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ORIGINAL ARTICLE

Prevalence of *Toxoplasma gondii* Antibodies in Stray Dogs from Various Locations in West and East Malaysia

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Abstract: Toxoplasmosis is caused by an obligate intracellular protozoan parasite; *Toxoplasma gondii*, which is one of the most important zoonotic parasite worldwide. In dogs, the sexual reproductive cycle of *T. gondii* is lacking, and the animals are not widely consumed as food, but they are vital in the mechanical transmission of the parasite. However, there is no present data on the exposure of stray dogs to *T. gondii* in Malaysia. The objective of this serological survey was to determine the prevalence of *T. gondii* antibodies (IgG) and associated factors in stray dogs in East and West Malaysia. Antibodies to *T. gondii* were determined in serum samples from 222 stray dogs from 6 different states in East and West Malaysia (Peninsular Malaysia) using an Indirect ELISA. The seroprevalence for *T. gondii* was 23.4% (Confidence interval: CI 17.8-29.2%). Stray dogs from Selangor and Kuala Lumpur had the highest seroprevalence (32.4%; CI 13.2-45.5%) and lowest in those from Penang and Kedah (12.5%; CI 1.3-23.5%). Gender and breed were not associated with *T. gondii* seropositivity. However, adult dogs were more likely to be seropositive for *T. gondii* (OR = 2.89; CI 1.1-7.7) compared with younger dogs. These results revealed that *T. gondii* is prevalent in stray dogs in the studied areas in Malaysia, and indicative of the level of environmental contamination of this parasite especially in urban areas.

Key words: *T. gondii*, stray dog, prevalence, ELISA, Malaysia

INTRODUCTION

Toxoplasmosis is caused by an obligate intracellular protozoan parasite; *Toxoplasma gondii* capable of infecting all warm-blooded animals including humans, mammals and birds [1,2]. Toxoplasmosis is one of the most important zoonotic parasitic diseases worldwide [1,3]. It is considered as one of the most effective parasitic infections with about 1/3 of the human population infected by the pathogen [3]. Although toxoplasmosis appears asymptomatic in most humans, the risks are severe in pregnant women as it may cause severe health implications to the fetus [1,2].

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Felids are the only definitive host of *T. gondii* with an infected cat capable of discharging millions of infective oocysts in feces for few days post-infection [4]. Humans, rodents and other animals serve as intermediate hosts and become infected through ingestion of oocysts from the environment either by consumption of contaminated food, undercooked meat containing *T. gondii* tissue cysts, or direct contact with oocysts excreted in cats feces [5,6]. While the distribution of the tissue cysts may vary among the intermediate hosts, the ingestion of tissue cysts from undercooked meat remains the major route of infection in humans [5,6].

The sexual reproductive phase of *T. gondii* is lacking in dogs, and the animals are not widely consumed as food, but they could serve as means of mechanical transmission of the parasite [7,8]. Dogs infected with *T. gondii* may not manifest clinical signs; however, they may occasionally show digestive, respiratory, and neurological signs and muscular anomalies, especially in immunocompromised patients [3]. Particularly, the risk of transmission of the *T. gondii* in dogs has been linked to their coprophagic and rolling behavior over grass or feces probably contaminated with oocysts [9,10].

The presence of *T. gondii* oocyst in the environment is an important portal for acquired toxoplasmosis [11]. Information on the environmental burden of *T. gondii* can be indirectly obtained from data on its seroprevalence in free-living animals. Stray dogs are considered as sentinels for indirect measurement of the burden of *T. gondii* in the environment, since they often roam about and are in close contact with the contaminated environment inhabited by humans [9]. The similarity in the prevalence of *T. gondii* infection in dogs and humans is also indicative of the former as an adequate sentinel of the parasite [12-14]. In comparison to domestic dogs, there is relatively fewer studies conducted in stray dogs to understand their role in the transmission of *T. gondii*. Most studies have focused on domestic dogs and cats probably due to their close interaction with humans [5,6].

In Malaysia, previous studies have reported the prevalence of *T. gondii* in domestic dogs, goats, poultry and wild boar and exotic meats [15-18]. A recent study also reported high seroprevalence of *T. gondii* (57.4%; 52.7-61.8%) among migrant workers in farms and plantations [19]. The increasing urbanization in the main cities may also influence *T. gondii* transmission and distribution in the environment [13]. However, there is no information about *T. gondii* prevalence in stray dogs in any parts of the nation. In view of this background, the objective of this study was to determine the prevalence of *T. gondii* antibodies in stray dogs and to identify the associated risk factors in various states in Malaysia.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee University Putra Malaysia (IACUC) (Approval code: R074/2013). Shelters were identified from the directory provided by the Department of Veterinary Services. The management officers in the respective shelters were briefed about the aim of the study and methodology, and those willing to participate were informed about the scheduled sampling dates. Upon visits to the shelters, the sample size was estimated based on the total number of stray dogs in each shelter. A random sample of stray dogs were selected from each shelter until the required sample size was reached. The sampled dogs were from the quarantine units and were newly introduced to the animal shelters. A total of 222 stray dogs were sampled from animal shelters in different states in Malaysia namely Selangor, Johor, Penang/Kedah, Pahang, Sabah and Sarawak, as well as three shelters located in Kuala Lumpur (KL) and Klang valley (Fig. 1).

The dogs were of different sex, age and breed and the sampling was conducted from May 2013 to June 2014. Dogs were categorized as young if within the age of 0 to 18 months, while those above 18 months were considered as adult. The breeds were categorized into pedigree (known breed) and local (no defined breed). Thereafter, the dogs were restrained appropriately and 3ml of blood was collected via venipuncture into plain tubes and separated for serology. Sera samples were kept in –20˚C till further analysis. The sera samples were subjected to a commercially available ELISA test kit (ID Screen® Toxoplasmosis-Indirect Multi-Species Test Kit, France). Briefly, the assay

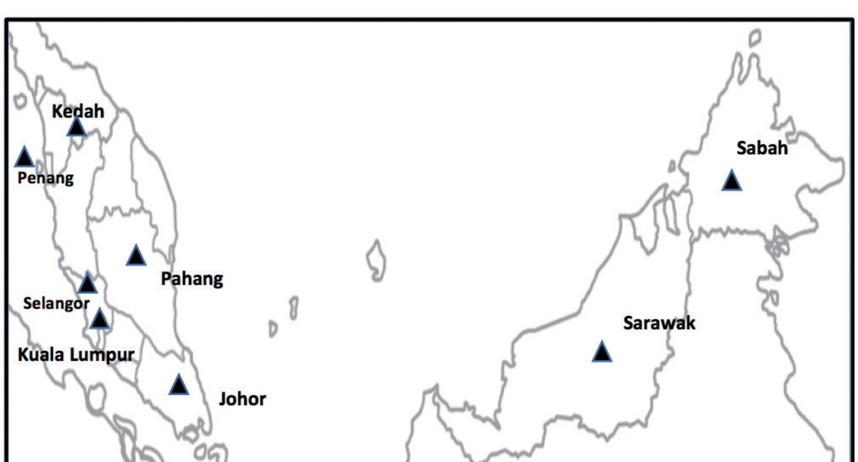

Fig. 1. A map showing serum sampling areas (▲) from stray dogs in East and West Malaysia.

utilizes *T. gondii* P30 antigen and anti-multi-specie IgG conjugates, which detects *T. gondii* antibodies from ruminants, cats, dogs and swine. The sample to positive ratio (S/P) were calculated for each sample (S/P %: [OD sample/OD positive control × 100]). As recommended by the manufacturer, samples with S/P of ≥50% were considered positive, S/P of 40 to ≤50% as doubtful and S/P ≤40% as negative. Samples with doubtful results were considered as negative in this study.

Data were analyzed using SPSS Version 25 (IBM, Armonk, New York, USA). Descriptive statistics were applied to summarize the data and to determine the seroprevalence of *T. gondii* in the studied population. Binary logistic regression models were applied to determine the factors associated with *T. gondii* seroprevalence. At univariable level, each factor was tested with the dependent variable, and $P<0.1$ was considered for any factor to be introduced into the next model. At multivariate level, $P<0.05$ was considered for any significant relationship, while odds ratio (OR) and 95% confidence interval (CI) were used to express the strength of the association.

RESULTS

Table 1 shows the characteristics of the study population and the corresponding seroprevalence. The proportion of female and male stray dogs was 52.3% and 47.3% respectively. Twenty percent of them were classified as young (0-18 months), whereas 80% were above 18-month-old (adults). Majority of the dogs were local breeds (83.3%) compared to those of specific breed type (16.7%). The number of sampled animals from each sampling site ranged from 34 to 40 dogs (Table 2). Among the 222 animals, 52 (23.4%; CI 17.8-29.2%) were found to be seropositive for *T. gondii* antibodies. The highest seroprevalence was observed in KL/Selangor (32.4%; 11/34), followed by Sabah (9/37; 24.3%), and lowest in Penang and Kedah (5/40; 12.5%).

Table 1. Seroprevalence of *Toxoplasma gondii* antibodies in stray dogs from various locations in East and West Malaysia

Factors	Frequency	%	Seroprevalence	%
Gender				
Male	106	47.7	22	20.8
Female	116	52.3	30	25.9
Age				
Young	45	20.3	5	11.1
Adults	177	79.7	47	26.6
Breed				
Pedigree	37	16.7	8	21.6
Local	185	83.3	44	23.8
Location				
KL/Selangor	34	15.3	11	32.4
Johor	37	16.7	9	24.3
Pinang and Kedah	40	18	5	12.5
Pahang	37	16.7	8	21.6
Sabah	37	16.7	11	29.7
Sarawak	37	16.7	9	24.3
Total	222	100	52	23.4

CI: confidence interval.

The association between the animal-based factors (breeds, age, and sex), location, and *T. gondii* seroprevalence is presented in Table 2. At Univariable level, only the age of the animals was associated with the outcome, as adult dogs were 2 times more likely to be seropositive for *T. gondii* (OR = 2.8; 95% 1.1-7.7) compared to young dogs. The seroprevalence between male and female stray dogs were 20.8% and 25.9% respectively, but gender was not associated with *T. gondii* seroprevalence ($P=0.37$). Likewise, breed and location were not associated with prevalence of *T. gondii* antibodies. However, marked difference in the seroprevalence estimates was observed between KL/Selangor (32.4%) and Pinang/Kedah (12.5%). At multivariable level, adult dogs were more likely to be seropositive (OR = 2.89; CI 1.07-7.7) compared with the young group.

DISCUSSION

This study is the first to report the seroprevalence of *T. gondii* among stray dogs in Malaysia. *T. gondii* antibodies were detected in 23% of the stray dogs examined, which may be a pointer of high oocyst environmental contamination in the studied locations. Humid tropical climates are conducive for the maintenance of soils and water where *T. gondii* oocysts are widely distributed [20]. This similar climatic condition in Malaysia coupled with the interaction between domestic and wild animals could contribute to the maintenance of transmission oocysts of the parasite. The seroprevalence rate in this study (23%) is higher than the one reported in pet dogs in Peninsular Malaysia (9.6%) by Chandrawathani et al. [15]. One possible reason for the high prevalence in stray dogs is the ingestion of food thrown in garbage cans and rubbish on the streets. Stray dogs are more exposed to *T. gondii* oocysts compared to owned dogs [33,38]. Moreover, stray dogs were observed roaming the streets, public places, and open markets in areas not too far from the sampled shelters.

Different serological techniques are used to detect *T. gondii*

Table 2. Analytics on association of *Toxoplasma gondii* infection factors in stray dogs in East and West Malaysia

	Univariable and multivariable model					
	B	S.E.	Wald	*P*-value	OR	95% CI
Gender						
Male	0.07	0.6	0.4	0.37	1.3	0.7-2.4
Female						
Age						
Young	-1.2	0.6	6.4	0.03*	2.8	1.1-7.7
Adults						
Age[a]						
Young	-1.2	0.5	7.8	0.01**	2.7	1.2-4.5
Adult						
Breed						
Pedigree	0.08	0.5	0.3	0.77	2.8	0.3-2.1
Local						
Location			0.4	0.51		
KL/Selangor	0.06	0.6	1.1	0.48	4.1	0.4-10.1
Johor	-0.9	0.8	0.7	0.62	4.2	0.2-2.7
Pinang and Kedah	-0.6	0.6	1.0	0.36	2.3	0.1-6.0
Pahang	-0.8	0.7	1.5	0.42	3.5	0.2-8.2
Sabah	-0.7	0.5	1.0	0.52	2.4	0.2-8.1
Sarawak						

CI, confidence interval; OR, odds ratio; S.E, standard error.
[a]Multivariable model for the factor "age".
*Significant at univariable model ($P < 0.1$).
**Significant at multivariable model ($P < 0.05$).

antibodies in dogs. This include modified agglutination test [21,22], latex agglutination test [23], indirect fluorescence antibody test [24] and ELISA [14]. In this study, ELISA was applied for detecting antibodies for *T. gondii*. Several researchers have used ELISA and few studies comparing the efficacy between various serological methods supported the use of ELISA for *T. gondii* antibodies detection [25,26]. The wide application of ELISA for epidemiological investigation of toxoplasmosis is due to its high sensitivity, cost-effectiveness, and practicality [2,5]. In addition, immunoglobulin G; IgG antibodies for *T. gondii* were assessed in the present study. This is supported by the fact that upon *T. gondii* infection, IgG antibodies often appears within the first or second week and reach a peak after a month [5]. Moreover, the assay used to identify the antibodies has a specificity and sensitivity of 98% and 95%, respectively [26]. In comparison to other studies where related immunodiagnostic method was applied, our result is similar to the seroprevalence reported among stray dogs in Czech Republic (25.9%) [27] and Turkey (19.8%) [28], lower compared to results from China (51.9%) [29], Brazil (70.9%) [30], Sri Lanka (67.4%) [31], and Portugal (38.0%) [32], but higher than results among stray dogs in Philippines (15.2%) [33] and Northwestern China (14.5%) [34]. Differences in the prevalence estimates in different regions result from the predominance of factors influencing exposure to the parasite. Specifically, comparison between these studies suggest that climatic conditions in various countries could influence *T. gondii* seroprevalence, with moist and warmer locations recording higher prevalence rates [5].

Based on the data analysis by location, results showed no significant difference in the frequency of *T. gondii*. However, 32.5% of the samples from KL/Selangor were seropositive compared to those from Penang/Kedah (12.5%). The seroprevalence rates in most of the locations suggest that similar factors may be influencing the exposure of stray dogs to *T. gondii* infection [35]. Nevertheless, the location–KL/Selangor with highest seroprevalence is relatively more urbanized and densely populated, which may increase the exposure of stray dogs to environment contaminated with oocysts. Saldanha-Elias et al. [36] reported that stray dogs from urban areas had higher risk of *T. gondii* seropositivity compared to those from rural areas. Specific factors common in urban areas such as high population of cats, human-related activities leading to more garbage cans in the street, and exposure to poorly cooked meat may heighten the risk of *T. gondii* infection in stray dogs [35]. Additionally, shelters in the sampled areas with high seroprevalence might have cats that readily interact with the quarantined dogs or share a common environment. Although, we did not assess the population of cats in the shelters, the role of cats in the transmission of this parasite in the sampling area is equally important. Other likely reasons for the high seroprevalence include: 1) the availability of preys such as rodents for stray dogs to hunt; 2) garbage consumption as majority of the dogs examined from the shelters were rescued from roads where they were exposed to contaminated garbage, infected small mammals and birds; 3) feeding on water and leftover food contaminated with *T. gondii* oocysts.

The present study showed that seroprevalence of *T. gondii* was not associated with breed and gender. This is similar to the findings of Yan et al. [14] in which no associations were observed in seroprevalence between various breeds of stray dogs. Also, the gender of stray and owned dogs was not associated with *T. gondii* infection in other studies [21,37]. This finding might be linked to the absence of marked behavioral difference between male and female stray dogs, which may influence exposure to the parasite [38]. There was a significant association between age and *T. gondii* seroprevalence, as adults were more likely to be infected compared with young dogs. This corroborates the results of Yan et al. [14] and Lopes et al. [21] where the levels of *T. gondii* antibody increased with age. Adult dogs are more likely to be exposed to *T. gondii* oocysts as they have greater roaming behavior and may come in contact with environment, garbage and leftover foods contaminated by the parasite.

This is the first report of *T. gondii* antibodies detected in stray dogs in Malaysia. The present result suggests high exposure of stray dogs to the parasite in East and West Malaysia. Adult stray dogs and those from high urban areas such as KL and Selangor had the higher risk of exposure to *T. gondii*. The high prevalence of *T. gondii* infection in stray dogs reflects the magnitude of the parasite contamination in the environment, and the need for preventive measures against their potential role in mechanical transmission of the parasite.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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