Veterinary Integrative Sciences 2021; 19(3): 557 - 566

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Toxoplasma gondii prevalence and risk factors in owned domestic cats from Nakhon Pathom Province, Thailand

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Abstract

Domestic cats are a potential source of *Toxoplasma gondii* infection for humans. This study was conducted to determine the seroprevalence and risk factors for *T. gondii* infection in domestic cats. Cat sera (n = 182) were tested for *T. gondii* IgG antibodies using the latex agglutination test (LAT) and the GRA7 of *T. gondii* (TgGRA7)-indirect enzyme-linked immunosorbent assay (iELISA). Univariable and multivariable logistic regression analyses were used to identify the factors associated with *T. gondii* infection. The overall prevalence rates were 18.1% (33/182) according to LAT, 19.2% (35/182) according to the iELISA and 17.0% (31/182) according to LAT and iELISA. Univariable analyses identified, outdoor access (P = 0.006), being a former stray cat (P = 0.001) and successful hunting behaviors (P = 0.04) as risk factors for *T. gondii* infection. Outdoor access (OR 2.63, 95% confidence interval (CI) 1.03–6.72) and is a former stray cat (OR 3.69, 95% CI 1.52–8.96) remained significant risk factors in multivariable analyses. This study indicated a relatively high seroprevalence of *T. gondii* among domestic cats. Cat owners can reduce the risk for *T. gondii* infection by not allowing their cats to roam free. Furthermore, education about the transmission of the parasite should be provided to prevent infection to the owners.

Keywords: Cat, Dense granule protein 7, Indirect enzyme-linked immunosorbent assay, Latex agglutination test, Risk factors, Serology, *Toxoplasma gondii*

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Article history; *received manuscript: 29 June 2021,*
revised manuscript: 14 July 2021,
accepted manuscript: 20 August 2021,
published online: 26 August 2021
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Toxoplasmosis is a widely distributed zoonotic infection caused by the intracellular parasite in Phylum Apicomplexa, *Toxoplasma gondii*. The parasite infections are widely prevalent in human beings and animals worldwide (Montoya and Liesenfeld, 2004; Stelzer et al., 2019). Members of the Felidae family are a source of infectious sporozoites in oocysts shed in their faeces. Infected cats can shed more than 100 million oocysts in their faeces (Zulpo et al., 2018). Ingestion of oocysts in contaminated food or water is the major source of infection for livestock, wildlife, and marine mammals (Shapiro et al., 2019). Carnivorous and omnivorous intermediate hosts, including humans, may also become infected with *T. gondii* through the ingestion of encysted bradyzoites in meat or organs of other intermediate hosts (Dubey, 2009).

Domestic cats are important reservoirs of *T. gondii* (Dubey, 1976). The prevalence of *T. gondii*-specific IgG in owned cats varies considerably between regions, being the lowest in Japan (6%–16%) (Nogami et al., 1998; Salman et al., 2018) and the highest in Northern Europe (50%–63%) (Jokelainen et al., 2012; Must et al., 2015). Common risk factors for *T. gondii* infection in cats include increasing age, outdoor access, hunting, and a diet containing raw meat (Jokelainen et al., 2012; Must et al., 2015; Salman et al., 2018). Serological studies of urban stray cats (unowned, free-roaming, and dependent on humans for food) conducted in Thailand have reported *T. gondii* seroprevalence rates ranging from 4.8% to 11% in Bangkok (Sukthana et al., 2003; Jittapalapong et al., 2007; Jittapalapong et al., 2010). However, the seroprevalence and risk factors for *T. gondii* infection among owned cats have been reported infrequently in Thailand. Beside feline toxoplasmosis, seroprevalence of *T. gondii* infection in human have been reported among different countries in Southeast Asia including Thailand. The seropositivity rate among Thai people ranged from 3.1 to 53.7% (Maruyama et al., 2000; Wanachiwanawin et al., 2001; Nissapatorn et al., 2003; Sukthana et al., 2003).

Serological methods play a primary role in the diagnosis of *T. gondii* infection in humans and animals (Zhang et al., 2016; Khan and Noordin, 2020). Frequently used diagnostic tests of Toxoplasma infection in humans and animals are based on the serological detection of specific antibodies, such as the enzyme-linked immunosorbent assay (ELISA), the latex agglutination test (LAT) and indirect fluorescent antibody test (IFAT). ELISA conducted using Toxoplasma lysate antigens (TLAs) has been used as a diagnostic method of *T. gondii* infection; however, for routine diagnostic screenings and seroepidemiological surveys, ELISA based on purified recombinant proteins is preferably used due to its easy test standardization and lesser production costs compared with TLAs (Holec-Gasior, 2013). Dense granule antigens of *T. gondii* (GRAs) are secreted in the parasitophorous vacuole and are involved in the survival and virulence of the parasite (Nam, 2009). The effectiveness of TgGRA7 as a serodiagnostic marker for *T. gondii* infection has already been confirmed using indirect ELISA (iELISA), with a sensitivity of 81%–98.9% and a specificity of 98%–100% (Jacob et al., 1999; Kotresha et al., 2012) in humans, and a sensitivity of 94.9% and a specificity of 97.9% in cats (Cai et al., 2015).

In Thailand, several studies are focused on the seroprevalence of *T. gondii* infection in stray cats. However, little information on *T. gondii* infection in owned cats and risk factors was available in Thailand. This study was conducted to determine the seroprevalence of *T. gondii* using TgGRA7 and the risk factors for infection among owned cats.

MATERIALS and METHODS

Animals and samples

The representative sample size was calculated based on an assumed prevalence of 11% (Jitapalapong et al., 2010) and was calculated with 95% confidence at an absolute precision of 5% assuming random sampling using online server (https://epitools.ausvet.com.au/oneproportion). Convenience sampling was conducted using serum samples collected prospectively from owned cats that were presented to Prasuarthon Small Animal Hospital, Faculty of Veterinary Science, Mahidol University, from January 2019 to October 2019. The study was approved by the Animal Care and Use Committee of the Faculty of Veterinary Science, Mahidol University, Thailand (Approval No. MUVS-2018-10-57). Informed consent was obtained from all cat owners involved in this study. Details, including age, sex, breed, neuter status, and health status, were obtained from medical records. Owners who consented to inclusion of their cats in the study completed a questionnaire inquiring information about their cat's domicile, diet, and lifestyle factors. The collected information included outdoor contact (yes or no), diet composition (commercial food, raw meat and/or home-cooked diets), observed hunting behaviors (yes or no), presence of other cats around the house (yes or no), being a former stray cat (yes or no) and availability and use of a litter tray (yes or no).

Indirect ELISA (iELISA)

Recombinant TgGRA7 was expressed from the dense granule proteins of *T. gondii* as described previously (Terkawi et al., 2013). The purified recombinant protein of TgGRA7 fused with glutathione S-transferase was prepared and used as the antigen. The iELISA protocol was performed as previously described (Abdelbaset et al., 2017) with slight modifications. Purified protein (100 µL) at a final concentration of 0.1 µg was coated onto MaxiSorp plates (Nunc, Roskilde, Denmark) overnight at 4 °C in a coating buffer (50 mM carbonate/bicarbonate buffer, pH 9.6). The plates were washed five times with phosphate-buffered saline (PBS) containing 0.01% Tween 20 (PBS/Tween) and blocked with 5% PBS-skimmed milk (PBS-SM) at 37 °C for 1 h. The plates were washed again five times with PBS/Tween, after which 50 µL cat serum diluted 1:250 with PBS-SM was added to each well. The microplates were incubated for 1 h at 37 °C. After, washing, 50 µL horseradish-peroxidase-conjugated anti-cat IgG antibodies (Invitrogen, CA, USA) diluted 1:8000 with PBS was added. The plates were incubated for 1 h at 37°C, and after washing, the colour was developed by the addition of the substrate 3,3′,5,5′-tetramethylbenzidine (Invitrogen, CA, USA). The reaction was then stopped by the addition of 0.1 M HCl. The optical density (OD) was measured

at 450 nm using a microplate reader (model ELx808, Biotex, VT, USA). iELISA results were determined in duplicate for each serum sample. The cut-off point for the OD value of a positive sample was set as the mean value of standard *T. gondii* -negative control cat sera plus five standard deviations. The negative and positive control of field cat sera were confirmed positive using MAST® TOXOREAGENT (Mast Group, Liverpool, UK).

Latex agglutination test

The commercial kit, MAST® TOXOREAGENT (Mast Group, Liverpool, UK) was used to detect *T. gondii* infection. The procedure was performed according to the manufacturer's instructions. Positive samples were considered when agglutination was observed at a dilution of ≥1:32.

Statistical analyses

Data analysis was performed using the SPSS version 25.0 software for Windows (SPSS Inc., IL, USA). The seroprevalence of *T. gondii* infection was calculated for all samples. Seropositive results in both LAT and TgGRA7-based iELISA were used for risk factor analysis. Univariable analysis was performed using the Chi-square test or Fisher's exact test to determine the association between the presence of seropositivity and exposure variables. Variables with a P- value of <0.05 were retained and selected for a multivariate logistic regression model performed via stepwise backward elimination. Model fit was evaluated using the Hosmer-–Lemeshow test (P > 0.05). The results of iELISA and LAT were calculated using online statistical analysis (http://vassarstats.net) to determine the percentage of agreement, the sensitivity and specificity, and the kappa values with a 95% confidence interval. The strength of agreement was graded with kappa values of a fair (0.21–0.40), moderate (0.41–0.60), and a substantial (0.61–0.80).

RESULTS

Demographic characteristics of study population

Of 182 cats sampled from 75 owners, female cats accounted for 48.4% of the population, whereas male cats comprised 51.6%. The majority (43.9%) of cats were adults (aged 2–10 years), followed by young (aged <1 year), comprising 30.7%, sub-adults (aged 1–2 years), comprising 21.4% and old cats (aged >10 years), comprising 3.8%. A purebred cat (13.7%) was not the dominant cat breed in this study. Most (64.3%) cats were neutered and vaccinated (72.5%). Litter trays were provided indoors for 83.5% of cats. A high proportion of cats (78.6%) were kept indoors, whereas the remainder (14.3%) had outdoor access. The rate of presence of other cats around the house was 84%. Being a former stray cat and hunting behaviors accounted for 47.8% and 42.8% of the sampled cats, respectively. All cats were fed with commercial food.

Seroprevalence and risk factor analysis

A total of 182 serum samples collected from cats were tested for anti-*T. gondii* antibodies using LAT and iELISA based on TgGRA7. The seropositivity rates of cat samples were 18.1% (33/182) in LAT, 19.2% (35/182) in iELISA based on TgGRA7 and 17.0% (31/182) in both LAT and iELISA. In this study 60% (45/75) of the owners have more than one cat in their household. Among them, the seropositivity in 2 techniques was 32.3% of cats (10/35)

The univariable analysis revealed significant differences ($P < 0.05$) in seropositivity for outdoor access ($P = 0.006$), being a former stray cat ($P = 0.001$) and successful hunting behaviors ($P = 0.04$) (Table 1). The final model for the multivariable logistic regression analysis (the Hosmer–Lemeshow goodness-of-fit test $P = 0.29$) is presented in Table 2. Outdoor access and being a former stray cat remained significant predictors of seropositivity.

Table 1 Univariable analysis of risk factors associated with *T. gondii* infection

Variables	Total test	Seropositive	P-value	OR (95% CI)
1. Sex				
Female	84	16	0.63	0.82 (0.38–1.80)
Male	92	15	Ref	
2. Age				
Young (≤ 1year old)	53	8	0.95	0.93 (0.09–8.86)
Sub-adult (1-2 years old)	39	9	0.60	0.55 (0.05–5.24)
Adult (2-10 years old)	77	13	0.86	0.82 (0.09–7.40)
Old (> 10 years old)	7	1	Ref	
3. Breed				
Mongrel breed	153	25	0.25	1.80 (0.64–5.03)
Pure breed	23	6	Ref	
4. Neutering				
Yes	112	19	0.76	0.88 (0.39–1.96)
No	64	12	Ref	
5. Vaccination				
Yes	126	25	0.21	1.81 (0.69–4.73)
No	50	6	Ref	
6. Use of litter tray				
Yes	147	23	0.12	0.48 (0.19–1.23)
No	29	8	Ref	
7. Cats around the house				
Yes	148	25	0.56	1.34 (0.49–3.64)
No	28	6	No	
8. Outdoor access				3.36 (1.36–8.26)
Yes	28	10	0.006	
No	148	21	Ref	
9. Hunting behaviors				
Yes	63	16	0.043	2.22 (1.01–4.87)
No	113	15	Ref	
10. Former stray cats				
Yes	82	23	0.001	4.19 (1.75–10.05)
No	94	8	Ref	

CI: Confidence interval
OR: Odds ratio

Table 2 The final multivariable logistic regression model for *T. gondii* seropositivity in owned cats

Variable	Total test	OR (95% CI)	P-value
1. Outdoor access			
Yes	28	2.63 (1.03–6.72)	0.04
No	148	Ref	
2. Former stray cats			
Yes	82	3.69 (1.52–8.96)	0.002
No	94	Ref	
3. Hunting behaviors			
Yes	63	1.40 (2.96-0.66)	0.38
No	113	Ref	

CI: Confidence interval
OR: Odds ratio

Comparison between iELISA and LAT

We compared the efficiency of TgGRA7-iELISA with that of commercial LAT, MAST® TOXOREAGENT (Mast Group, Liverpool, UK). The TgGRA7-iELISA had a sensitivity of 92.6% and a specificity of 92.9% for the detection of IgG antibodies. A substantial agreement between the two methods was indicated by $\kappa = 0.89$ (Table 3).

Table 3 Comparison of LAT and TgGRA7 recombinant protein-based iELISA for the detection of IgG antibodies against *T. gondii* infection

TgGRA7 iELISA	LAT			Sensitivity (95% CI)	Specificity (95% CI)	Kappa value
	Positive	Negative	Total			
Positive	31	4	35	93.93	97.31	0.89
Negative	145	2	147	(78.37–98.94)	(92.84–99.13)	
Total	176	6	182			

CI: Confidence interval

DISCUSSION

Cats are one of the most common companion animals and are frequently in close contact with humans (Overgaauw et al., 2020). As a definitive host of *T. gondii*, cats play a vital role in the transmission of oocysts to humans and other animals (Dubey, 1976). The global seroprevalence of *T. gondii* in domestic cats has been estimated to be approximate 30%–40% (Dubey, 2010). The seroprevalence of this parasite has also been investigated in Asian countries, where a high rate of infection was reported in Myanmar (41.3%) (Bawm et al., 2020), China (25.2%) (Zhang et al., 2009), Singapore (30.7%) (Chong et al., 1993), Indonesia (59.4%) (Durfee et al., 1976), Iran (63%) (Haddadzadeh et al., 2006) and Vietnam (72.3%) (Hosono et al., 2009) whereas the low prevalence rate was found in Malasia (5.5%) (Tan et al., 2020). In the present study, the seroprevalence of *T. gondii* infection in owned cats was found to be 17% (31/182). The seropositivity found in cat samples was similar to the value

of 18.7% reported in a previous study conducted in cats in Bangkok (Huertas-López et al., 2021). However, the seroprevalence of *T. gondii* in cats in this study was higher than that of previous reports in stray cats from Bangkok, wherein the seroprevalence ranged from 4.8% to 11% (Jittapalapong et al., 2007, 2010). This result indicated that the owned cats are exposed to *T. gondii* and possibly play a significant role in maintaining the parasite. However, the different methodologies used, different sample sizes and sample populations in the regions surveyed may have contributed to these differences. The infected cats shed oocysts for a short time before their immune response stops oocyst production (Dubey, 1995). However, infected cats can excrete millions of oocysts through their faeces that contaminate the environment and become a source of infection for humans and other warm-blooded animals (Hill and Dubey, 2002). In previous research conducted using Thai human sera, the seroprevalence of *T. gondii* was 6.4%, and the seropositivity was associated with people living with seropositive cats (Sukthana et al., 2003). Because the seropositive cats in this study are in close contact with their owner, the detection of antibodies to *T. gondii* among pet owners requires further investigation.

Various serological tests have been used to detect *T. gondii* infection (Liu et al., 2015). Modified agglutination test (MAT) and immunofluorescence assay (IFA) were frequently used for serodiagnosis of feline toxoplasmosis (Dubey et al., 2020). Fernandes et al., 2019 has been reported that the commercial LAT has low sensitivity and specificity compared with MAT. However, previous studies have been used LAT as a reference test of *T. gondii* infection in cats (Abdelbaset et al., 2017; Ybañez et al., 2019; Ybañez et al., 2020). Recombinant TgGRA7 has been used in other studies and demonstrated to be a good serological marker for the detection of *T. gondii* infection in human and animal samples (Holec-Gasior, 2013; Fereig et al., 2016; Udonsom et al., 2021). In the present study, we used the recombinant TgGRA7-iELISA to detect *T. gondii* infection. Our results support those of previous studies showing high specificity (93.93%) and sensitivity (97.31%) for the detection of IgG in cat sera (Cai et al., 2015; Ybañez et al., 2020). Therefore, this method could be used as an alternative test for the serological testing of *T. gondii* in cats.

Outdoor access and hunting behaviors were identified as risk factors associated with *T. gondii* infection in this study. Outdoor cats were 2.63 times more likely to be infected with the parasite than indoor cats. This result is consistent with that of previous reports showing that outdoor cats are at a higher risk of being exposed to *T. gondii* than cats exclusively kept indoors (Nagomi et al., 1998; Salman et al., 2018). The high seroprevalence in those cats could be explained by an increased probability of the cats ingesting oocyst-contaminated food or primarily tissue cysts in intermediate hosts. Cats living outdoors could hunt and eat infected small mammals or birds (Gauss et al., 2003). This implied their higher seroprevalence in cats with hunting habits. Most cats in this study (84%) were kept indoors, but 25% of them were hunters. Small rodents entering residential buildings could be a potential source of *T. gondii* infection in the cats. In addition to outdoor access and hunting behaviors, being a former stray cat was identified as a predisposing factor for *T. gondii* infection. This could be explained by the high level of hunting in the past or exposure to *T. gondii* oocysts contaminated in the environment of a

former stray cat. In Thailand, there is an increasing trend of adoption of pet cats from shelters or monasteries. Stray cats comprise an important source of several zoonotic diseases, including *T. gondii* infection (Gerhold and Jessup, 2012). Therefore, education about zoonotic diseases and their prevention should be provided to the owners in the near future.

Most cats become infected soon after weaning. However, in our study, age was not identified as a risk factor in cats. This result is in contrast to a previous study that reported the seropositivity with increasing age was considered as a risk factor for *T. gondii* infection in cats (Must et al., 2017). This could be due to a possible bias in the present study and limited amount of cat samples. However, our results demonstrated that the older cats were not aged > 1 year had a higher prevalence than younger cats (aged <1 year). We did not find an association between sex and *T. gondii* seropositivity, suggesting that both female and male cats are equally exposed and susceptible to *T. gondii* infection (Must et al., 2015). The presence of stray cats around the house was not identified as a risk factor for seropositivity. This might be explained by the fact that most cats in this study were strictly maintained indoors. However, this finding is consistent with that reported by Opsteegh et al. (2012) who found that contact with stray cats did not confer an increased risk for *T. gondii* infection.

ACKNOWLEDGEMENT

We would like to thank all the cat owners and veterinarian who participated in this study for their cooperation. The authors thank Dr. Phirom Prompiram for technical assistance. This research project is supported by the Faculty of Veterinary Science, Mahidol University.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: CJ. Performed the experiments: RU, RB, CJ. Analyzed the data: CJ. Contributed reagents, materials, and analysis tools: RMF, YN. Project supervisor: YN. Wrote the manuscript: RU, CJ. All authors read and approved the final manuscript

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How to cite this article;

Ruenruetai Udonsom, Ruangrat Buddhirongawatr, Yoshifumi Nishikawa, Ragab M. Fereig and Charoonluk Jirapattharasate. *Toxoplasma gondii* prevalence and risk factors in owned domestic cats from Nakhon Pathom Province, Thailand. Veterinary Integrative Sciences. 2021; 19(3): 557 - 566.