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RESEARCH

Seroprevalence of *Toxoplasma gondii* from stray cats residing in temples, Bangkok, Thailand

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ABSTRACT

Toxoplasma gondii are zoonotic protozoa that cause serious problems in both of human and animals. This organism infects human population worldwide and is a threat to pregnant women and their fetuses. *T. gondii* may infect many species of animals, especially cats as the definitive host. In Thailand, the stray cats are often living in close proximity to humans. The objectives of this study are to detect antibodies against *T. gondii* infection in stray cats living in the temples in Bangkok by using the indirect fluorescent antibody test (IFAT) and to identify risk factors associated with such *T. gondii* infection. The sera of 458 stray cats were collected from temples in 19 districts of Bangkok between June and December 2015 and tested by IFAT. An overall positive to *T. gondii* were 9.0% (41/458). Moreover, Bangkok Noi District had the highest prevalence of 50%. The highest rate of infection was observed in stray cats aged more than 5 years old at 17.9% (7/39) while the infection rates of cats between 1-5 years old and younger than 1 year old were 10.1% (32/318) and 2.0% (2/101), respectively. The seroprevalence in male 9.3% (18/193) is higher than that in female 8.7% (23/265). However, there was no significant difference regarding sex of the stray cats. The result of this study showed that stray cats infected with *T. gondii* may be an important potential source of human and animal toxoplasmosis, predominantly when they are free-roaming and may themselves be exposed to *T. gondii* parasites in their respective habitats.

Keywords: *Toxoplasma gondii*, stray cats, temple, Thailand

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan that infects a wide variety of animals including humans (Edelhofer and Prossinger, 2010). Toxoplasmosis is an important zoonosis which causes serious disease worldwide (Salant et al., 2010). *T. gondii*, infects human population and can lead to lymphadenitis, encephalitis and retinochroiditis (Jung et al., 2015), particularly being a threat to pregnant women and their fetuses (Edelhofer and Prossinger 2010). Humans contracts *T. gondii* infection by ingestion of tissue cysts in raw or undercooked meat of infected animals, or ingesting food or water contaminated with oocysts from feces of infected cat, the definitive host (Jung et al., 2015). Diagnosis of *T. gondii* infection in cats is based on detection of oocysts in feces by microscopy, bioassay, serology or PCR. Nevertheless, definitive diagnosis of toxoplasmosis in cats is difficult to establish (Györke et al., 2011). Infected cats only spread

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T. gondii oocysts in their feces for a few weeks after infection and rarely show any symptoms or other nonspecific signs (Liu et al., 2015). Many epidemiological studies on *T. gondii* in cats have been published. Serologic diagnosis commonly relies on the detection of specific antibodies that are produced by the hosts rather than the detection of oocysts shedding, as the chance of finding a cat shedding is low whereas antibodies persist and indicate prior exposure to *T. gondii*. Nowadays, most clinical laboratories use an ELISA for the screening of specific IgG whereas other techniques are mostly reserved for reference laboratories. However, the performance of the different methods used varies and the derived IgG titer results cannot be compared and evaluated in relation to each other (Robert-Gangneux and Dardé, 2012). The indirect fluorescent antibody test (IFAT) is a simple test detecting antibodies, and has been widely used in detection of *T. gondii* antibodies in humans and animals and shows sensitivities 80.4-100.0% and specificities of 91.4-95.8% (Liu et al., 2015) while previous study in cats shows 94.2% sensitivity and 93.7% specificity (Dabritz et al., 2007). In Thailand, there were a few studies on *T. gondii* infection in cats such as the studes in Bangkok where a range of 7.3-10.1% infection was reported in pet cats (Sukthana et al., 2003, Sukhumavasi et al., 2012), and a range of 4.8-11.0% was reported in stray cats (Jittapalapong et al., 2007, Jittapalapong et al., 2010). Another study completed in the west of Thailand established a 8.3% infection in farm cats (Arunvipas et al., 2013). Bangkok occupies a total area of 1,568.74 km^2 on both sides of the Chao Phraya River and it has a population of over 8 million which is more than 10% of the total population of Thailand. As expected from a modern metropolis such as Bangkok, the population density varies across the area of the city, with the highest population density in the city center and inner suburbs and the lowest in the outer suburbs. There are nearly 500 temples in Bangkok (National Office of Buddhism, 2015) and there are numerous cats living in the temples. The temples are not only the monks' residences, but also centers for religious events during which people may be exposed to the infected cats directly or indirectly. Recently, the number of stray cats has been gradually increasing in Thailand. Thus, food and water contaminated with oocysts from stray cat feces may be a risk factor of increasing importance for *T. gondii* infection. The objectives in this study are to detect antibodies against *T. gondii* infection of stray cats residing in temples in Bangkok by using the indirect fluorescent antibody test (IFAT) and to identify risk factors associated with *T. gondii* infection of stray cats in Bangkok.

MATERIALS AND METHODS

Study area and animal samples

A total of 458 sera samples were collected from stray cats in 19 selected districts (5-15 cats/temple and 1-3 temples/district) between June and December 2015 (Fig. 1). One hundred and ninety-three male and 265 female cats were sampled. Individual blood samples were collected from the jugular veins. For sera separation, blood samples collected in sterile tubes without anticoagulant were centrifuged at 3,000 rpm for 10 minutes; serum fractions were stored at -20°C until analysis. The randomly selected cats were determined for their age and divided into three age groups: ≤ 1 years (101 animals), 1 to 5 years (318 animals) and >5 years (39 animals). Factors including sex, and age of animals associated with *T. gondii* infection were established for analysis based on questionnaires filled in by monks, nuns or animal caretakers. The study protocol was approved by the Animal Ethics Committee of Faculty of Veterinary Medicine, Kasetsart University, based on the Ethics of Animal Experimentation of the National Research Council of Thailand. The certificate of approval number is ACKU60-VET-007.

Detection of antibodies to *T. gondii*

IFAT was used to examine the reactivity of each sample serum to the isolated parasite. *T. gondii* tachyzoites (RH strain) were maintained in African green monkey kidney (Vero) cells and cultured in the minimum essential medium

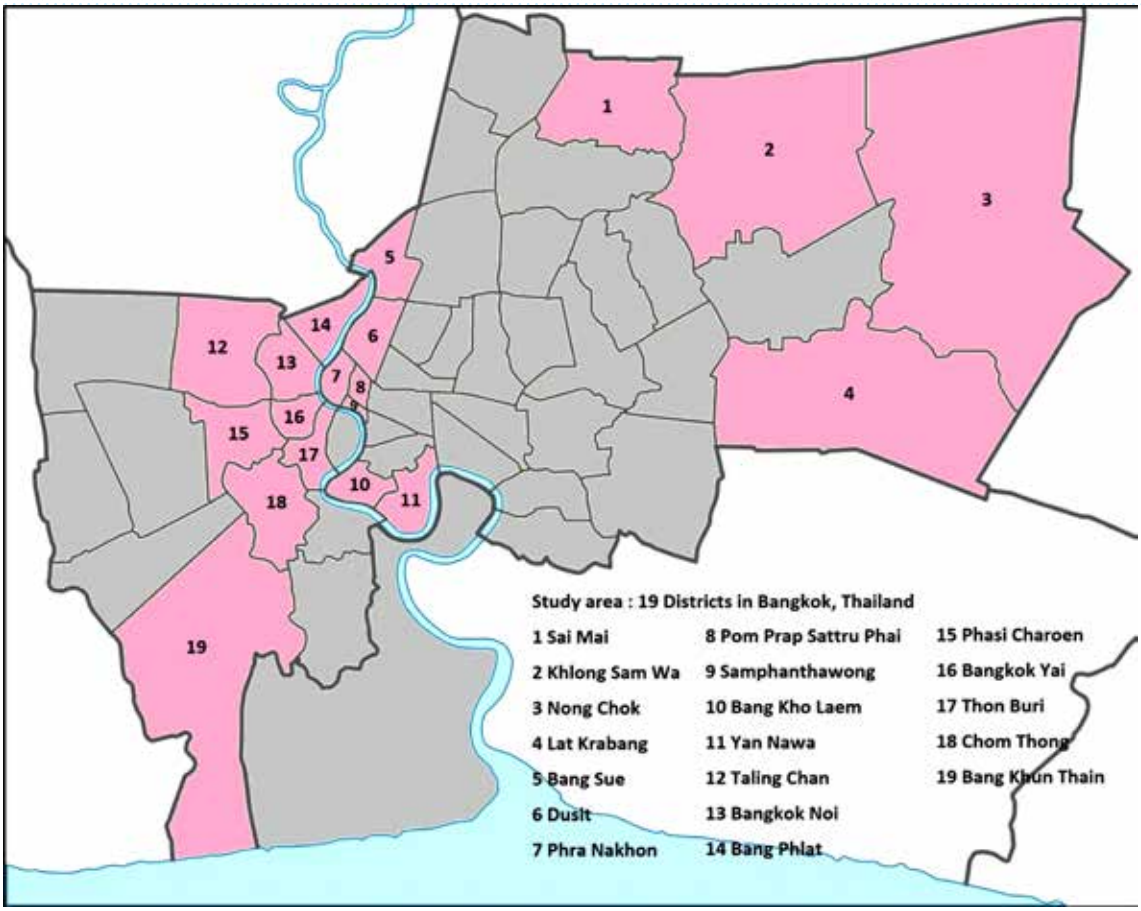

Fig 1 Study sites in Bangkok, Thailand (Pink color refers to the 19 districts where the blood samples were collected from stray cats).

(MEM, Sigma, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS), L-glutamine and penicillin-streptomycin at 37°C in 5% CO_2 air environment. Infected host cells were scraped from flasks and disrupted by three passages through 25 and 27-gauge needles, followed by 5.0 µm ultrafiltration (Millipore, USA), filtrate centrifugation at 3,000 rpm for 5 minutes. The pellets were washed with 10 ml of PBS, and re-centrifuged before pelleted parasites were counted and diluted to 1×10^4 tachyzoites/ml. The tachyzoite suspension was dispensed into 5-mm wells (10 µl/well) on teflon-coated slides (Cel-Line Associates, Newfield, NJ), which were then air dried at room temperature, fixed with acetone for 30 minutes and stored at -20 °C.

Samples sera were diluted to 1:100 (Macri et al., 2009) in PBS with 4% bovine serum albumin, placed onto 5-mm well of coated antigen slides, and incubated at 37 °C for 30 minutes. These slides were then washed three times with PBS, and incubated with 10 µl/well of caprine anti-feline IgG FITC conjugate (VMRD, Pullman, WA, USA) at 37°C for 30 minutes. After incubation with secondary antibody conjugate, these slides were again washed three times with PBS, covered with cover slips and examined with a fluorescence microscope. *T. gondii* positive and negative control sera were used from IgG FA Positive and FA Negative control Feline origin (VMRD, Pullman, WA, USA).

Statistical analysis

Characteristics of individual stray cats such as confined or free-roaming and information about the different gender and age were analyzed in relation to seroreactivity to identify putative risk factors associated with stray cat exposure to *T. gondii*. The relationship between the seropositivity and possible associated factors was tested with the Chi-square ($\chi 2$) test in Number Cruncher Statistical System (NCSS) version 2000 (Kaysville, Utah, USA) programs.

RESULTS

Seroprevalence of *T. gondii* infection in stray cats

This study revealed a seroprevalence of *T. gondii* infection in stray cats in Bangkok, Thailand as shown in Table 1. An overall seropositive in stray cats to *T. gondii* was 9.0% (41/458) and ranged from 0.0% to 50.0% among 19 districts sampled. Of 19 districts, *T. gondii* infection was found in 15 districts (78.9%). Moreover, Bangkok Noi district had the highest prevalence of 50.0% (10/20). The difference of prevalence was varied by host age and gender shown in Table 2. Stray cats aged more than 5 years old had an infection of 17.9% (7/39), which is significantly higher than other age groups, i.e., 10.1% (32/318) in cats aged 1-5 years old and 2.0% (2/101) in cats aged less than 1 year ($p < 0.05$). The 9.3% (18/193) seroprevalence in male is higher than that of 8.7% (23/265) in female. However, there were no significant differences regarding sex ($p = 0.8$) and other risk factors in this study.

Table 1 Detection of antibodies to *T. gondii* from stray cats in 19 districts of Bangkok, Thailand.

District	No. of IFAT positive/No. of samples	Percentage
Bangkok Noi	10 / 20	50.0
Phra Nakhon	2 / 5	40.0
Bang Kho Laem	8 / 26	30.8
Bang Phlat	5 / 22	22.7
Taling Chan	4 / 23	17.4
Chom Thong	2 / 21	9.5
Bang Khun Thain	2 / 31	6.5
Bangkok Yai	1 / 16	6.3
Pom Prap Sattru Phai	2 / 33	6.1
Thon Buri	1 / 23	4.4
Dusit	1 / 26	3.9
Phasi Charoen	1 / 30	3.3
Lat Krabang	1 / 32	3.1
Sai Mai	1 / 33	3.0
Yan Nawa	1 / 32	3.1
Samphanthawong	0 / 28	0
Khlong Sam Wa	0 / 30	0
Bang Sue	0 / 12	0
Nong Chok	0 / 28	0

Table 2 Seroprevalence of *T. gondii* infection from stray cats in Bangkok, Thailand.

Parameter	IFAT+ / Total (%)	*p*-value	$\chi 2$	df
Sex		0.8	0.06	1
Male	18 / 193 (9.3%)			
Female	23 / 265 (8.7%)			
Age		0.006	10.4	2
≤ 1 year	2 / 101 (2.0%)			
1-5 years	32 / 318 (10.1%)			
> 5 years	7 / 39 (17.9%)			
Total	**41 / 458 (9.0%)**			

DISCUSSION

The worldwide estimation of *T. gondii* seroprevalence in cats varied between 6-74% (Tenter et al., 2000). Some studies in Asia showed 4.7-62.8% seropositive (Nogami et al., 1998; Ahmad et al., 2001; Maruyama et al., 2003; Zhang et al., 2009; Xie et al., 2010; Lee et al., 2010; Raeghi et al., 2011; Al-Mohammed 2011; Lee et al., 2011; Hong et al., 2013; Erkılıç et al., 2016). Other studies undertaken in Southeast Asia revealed various degrees of infection, i.e., 14.5% in Malaysia (Chandrawathani et al., 2008), 59.4% in Indonesia (Durfee et al., 1976), 30.7% in Singapore (Chong et al., 1993), 72.3% in Vietnam (Hosono et al., 2009) and 4.8-11% in Thailand (Sukhana et al., 2003; Jittapalapong et al., 2007; Jittapalapong et al., 2010). The seroprevalence of *T. gondii* in stray cats in this study was 9.0%. This result is close to the results demonstrated by Sukhana et al. in 2003 (7.3%), Jittapalapong et al. in 2007 (11.0%) and Sukhumavasi et al. in 2012 (10.1%) but higher than that of Jittapalapong et al. in 2010 when the seropositive was only 4.8%. The prevalence of *T. gondii* in cats might vary on type of cat, age, diagnostic test and location (Dubey et al., 2002). This study collected cat samples from 19 districts in Bangkok and positivity was found in 15 districts (78.9%) which is higher than the previous study by Jittapalapong et al. (2010) who reported a 56.0% of positive study sites. In this study, the infection rate was found to be higher in dense population. Since the population of Bangkok ranges between 1,464-25,160 per sq km (The Bureau of Registration Adminstration, 2016), so it is possible that the increased number or residents might lead to the increase of number of cats in those areas and subsequently cause the further spreading of the parasite.

As indicated by the current study and also by the report of Jittapalapong et al. in 2010, the age group could be a factor influencing the risk of exposure to *T. gondii* infection in cats by postnatal transmission of *T. gondii*. This observation could be attributed to the fact that adult cats can go out freely for food or mating. They not only defecate anywhere, but also get tissue cyst or oocysts from the environment. In our study, male cats (9.3%) showed higher prevalence than females (8.7%) which is similar to the study in Korea (Lee et al., 2011; Hong et al., 2013), Africa (Hammond-Aryee et al., 2015) and in Pakistan (Ahmad et al., 2001). Hall et al. (2016) found that male cats have statistically larger home range than females. Therefore, the risk of infection in male cats was higher than female because they could roam unrestrictedly as mentioned above. Moreover, *T. gondii* oocysts can be transmitted by these cats to livestock, pet cats and humans (Moon et al., 2013). In conclusion, the result of this study showed similar seroprevalence of *T. gondii* infection as

compared to those previous studies in Thailand and indicated that stray cats infected with *T. gondii* could be an important source of human and animal toxoplasmosis, mainly when they are free-roaming and may themselves be exposed to *T. gondii* parasites in their respective habitats.

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