CANINE PARASITIC ZOONOSES IN BANGKOK TEMPLES

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Abstract. Fecal samples were collected from 204 humans and 229 dogs from 20 different temples in Bangkok, as well as communities in the surrounding temple ground areas. Human and dog stool samples were examined for intestinal parasites including *Giardia* using zinc sulfate flotation and microscopy. Hookworms were the most common parasite in dogs (58.1%) followed by *Trichuris* (20.5%), *Isospora* (10%), *Giardia* (7.9%), *Toxocara* (7.4%), *Dipylidium caninum* (4.4%) and *Spirometra* (3.1%). *Blastocystis hominis* (5.9%) was the most common parasite in humans followed by hookworms (3.4%), *Giardia* (2.5%), *Strongyloides* (2%) and *Cryptosporidium* (1.5%). All samples microscopy-positive for *Giardia* were genotyped. The majority of *Giardia* isolated from the dog population was placed in Assemblage A, followed by Assemblages D, B and C, respectively, while human isolates were placed in Assemblages A and B. Therefore, dogs in temple communities posed a potential zoonotic risk to humans for transmission of hookworms, *Giardia* (especially Assemblage A genotypes) and *Toxocara canis*.

INTRODUCTION

It is common in Thailand to leave or abandon unwanted dogs in temple grounds. Since temples are public places of worship and the donation of gifts, the owners trust that their animals will be fed and will be taken cared of by monks and nuns, as well as good-hearted temple visitors such as people who live nearby the temple grounds or people who love animals and come to temple to feed them. Temple grounds in Thailand are generally sizeable with free access to the public and therefore seem to be a perfect place for unwanted animals. At a minimum, the dogs can live on the left-over food of the monks. Under these circumstances, the population of semi-domesticated and stray dogs is high in temple communities. This coupled with poor hygienic practices and overcrowded conditions, places the monks, nuns and people living in the surrounding communities at a high risk of acquiring zoonotic parasites either directly through close contact with the dogs, or indirectly through the highly contaminated environment.

Surveillance data with regard to prevalence of zoonotic canine gastrointestinal parasites in Thailand is largely lacking. Previous studies conducted by Hinz (1980) and Rojekittikhun *et al* (1998) found hookworms, *Trichuris vulpis* and *Toxocara canis* to be the most common parasites in stray dogs from Bangkok. Another study by Wiwanitkit and Waenlor (2004) found 5.7% of soil samples collected from Bangkok to be contaminated with *Toxocara* eggs. Semi-domesticated dogs in rural communities in Thailand have also

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shown to act as potential reservoir hosts for the fish-borne parasites such as *Gnathostoma spinigerum* (Maleewong *et al,* 1992) and *Opisthorchis viverrini*. No data exist with regards to the prevalence of *Giardia* in dogs in Thailand.

Giardia duodenalis is a flagellated protozoan that inhabits the small intestine of humans and other mammals. It is distributed worldwide and considered the most commonly detected intestinal parasite in humans in developed countries (Schantz, 1991). In developing regions of the world, *Giardia* constitutes part of the complex group of parasitic, bacterial and viral diseases that impair the ability to achieve full potential and impair development and socio-economic improvement. All diseases included in the WHO Neglected Diseases Initiative have a common link with poverty and as the current view is to take a comprehensive approach to all these diseases, *Giardia* was included (Savioli *et al*, 2006). *Giardia* is also considered a re-emerging infectious agent because of its role in outbreaks of diarrhea in child-care centers (Thompson, 2000). *Giardia* is a zoonotic agent (Milstein and Goldsmid, 1997: Thompson *et al*, 2000); the organism is transmitted fecal-orally producing environmentally resistant cysts that are voided in the feces and may be transmitted directly via person-to-person or animal-to-person contact or indirectly via contaminated food and water. There is increasing evidence in support of the zoonotic potential of canine *Giardia* with a growing number of studies identifying potentially zoonotic genotypes of *Giardia* in dogs and humans from both developed and disadvantaged communities worldwide (Traub *et al*, 2004; Eligio-Garcia *et al*, 2005; Lalle *et al*, 2005). In Thailand, recent surveys addressed *Giardia* infection only in humans with a prevalence of 5.3-14.36% (Nuchprayoon *et al*, 2002; Waikagul *et al*, 2002; Sirivichayakul *et al*, 2003). The prevalence of *Giardia* in animals, however, is unknown. Information regarding the epidemiological status of zoonotic canine *Giardia* infection in dogs and humans in Thailand is needed. In this study, we aim to determine the prevalence of canine gastrointestinal parasites in canine and human populations in temples and their surrounding communities in Bangkok, with particular emphasis on the zoonotic potential of *Giardia.*

MATERIALS AND METHODS

Collection of fecal samples

Single fecal samples were collected from 204 humans and 229 dogs from 20 temples and their surrounding communities in the city of Bangkok. After informed consent, the questionnaires were administered to the appropriate personnel with regard to risk factors for parasitic infection, including socio-economic status, crowding, age, gender, defecation practices, dog ownership and current signs of diarrhea. Fecal samples were preserved separately in 5% formalin for microscopic screening and 20% dimethyl sulfoxide for molecular testing.

Parasitological techniques

Human and dog fecal samples were examined for the presence of parasites and *Giardia* cyst using zinc sulfate and sodium nitrate flotation and microscopy (Faust *et al*, 1938). *Giardia* cyst were then concentrated and purified from microscopically positive human and dog samples using a saturated salt and glucose method (Meloni and Thompson, 1987).

Molecular methods

DNA extraction. The purified *Giardia* cysts were transferred into a 1.5 ml Eppendorff tube and centrifuged at 20,000*g*. Extraction of the *Giardia* cysts was then carried out using QiAMP DNA Mini Stool Kit (Qiagen GMBH, Hilden, Germany).

The SSU-rDNA gene. A nested PCR was used to amplify a 300 bp region of SSU-rDNA gene using primer AL4303, AL4305 and AL4304 and AL4306. Amplification conditions were carried out as previously described by Sulaiman *et al* (2003) and was used to genotype all microscopic positive samples in this study.

Sequencing of PCR product. All PCR-positive samples were subjected to sequencing. PCR products were purified using Qiagen spin columns (Qiagen, Hilden, Germany) and sequenced using an ABI Terminator Cycle Sequencing Kit (Applied Biosystems, CA, USA) according to manufacturer's instructions (GeneWorks, Australia). Sequences were analysed using SeqEd (Applied Biosystems, CA, USA) and aligned with each other as well as previously published sequences for *G. duodenalis* isolates using Clustal W (Thompson *et al*, 1994).

Molecular characterization and phylogenic analysis. Distance-based analyses were performed using MEGA version 2.1 (Kumar *et al*, 2001). Distance-based analysed were conducted using Tamura-Nei distance estimates and trees were constructed using the Neighbor-Joining algorithm.

Statistical analysis

Univariate associations between the prevalence of *Giardia* in humans and dogs utilizing microscopic examination and PCR and host, behavioral and environmental factors were initially made using chi-square results for independence and ANOVA (continuous variables). Logistic multiple regression was used to quantify the association between the prevalence of *Giardia* using each test and each variable after adjusting for other variables. Pearson's correlation was utilized to determine the subfactors or variables that were highly associated with whether individual humans and animals belonged to the monastery or individual households and these variables were tested for significance. Only variables significant at $p \leq 0.25$ in the univariate analyses were considered eligible for inclusion in the logistic multiple regression (Hosmer and Lemeshow, 1989; Frankena and Graat, 1997). Backward elimination was used to determine which factors could be dropped from the multivariable model. The likelihood- ratio chi-squared statistic was calculated to determine the significance at each step of the model building. The level of significance for a factor to remain in the final model was set at 10%. The goodness of fit of the model was assessed with the Hosmer-Lemeshow statistic (Lemeshow and Hosmer, 1982). Data were analysed and statistical comparisons were performed using SPSS (SPSS for Windows, Version 14.0, Rainbow Technologies) and Excel 2002 (Microsoft).

RESULTS

Survey results and intestinal parasites prevalence

Eighty percent of dogs belonged to monks in the temple, and 20% of dogs belonged to households surrounding the temple. Ten and a half percent of dogs were puppies < 4 months, 23.5% young adult dogs between 5-12 months of age, 56% adult dogs between 1-7 years of age and 10% geriatric dogs > 7 years old. Thirty-three percent of dogs were entire males, 6% sterilized males, 35.5% entire females, 14.0% sterilized females, 2% lactating females and 9.5% females of unknown sterilization or pregnancy status. Ninety-three percent of dogs were of mixed breed and the rest pure bred dogs. The majority of dogs (79.5%) had been vaccinated against rabies.

Fifty-eight percent of participants were monks or nuns belonging to the monastery, and the rest were families from households surrounding the Temple grounds. The mean age of individuals was 32.7 years with 18% being < 10 years of age, 10% between 11-20 years of age, 22.5% between 21-30 years of age, 10.3% between 31-40 years of age, 17.7% between 41-50 years of age and 21.5%

above the age of 50 years. Sixty-six percent of participants were male and 34% female. The majority of people defecated in indoor latrines (95.6%), 3.4% defecated in indoor latrines that directly emptied into the Chao Phraya River and 1% admitted to defecating outdoors. Commercial bottled water was drunk by 58% of participants, 28% drunk boiled or filtered tap water and 14% drunk untreated tap water.

Hookworms (58.1%, 95% CI = 51.7-64.5) were the most common parasite of dogs followed by *Trichuris* (20.5%, 95% CI = 15.3-25.8), *Isospora* spp (10%, 95% CI = 6.2-13.9), *Giardia* spp (7.9%, 95%CI = 4.4-11.3), *Toxocara canis* (7.4%, 95%CI = 4-10.8), *Dipylidium caninum* (4.4%, 95%CI = 1.7-7) and *Spirometra* (3.1%, 95%CI = 0.8-5.3). In humans, *Blastocystis hominis* was the most common parasite (5.9%, 95% CI = 2.7- 9.1), hookworms–(3.4%, 95%CI = 0.9-5.9) followed by *Giardia* (2.5%, 95% CI = 0.9-5.9), *Strongyloides* (2%, 95% CI = 0.1-3.9) and *Cryptosporidium* (1.5%, 95%CI = 0-3.1). Most individuals defecated in proper indoor latrines (96%) and wore footwear while outdoors (92.5%).

Age was found to be the only significant risk factor for infection with *Giardia* and *Toxocara* in dogs. Dogs less than 4 months of age were more likely to be infected with *Giardia* than dogs that were older (OR= 4.6, 95% CI = 1.5- 13.7, p = 0.01). Similarly, dogs less than one year were more likely to be infected with *Toxocara canis* than older dogs (OR= 5.2, 95% CI = 1.7-15.3, p = 0.01). Dogs less than one year (OR 2.1, 95% CI, 1.0-4.1, p = 0.04), those living in the immediate surroundings of more than 10 dogs (OR 2.8, 95% CI, 1.0-7.4, p = 0.04), those that were not dewormed at least once in the past 12 months (OR 7.2, 95% CI, 2.7-19.2, p = 0.00), and those that were entire animal (OR 3.1, 95% CI, 1.5-6.7, p = 0.00) were more likely to be infected with hookworms. Finally, dewormed dogs (OR 3.9, 95% CI 0.9-17.5, p = 0.07) and those living in the immediate surroundings of less than 10 dogs (OR 4.8, 95% CI 1.1-20.0, p = 0.04) were also more likely to harbor *Trichuris vulpis*.

In humans, risk factors of significance were only found for infection with *Giardia.* All *Giardia* positive individuals were children below 6 years old. Apart from one child suffering from anorexia no other child complained of any associated clinical symptoms of giardiasis. Only univariate analysis was conducted for humans positive for *Giardia* by ZME as there were too few samples positive for *Giardia* to enable multivariate analysis. The mean age of *Giardia* positive individuals was 4.36 years compared to 33.4 years for *Giardia* negative individuals (p = 0.002). Individuals drinking untreated tap water were 2.46 times (95% CI = 0.8-2.91) more likely to be positive for *Giardia* using ZME than those drinking either treated tap water or commercially bottled water (p = 0.012).

Molecular characterization and phylogenetic analysis of *Giardia* isolates found in humans and dogs

In total, 3/5 and 13/18 microscopy positive *Giardia* samples from humans and dogs respectively, were successfully amplified by PCR and sequenced at the SSU-rDNA gene. Phylogenetic analysis of the 290 bp region of the SSU-rDNA data placed all of the dog isolates into the Assemblages A, B, C and D clusters and human isolates into Assemblages A and B clusters but was poor at resolving the relationships (Fig 1) between Assemblages B, C and D. The majority of *Giardia* isolates recovered from the dog population were Assemblage A followed by Assemblages D, then B and C, respectively. Three dogs had mixed infection with Assemblages A and BIII. Similar genotypes of *G. duodenalis* (Assemblage A) were recovered from a temple dog (T8D10) and monks (T8P7, T8P) within the same monastery. The majority of human *Giardia* isolates clustered into Assemblage A, while only one human (T8P8) had mixed infection with Assem-

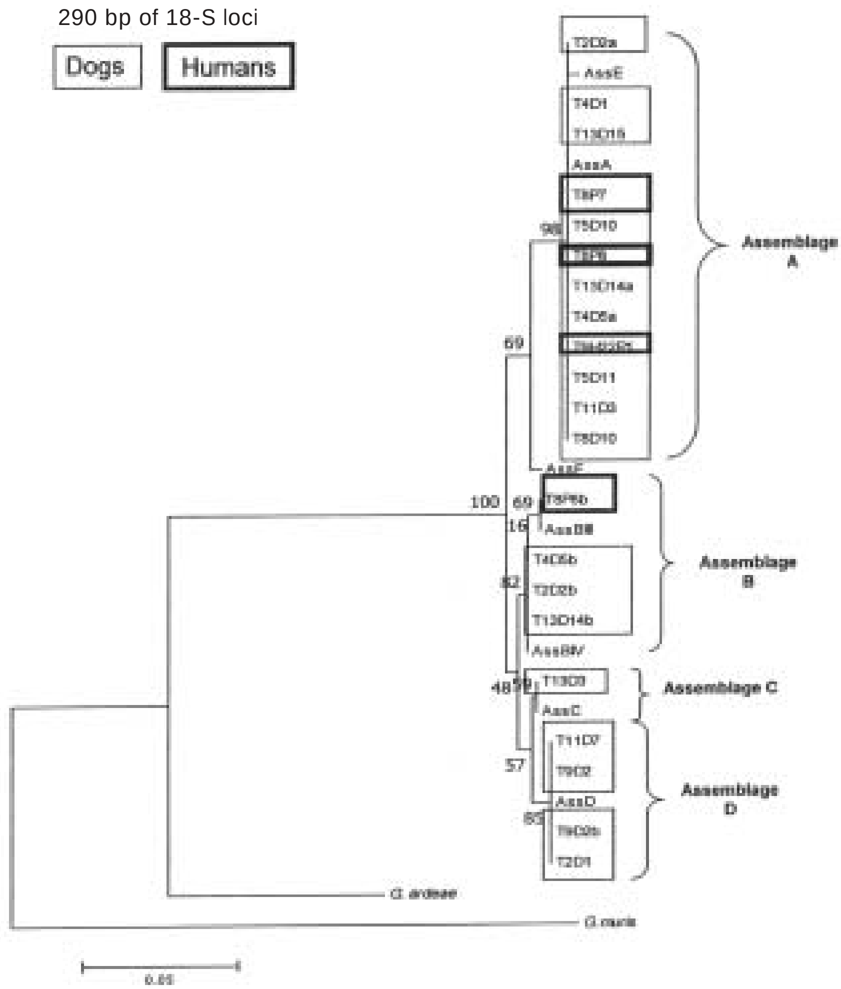

Fig 1–Phylogeny of the *Giardia* isolates inferred by distance based analysis using Tamura-Nei distance estimates of alined nucleotide sequences derived from the PCR products of the SSU-rDNA gene (T= temple number, H= house number, D= dog number, P= human number) and boxes with bold outlines are humans isolates, those with normal outlines are dog isolates.

blages A and BIII.

DISCUSSION

The results of this study strongly support previous conclusions of Hinz (1980) and Rojekittikhun *et al* (1998), showing that dogs in temple communities of Bangkok pose an important zoonotic risk with regards to the transmission of hookworms and *Toxocara canis*. Humans become infected with *Toxocara* when they accidently ingest embryonated eggs, usually through contaminated soil. Although most people infected with *T. canis* do not develop overt clinical disease, three clinical syndromes, namely visceral larva migrans, ocular larva migrans and covert toxocariasis in humans have been reported (reviewed by Irwin and Traub, 2006). Although the prevalence of *T. canis* was lower than the other helminths (7.5%) the ability of these highly resistant eggs to withstand adverse conditions means that they may remain infective and accumulate to high intensities in the environment for a period of years. Similarly, larval stages of hookworms in soil may infect humans with exposed unprotected skin, manifesting primarily as cutaneous larva migrans or "creeping eruptions". The majority of individuals in this community admitted to using footwear while outdoors. This method of control will need to be re-enforced within the community, especially among children, given the high prevalence of hookworms in dogs in this community coupled with poor environmental standards of hygiene and widespread environmental contamination.

This is the first study to reveal the prevalence, transmission cycles and zoonotic potential of *Giardia* in humans and dogs in Thailand. However, it has been suggested that the prevalence of *Giardia* using conventional methods such as zinc flotation and microscopy is often underestimated because of the low sensitivity of this diagnostic method. This may be exacerbated due to the intermittent nature of cyst excretion and poor technical training of laboratory personnel (Dryden *et al*, 2006). Therefore microscopy negative samples should, in the future, also be screened using molecular as well as immunodiagnostic tests such as immunofluorescence antibody testing (IFAT) against the cyst wall proteins and the coproantigen capture enzyme-linked immunosorbent assay (CELISA). Moreover, the diagnostic methods utilized depend highly on the purpose of the study. If the aim of study is to determine the morbidity of giardiasis in clinically affected individuals (*eg*, children, elderly, immunologically naive) then the intensity of *Giardia* cysts may be a better indicator and microscopic screening would be a more appropriate diagnostic tool.

The genetic characterization of *Giardia* isolates recovered from dogs and humans provides supporting evidence for the occurrence of both zoonotic and non-zoonotic transmission in this localized endemic focus. Analysis of the SSU-rDNA sequence data shows dog populations to have two cycles of *Giardia* transmission; the first with a zoonotic cycle in which *Giardia* isolates belonging to Assemblages A and B have a cycle among dogs and presumably humans, similar to a previous study in the tea growing communities in India, where it was found that Assemblages A and B predominated in dogs (Traub *et al,* 2004). The second with a dog specific cycle in which dogs isolates of *Giardia* placed within Assemblages C and D have a cycle among dogs, similar to a previous study in aboriginal communities in Australia (Hopkins *et al*, 1997). In temple communities in Bangkok, both zoonotic and non-zoonotic cycles of *Giardia* were circulating among dogs. The majority of dogs within the temple roam within packs with a high level of dog to dog contact. Dogs in this group were therefore likely to be shedding and transmitting *Giardia* Assemblages C and D from dog to dog. At the same time, these dogs and more so dogs from surrounding households also had close contact with human populations and may be cycling *Giardia* isolates from Assemblages A and B both to and from humans and among each other. The majority of dog isolates of *G. duodenalis* in dogs clustered into Assemblage A followed by Assemblages D, B and C, while human isolates were placed into Assemblages A or B. Consequently dogs in temple communities pose a moderate zoonotic risk to humans with regards to transmission of *Giardia,* especially with Assemblage A. Traub *et al* (2004), Eligio-Garcia *et al* (2005), Itagaki *et al* (2005) and Lalle *et al* (2005) also found *Giardia* isolates from dogs within Assemblage A to be the most common and significant in terms of zoonotic risk. However, the question as to whether humans or dogs are the primary source of *G. duodenalis* Assemblages A and B isolates still remain unanswered. Even so, dogs act as reservoirs for human infection.

The majority of *Giardia* positive individuals in this study, regardless of the method of detection, were clinically asymptomatic. Previous studies have shown that most humans positive for *Giardia* were suffering from chronic malabsorption (Jahidi, 1978). Malabsorption syndrome is especially significant in children with low nutritional status. In another study, *Giardia* infection in children was demonstrated to not necessarily be accompanied by diarrhea (Read *et al*, 2002). In the present study the majority of participants were adult males, therefore they may already have immunity against *Giardia*, which explains why they did not show any clinical symptoms. However, when looking at the microscopy results, all *Giardia* positive individuals were children below the age of 6 years old, emphasizing the high intensity of this parasite in children which could be detected by microscopy. All of the participants in this study were clinically asymptomatic, so antiparasitic treatment is not necessarily advocated, however, advice for preventing further transmission is recommended.

In this study, multivariate risk factor analysis revealed that younger dogs were consistently more likely to be positive for parasites such as hookworms, *Toxocara* and *Giardia*. Age related immunity could account for this finding; however, it must be noted that microscopy alone was utilized to establish prevalence. It is likely that the lower sensitivity of conventional flotation and microscopy compared to immunodiagnostic and molecular methods (McGlade *et al*, 2003; Cirak and Bauer, 2004) is only detecting high intensities of infection which again, is likely to be encountered in younger animals, therefore putting bias on these risk factors for prevalence. Nevertheless, the high intensities of parasite stages shed by younger animals and those animals in overcrowded environments, as well as animals that were not de-wormed, make them a significant source of infection both directly and through environmental contamination. A majority of these dogs in temple communities in Bangkok are vaccinated against rabies by government veterinary assistants. Volunteer veterinarians also offer their services in some temple communities by mass treating the temple dogs with ivermectin injections every 6 months and de-sexing the male dogs. Increased commitment from the government to ensure that this service is offered free across all 500 odd temples in Bangkok will significantly aid the control of canine parasitic zoonoses in these communities.

ACKNOWLEDGEMENTS

Authors would like to thank Miss Chantira Sutthikornchai, Dr Ryan O'Handley, Dr Carlyse Palmer, Associate Professor Ian Robertson and Dr Hannes Wickert for their help, suggestion and support. We also would like to thank all the dog owners, monks, nuns and people who cooperated in this study. This project was financially supported by the Australian Research Council, Bayer Animal Health, Leverkusen, Germany and Faculty of Tropical Medicine Research fund, Mahidol University.

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