study were expected, since sampling intensity was low. The detection rate for *T. gondii* oocysts in soil using molecular methods has been low in other studies (18.3% in Afonso et al. 2008; 17.8% in Du et al. 2012, and 17.8% in Lass et al. 2009) and those studies each included large samples sizes. It will be important to increase the sampling intensity for each site and include more cat colony locations in the study.

The presence of *T. gondii* in soils has the potential to pose a health threat to the staff, faculty, students, and cat

caregivers at the University if they come into direct contact with infected soil and ingest the oocysts. The University administration will need to consider the implications of toxoplasmosis infection in its community if *T. gondii* oocysts are detected on the campus. An important step would be to test landscaping personnel for toxoplasmosis infection. Though there is no guarantee that any antibodies present for toxoplasmosis is related to the University, a high enough occurrence in personnel may suggest that the infections came from the University.

Managing for any toxoplasmosis infection on the campus may prove difficult. Removal of cats from the campus may stop *T. gondii* oocyst inputs into soil, but oocysts can persist in the environment for years. Social impacts in the University community should also be considered. It will be very important to properly educate the community on the true risk of contracting toxoplasmosis on the University campus, and cat caregivers may be resistant to removal of cats from campus.

The University is also located along the Mānoa stream which follows the Mānoa-Ala Wai watershed and empties into the near shore area near Waikīkī. With infections documented in the endangered Hawaiian monk seal, it can be assumed that *T. gondii* oocysts are reaching and persisting in the coastal area around the Hawaiian Islands. This may also pose a risk to recreational ocean users in the state. If *T. gondii* oocysts are detected in the soil at the University of Hawai'i at Mānoa, it may be important to test other cat colony locations in areas identified with substantial soil runoff.

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LITERATURE CITED

- AFONSO, E., M. LEMOINE, M.L. POULLE, M.C. RAVAT, S. ROMAND, P. THULLIEZ, I. VILLENA, D. AUBERT, M. RABILLOUD, B. RICHE, and E. GILOT-FROMONT. 2008. Spatial distribution of soil contamination by *Toxoplasma gondii* in relation to cat defecation behaviour in an urban area. *Intl. J. Parasitol.* 38:1017-1023.
- AMARASEKARE, P. 1994. Ecology of introduced small mammals on Western Mauna Kea, Hawaii. *J. Mammal.* 75(1):24-38.
- BEAZLEY, D. M., and R. S. EGERMAN. 1998. Toxoplasmosis. *Seminars in Perinatol.* 22(4):332-338.
- DANNER, R. M., D. M. GOLTZ, S. C. HESS, and P. C. BANKO. 2007. Evidence of feline immunodeficiency virus, feline leukemia virus, and *Toxoplasma gondii* in feral cats on Mauna Kea, Hawaii. *J. Wildl. Dis.* 43(2):315-318.
- DAVIS, A., and C. A. LEPczyk. 2010. Feral cat population surveys and management options for the University of Hawai'i at Mānoa. *Proc. Vertebr. Pest Conf.* 24:68-71.

- DU, F., H. L. FENG, H. NIE, P. TU, Q. L. ZHANG, M. HU, Y. Q. ZHOU, and J. L. ZHAO. 2012. Survey on the contamination of *Toxoplasma gondii* oocysts in the soil of public parks of Wuhan, China. *Vet. Parasitol.* 184(2-4):141-146.
- DUBEY, J. P. 1998. *Toxoplasma gondii* oocyst survival under defined temperatures. *J. Parasitol.* 84:862-865.
- DUBEY, J. P. 2010. *Toxoplasmosis of Animals and Humans*, 2nd Ed. CRC Press, Boca Raton, FL. 336 pp.
- DUBEY, J. P., and J. L. JONES. 2008. *Toxoplasma gondii* infection in humans and animals in the United States. *Intl. J. Parasitol.* 38:1257-1278.
- DUMETRE, A., and M. L. DARDE. 2003. How to detect *Toxoplasma gondii* oocysts in environmental samples? *FEMS Microbiol. Rev.* 27:651-661.
- FAN, P. F., R. NG, and D. REMULAR. 1995. Mineral assemblages of the sediments of the Ala Wai Canal and its drainage basins, O'ahu, Hawai'i. *Pac. Sci.* 49(4):400-411.
- FLEGR, J. 2007. Effects of *Toxoplasma* on human behavior. *Schizophrenia Bull.* 33(3):757-760.
- FLEGR, J., and J. HAVLICEK. 1999. Changes in the personality profile of young women with latent toxoplasmosis. *Folia Parasitol.* 46:22-28.
- FLEGR, J., J. HAVLICEK, P. KODYM, M. MALY, and Z. SMAHEL. 2002. Increased risk of traffic accidents in subjects with latent toxoplasmosis: A retrospective case-control study. *BMC Infect. Dis.* 2:11.
- FRENKEL, J. K., A. RUIZ, and M. CHINCHILLA. 1975. Soil survival of *Toxoplasma* oocysts in Kansas and Costa Rica. *Am. J. Trop. Med. Hyg.* 24(3):439-443.
- GARCIA, J. L., S. M. GENNARI, I. T. NAVARRO, R. Z. MACHADO, S. A. HEADLEY, O. VIDOTTO, J. D. S. GUIMARAES, JR., F. M. BUGNI, and M. IGARASHI. 2008. Evaluation of IFA, MAT, ELISAs and immunoblotting for detection of anti-*Toxoplasma gondii* antibodies in paired serum and aqueous humour samples from experimentally infected pigs. *Res. Vet. Sci.* 84:237-242.
- HOMAN, W. L., M. VERCAMMEN, J. DE BRAEKELEER, and H. VERSCHUEREN. 2000. Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii* and its use for diagnostic and quantitative PCR. *Intl. J. Parasitol.* 30:69-75.
- HONNOLD, S. P., R. BRAUN, D. P. SCOTT, C. SREEKUMAR, and J. P. DUBEY. 2005. Toxoplasmosis in a Hawaiian monk seal (*Monachus schauinslandi*) *J. Parasitol.* 91(3):695-697.
- IKEDA, J. K. 2000. Toxoplasmosis in Hawai'i – A review and evaluation of its implications. Unpubl. ms., Environmental Health Administration, Department of Health, Honolulu, HI.
- LASS, A., H. PLETKIEWICZ, E. MODZELEWSKA, A. DUMÈTRE, B. SZOSTAKOWSKA, and P. MYJAK. 2009. Detection of *Toxoplasma gondii* oocysts in environmental soil samples using molecular methods. *Eur. J. Clin. Microbiol. Infect. Dis.* 28:599-605.
- LELU, M., E. GILOT-FROMONT, D. AUBERT, A. RICHAUME, E. AFONSO, E. DUPUIS, C. GOTTELAND, F. MARNEF, M. L. POULLE, A. DUMETRE, P. THULLIEZ, M. L. DARDE, and I. VILLENA. 2011. Development of a sensitive method for *Toxoplasma gondii* oocysts extraction in soil. *Vet. Parasitol.* 183(1-2):59-67.
- MANCIANTI, F., S. NARDONI, G. ARITI, D. PARLANTI, G. GIULIANI, and R. A. PAPINI. 2010. Cross-sectional survey of *Toxoplasma gondii* infection in colony cats from urban Florence (Italy). *J. Feline Med. Surg.* 12:351-354.
- NOVOTNA, M., J. HANUSOVA, J. KLOSE, M. PREISS, J. HAVLICEK, K. ROUBALVA, and J. FLEGR. 2005. Probable neuroimmunological link between *Toxoplasma* and cytomegalovirus infections and personality changes in the human host. *BMC Infect. Dis.* 5:54.
- NRCS (NATURAL RESOURCE CONSERVATION SERVICE). 2009. Web Soil Survey. U.S. Department of Agriculture. Updated Nov. 2011 2009. <http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm>.
- PRANDOVSKY, E., E. GASKELL, H. MARTIN, J. P. JUBEY, J. P. WEBSTER, and G. A. McCONKEY. 2011. The neurotrophic parasite *Toxoplasma gondii* increases dopamine metabolism. *PLoS ONE* 6(9):9.
- QIAGEN. 2010. QIAamp DNA Stool Handbook, 2nd Ed. QIAGEN GmbH, Hilden, Germany.
- QIAGEN. 2012. Sample and assay technologies. QIAGEN Inc., Valencia, CA. <http://www.qiagen.com>.
- SALANT, H., A. MAKROVICS, D. T. SPIRA, and J. HAMBURGER. 2007. The development of a molecular approach for coprodiagnosis of *Toxoplasma gondii*. *Vet. Parasitol.* 146:214-220.
- SALANT, H., D. T. SPIRA, and J. HAMBURGER. 2010. A comparative analysis of coprologic diagnostic methods for detection of *Toxoplasma gondii* in cats. *Am. J. Trop. Med. Hyg.* 82(5):865-870.
- SU, C., X. ZHANG, and J. P. DUBEY. 2006. Genotyping of *Toxoplasma gondii* by multilocus PCR-RFLP markers: A high resolution and simple method for identification of parasites. *Intl. J. Parasitol.* 36:841-848.
- TENTER, A. M., A. R. HECKEROTH, and L. M. WEISS. 2000. *Toxoplasma gondii*: From animals to humans. *Intl. J. Parasitol.* 30:1217-1258.
- TYNER, E. H. 1939. The use of sodium metaphosphate for dispersion of soils for mechanical analysis. *Soil Sci. Soc. Am. Proc.* 4:106-113.
- UHM (UNIVERSITY OF HAWAII MĀNOA). 2011. About UHM. University of Hawai'i at Mānoa, Honolulu, HI. Updated Apr. 4, 2011. <http://manoa.hawaii.edu/about/>.
- WEBSTER, J. P., and G. A. McCONKEY. 2010. *Toxoplasma gondii*-altered host behavior: Clues as to mechanism of action. *Folia Parasitol.* 57(2):95-104.
- WORK, T. M., J. G. MASSEY, D. S. LINDSAY, and J. P. DUBEY. 2002. Toxoplasmosis in three species of native and introduced Hawaiian birds. *J. Parasitol.* 88(5):1040-1042.
- WORK, T. M., J. G. MASSEY, B. A. RIDEOUT, C. H. GARDINER, D. B. LEDIG, O. C. H. KWOK, and J. P. DUBEY. 2000. Fatal toxoplasmosis in free-ranging endangered 'alalā from Hawai'i. *J. Wildl. Dis.* 36(2):205-212.
- YAGMUR, F., S. YAZAR, H. O. TEMEL, and M. CAVUSOGLU. 2010. May *Toxoplasma gondii* increase suicide attempt-preliminary results in Turkish subjects? *Forensic Sci. Intl.* 199:15-17.