Seroprevalence of *Toxoplasma gondii* in outdoor dogs and cats in Bangkok, Thailand

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Research Article

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Abstract

The aim of this study was to estimate the seroprevalence and risk factors associated with *Toxoplasma gondii* exposure in dogs and cats from Bangkok, Thailand. Blood samples from 318 dogs and 321 cats were tested for *T. gondii* antibodies by modified agglutination test (cut-off 1:25). Additionally, 18 dogs and 20 cats were longitudinally sampled for *T. gondii* antibodies during the same study period, between June and July 2019. The overall seroprevalence in dogs and cats was 7.9% (25/318; 95% CI 4.9–10.8%) and 18.7% (95% CI 14.4–23.0%), respectively. For dogs, risk factors identified were being a mixed-breed animal and living totally outdoors, while increasing age was shown to be a risk factor for cats. Seroconversion was not detected and titres from positive animals remained constant over longitudinal study. The present study indicates that there is a prominent presence of *T. gondii* in urban and peri-urban areas of Bangkok, suggesting that outdoor dogs and cats should be considered as a possible risk factor for humans.

Introduction

Toxoplasmosis is a worldwide distributed zoonosis caused by the protozoan *Toxoplasma gondii* (Dubey, 2010). Felids are the only definitive hosts of this parasite, with a variety of warm-blooded vertebrates acting as intermediate hosts. Felids excrete oocysts by feces, which can be a source of infection for other animals. Dogs can act as mechanical vectors of *T. gondii*, as it has been shown that oocysts are present on their fur due to the habit of rubbing on the ground where feces are present (Frenkel et al., 2003). Dogs can also act as mechanical disseminators because of their coprophagous behaviour, resulting in the risk of ingestion of oocysts that remain viable until they appear in the dog’s feces (Lindsay et al., 1997; Schares et al., 2005; Cong et al., 2018).

According to the World Animal Health Information Database (OIE, 2019), the dog and cat population in Thailand has increased markedly during the last decade. Since 2009, the census increased from about 6.1 and 1.9 million dogs and cats, respectively, to 8.2 and 2.5 million dogs and cats in 2019. In addition to the traditional pet ownership, community dog and cat keeping is a common practice in Thailand and more specifically in Bangkok, which consists of providing food and water to stray animals, but not housing, so they maintain a life completely outdoors and do not usually receive veterinary care (Savvides, 2013; Toukhsati et al., 2015). Both household and free-roaming cats could act as a reservoir of *T. gondii*, which may imply a risk for people having contact with cat’s feces or contaminated environment.

In this context, previous studies have shown that 6.4% of cat owners in Bangkok were seropositive (Sukthana et al., 2003). Moreover, *T. gondii* has been found to be widely distributed in the human population in Thailand, as indicated by recent studies in which seropositivity of 17.3% was estimated in 5101 pregnant women (Rostami et al., 2020) and the presence of acute toxoplasmosis infection in 2.8% of seropositive women (Rostami et al., 2019). Drinking water was identified as a risk factor for *T. gondii* infections in 190 of 760 (25%) pregnant women tested in a hospital in southern Thailand, suggesting contamination of water with oocysts excreted by cats (Andiappan et al., 2014).

Considering that dogs and cats can be involved in the maintenance of the urban and peri-urban life cycle of *T. gondii*, and that they usually share the same areas within cities, serological...
surveys in both species are the key aspect to address control strategies of this zoonotic pathogen, especially in areas where there are colonies of free-roaming dogs and cats in close contact with humans (Fábrega et al., 2020; Park et al., 2020). In fact, this health concern is reflected in various studies of *T. gondii* seroprevalence in dogs and cats previously conducted in several areas around the world from a One Health perspective (Rengifo-Herrera et al., 2017; Cong et al., 2018; de Oliveira et al., 2019; Park et al., 2020). In this respect, serological survey studies provide valuable information that allows us to know the contact of host species with *T. gondii* and, indirectly, to interpret the epidemiological risk that the presence of these animals implies for the human population (Ding et al., 2017). In the specific case of Bangkok, this is a very interesting epidemiological scenario for studying shared diseases between humans and domestic animals, due to the aforementioned community pet keeping, which results in a close contact between humans and free-roaming dogs and cats (Jittapalapong et al., 2007). In Bangkok, the seroprevalence of *T. gondii* infection has been previously assessed in cats (Jittapalapong et al., 2010; Sukhumavasi et al., 2012) and dogs (Jittapalapong et al., 2009) separately. *Toxoplasma gondii* antibodies were detected in 7 of 114 (6.1%) dogs from dairy farms previously described by Dubey and Desmonts (1987). Serum diluted for a longitudinal study (Table 2). Blood samples were collected from 18 dogs and 20 cats that had been sampled several consecutive times with a minimum of 3 weeks of difference between blood draws were also used for a *T. gondii* longitudinal study (Table 2). Blood samples were collected by puncture of jugular or cephalic veins using sterile tubes with an anticoagulant (EDTA or heparin). Samples were transported to the laboratory under refrigeration conditions (4°C).

### Materials and methods

#### Animals surveyed and sample collection

A total of 639 (318 dogs, 321 cats) blood samples were obtained from Bangkok (Table 1). Selected animals had in common a history of outdoor life, irrespective of being owned, stray or community pet animals. All samples were opportunistically collected from animals admitted to the CU Small Animal Teaching Hospital, Chulalongkorn University (Bangkok), or to Neutering Service of Rabies Control Group, Department of Health, Din Daeng (Bangkok) between June and July 2019. Additionally, blood samples from 18 dogs and 20 cats that had been sampled several consecutive times with a minimum of 3 weeks of difference between blood draws were also used for a *T. gondii* longitudinal study (Table 2). Blood samples were collected by puncture of jugular or cephalic veins using sterile tubes with an anticoagulant (EDTA or heparin). Samples were transported to the laboratory under refrigeration conditions (4°C).

#### Serological testing

Blood samples were centrifuged for 5 min at 2370 g and plasma was stored at −20°C. Modified agglutination test (MAT) was used to assay the presence of antibodies against *T. gondii* as previously described by Dubey and Desmonts (1987). Serum diluted from 1:25 to 1:3200 (with a minimum titre of 1:200) from a naturally infected pig was included in each test as a positive control. PBS was used as a negative control. Samples were tested at 1:25, 1:50, 1:100 and ≥1:500. Samples with a 1:25 titre or higher were considered positive. MAT has been extensively used for the diagnosis of toxoplasmosis in both species (El Behairy et al., 2013; Dubey et al., 2013a, 2013b; de Almeida et al., 2016). Although there is no validation of MAT for the detection of *T. gondii* antibodies in cats and dogs, a good correlation has been found between the results of bioassays in mice and serology results at 1:25 dilution (Dubey, 2010; Dubey et al., 2020a, 2020b).

#### Table 1. Seroprevalence of *Toxoplasma gondii* (MAT; titre ≥1:25) in outdoor dogs and cats in Bangkok, Thailand

| Breed                      | Positive/overall (% MAT positive) | P value | Breed                      | Positive/overall (% MAT positive) | P value |
|----------------------------|----------------------------------|---------|----------------------------|----------------------------------|---------|
| Mixed-breed                | 16/104 (15.4%)                  | 0.001   | Male                       | 12/175 (6.9%)                   | 0.385   |
| Purebred                   | 9/198 (4.5%)                    |         | Not spayed/neutered        | 13/164 (7.9%)                   |         |
| Female                     | 13/136 (9.6%)                   |         | Male                       | 12/175 (6.9%)                   | 0.385   |
| Male                       | 13/136 (9.6%)                   | 0.385   | Sub-adult                  | 1/25 (4%)                       | 0.113   |
| Spayed or neutered         | 11/125 (8.8%)                   | 0.790   | Adult                      | 13/106 (12.3%)                  |         |
| Spayed/neutered            | 11/125 (8.8%)                   |         | Old                        | 11/187 (5.9%)                   |         |
| Not spayed/neutered        | 13/164 (7.9%)                   |         | Outdoor contact            |                                  |         |
| Kitten                     | 8/80 (10%)                      | 0.010   | Total                      | 7/31 (22.6%)                    | 0.001   |
| Sub-adult                  | 10/62 (16.1%)                   |         | Partial                    | 25/287 (7.9%)                   | 0.984   |
| Adult                      | 18/88 (20.5%)                   |         |                             |                                  |         |
| Old                        | 7/16 (43.8%)                    |         |                             |                                  |         |
| Purebred                   | 1/9 (11.1%)                     |         |                             |                                  |         |
| Not spayed/neutered        | 19/79 (24.1%)                   |         |                             |                                  |         |
| Male                       | 23/135 (17%)                    |         |                             |                                  |         |
| Female                     | 35/217 (16.1%)                  |         |                             |                                  |         |

MAT, modified agglutination test.
Data collection
Whenever possible, data were collected from the animals by completing a clinical form. Animals were grouped according to species (cat or dog), sex (male or female), breed (purebred or mixed-breed), spayed/neutered animals (yes or no) and degree of outdoor contact (animals living permanently outdoors or animals living partially enclosed, which are those animals who live in a house but also, to a greater or lesser degree, have outdoor access). With regard to the estimated age, pets were grouped into several categories, depending on whether they were dogs (sub-adult 10–36 months old, adult 3–8 years old and old individuals >8 years old) or cats (kitten ≤1 year old, sub-adult 1–2 years old, adult 2–10 years old and old cats >10 years old).

Statistical analysis
Seroprevalence of *T. gondii* was estimated from the ratio of positive samples to the total number of samples tested, with the exact

| ID | Animal | Species | Age       | First day | <1 week | 1-2 weeks | 2-3 weeks | 3-4 weeks | 4-6 weeks | >6 weeks |
|----|--------|---------|-----------|-----------|---------|-----------|-----------|-----------|-----------|---------|
| P2 | Dog    | Old     | Negative  | NP        | Negative| NP        | NP        | Negative  | NP        | NP      |
| P19| Dog    | Old     | Negative  | NP        | Negative| NP        | NP        | Negative  | NP        | NP      |
| P24| Dog    | Old     | Negative  | NP        | NP      | NP        | NP        | Negative  | NP        | NP      |
| P27| Dog    | Old     | Negative  | NP        | NP      | NP        | NP        | Negative  | NP        | NP      |
| P29| Dog    | Old     | Negative  | NP        | NP      | NP        | Negative  | NP        | NP        | NP      |
| P34| Dog    | Adult   | Negative  | NP        | Negative| Negative  | Negative  | Negative  | NP        | NP      |
| P36| Dog    | Old     | Negative  | NP        | NP      | NP        | Negative  | Negative  | NP        | NP      |
| P41| Dog    | Sub-adult | Negative | NP        | NP      | NP        | NP        | Negative  | NP        | NP      |
| P44| Dog    | Sub-adult | Negative | Negative  | NP      | NP        | Negative  | NP        | NP        | NP      |
| P49| Dog    | Sub-adult | Negative  | Negative  | Negative| Negative  | Negative  | NP        | NP        | NP      |
| P53| Dog    | Adult   | Negative  | NP        | NP      | NP        | NP        | Negative  | NP        | NP      |
| P58| Dog    | Adult   | Negative  | NP        | NP      | NP        | Negative  | NP        | NP        | NP      |
| P69| Dog    | Old     | Negative  | NP        | NP      | NP        | Negative  | NP        | NP        | NP      |
| P71| Dog    | Adult   | Negative  | NP        | NP      | NP        | Negative  | NP        | NP        | NP      |
| P88| Dog    | Old     | 1:25      | NP        | NP      | NP        | 1:25      | NP        | NP        | NP      |
| P107| Dog   | Old     | Negative  | NP        | NP      | NP        | Negative  | NP        | NP        | NP      |
| P172| Dog  | Adult   | Negative  | NP        | Negative| NP        | Negative  | NP        | NP        | NP      |
| P318| Dog  | Old     | Negative  | NP        | NP      | NP        | Negative  | NP        | NP        | NP      |
| G4  | Cat    | Old     | Negative  | NP        | NP      | NP        | NP        | Negative  | NP        | Negative|
| G9  | Cat    | Adult   | Negative  | NP        | NP      | NP        | NP        | NP        | Negative  | NP      |
| G14 | Cat    | Adult   | Negative  | NP        | NP      | NP        | NP        | Negative  | NP        | NP      |
| G15 | Cat    | Adult   | Negative  | NP        | Negative| Negative  | Negative  | NP        | NP        | NP      |
| G37 | Cat    | Adult   | Negative  | NP        | Negative| NP        | Negative  | NP        | NP        | NP      |
| G38 | Cat    | Old     | 1:25      | NP        | NP      | NP        | Negative  | NP        | NP        | NP      |
| G50 | Cat    | Sub-adult | Negative | NP        | NP      | NP        | Negative  | NP        | NP        | NP      |
| G55 | Cat    | Sub-adult | Negative  | NP        | Negative| Negative  | Negative  | NP        | NP        | NP      |
| G58 | Cat    | Old     | Negative  | NP        | NP      | NP        | Negative  | NP        | NP        | NP      |
| G59 | Cat    | Adult   | Negative  | NP        | NP      | NP        | Negative  | NP        | NP        | NP      |
| G60 | Cat    | Adult   | Negative  | NP        | Negative| NP        | Negative  | NP        | Negative  | NP      |
| G68 | Cat    | Adult   | Negative  | NP        | NP      | Negative  | NP        | NP        | Negative  | NP      |
| G72 | Cat    | Sub-adult | Negative  | NP        | Negative| Negative  | Negative  | Negative  | Negative  | NP      |
| G79 | Cat    | Old     | 1:25      | NP        | NP      | NP        | 1:25      | NP        | NP        | NP      |
| G103| Cat   | Adult   | Negative  | NP        | NP      | NP        | Negative  | NP        | NP        | NP      |
| G124| Cat   | Adult   | Negative  | NP        | NP      | NP        | Negative  | Negative  | NP        | NP      |
| G159| Cat   | Adult   | Negative  | NP        | NP      | NP        | Negative  | NP        | NP        | NP      |
| G202| Cat   | Adult   | 1:500     | NP        | NP      | NP        | 1:500     | NP        | NP        | NP      |
| G227| Cat   | Adult   | Negative  | NP        | NP      | NP        | Negative  | NP        | NP        | NP      |
| G236| Cat   | Kitten  | 1:500     | NP        | NP      | NP        | 1:500     | NP        | NP        | NP      |

NP, not performed; 1:25, positive with 1:25 titre; 1:500, positive with 1:500 titre.
binomial confidence intervals of 95% (95% CI). Associations between seropositivity to *T. gondii* and epidemiological variables were assessed by Pearson’s $\chi^2$ test or Fisher’s exact test, as appropriate. Variables with a $P$ value <0.20 in bivariate analysis were selected for inclusion in the multivariate analysis. Cramer’s $V$ correlation coefficient between pairs of variables was computed to prevent collinearity. Finally, the effect of the exploratory variables selected on *T. gondii* seropositivity was investigated using a multiple logistic regression analysis (likelihood-ratio Wald’s test, $P < 0.05$) (Hosmer and Lemeshow, 2000). For forward model building, variables were included one at a time, starting with the variable with the lowest $P$ value in bivariate analysis. At each step, the confounding effect of the included variable was assessed by computing the change in the odds ratio. Confounding variables were those that, when added to the model, changed the OR by more than 30%, and were forced into the final model regardless of their significance level. Potential two-way interactions between all the variables were also tested for significance in the models. The goodness of fit was assessed using the Hosmer–Lemeshow goodness-of-fit test. Statistical analyses were carried out using the SPSS software (Statistical Package for Social Sciences – SPSS, Version 22.0, IBM Corp., Armonk, NY, USA).

Results

Seroprevalence of *T. gondii* in dogs from Bangkok was 7.9% (25/318; 95% CI 4.9–10.8%), and 18.7% in cats (60/321; 95% CI 14.4–23.0%). Statistically significant differences were found in seroprevalence between species ($P < 0.001$). The distribution of antibody titres is represented in Fig. 1. Titres of 1/500 were more frequently found in cats (28%) than in dogs (4%), while low titres (1:25) were detected in 46% of cats and 64% of dogs. However, there were no significant differences in titres between both species (Pearson’s $\chi^2$, $P = 0.09$).

The frequency of seropositive and seronegative animals detected according to the variables analysed in dogs and cats is shown in Table 1. The logistic regression model showed that the main risk factors associated with *T. gondii* exposure in dogs were breed and degree of outdoor contact, while age class was the main risk factor in cats (Table 3). Seroprevalence in dogs was significantly higher in mixed-breed compared to purebred. In addition, dogs living permanently outdoors had a significantly higher seroprevalence than those that lived partially enclosed. Regarding cats, significantly higher seropositivity was detected in old and adults compared to kittens.

Serconversion was not detected in the seronegative individuals longitudinally sampled. One of the 18 dogs and four of the 20 cats were seropositive at the first sampling (Table 2). All seropositive animals maintained the same titre after 4–6 weeks, except one cat, which became seronegative after 36 days from a titre of 1:25.

Discussion

Results of the present study indicate that both dogs and cats in Bangkok are exposed to *T. gondii*. Our results highlight differences in seroprevalence between the studied host species despite sharing the same habitat, being significantly higher in cats than in dogs. However, regarding antibody titres, there were no statistically significant differences between both species, which agrees with the results obtained by Cong et al. (2018). The seroprevalence found in dogs in our study was similar (7.9%) to the 9.4–9.6% reported in previous studies performed in stray dogs in Bangkok (Jittapalapong et al., 2007, 2009). This result indicates that there is an urban and peri-urban cycle of *T. gondii* in which outdoor dogs are involved, either through consumption of infected prey or via ingestion of oocysts (Lindsay et al., 1997; Cong et al., 2018). Our results indicate that a considerable percentage of Bangkok’s stray dogs are seropositive, and therefore they could be considered as sentinels of environmental contamination with *T. gondii* by health authorities. The seropositivity found in cats (18.7%) was higher than in other studies conducted in stray cats from Bangkok, whose seroprevalence values ranged between 4.8 and 11% (Jittapalapong et al., 2007, 2010), and in pet cats, in which a prevalence of anti-*T. gondii* antibodies of 10.1% was reported (Sukhumavasi et al., 2012). This finding is especially interesting from an epidemiological point of view, since seropositive cats may have eliminated oocysts at some point in their lives, contaminating the soil and being a source of infection in the urban and peri-urban environment that is shared with humans and other warm-blooded vertebrate species, including birds, rodents, dogs and other synanthropic species (Dabritz and Conrad, 2010; Torrey and Yoklen, 2013; de Wit et al., 2020). Cats can excrete *T. gondii* oocysts more than once in their life (Zulpo et al., 2018). Thus, taking into account the growth of the dog and cat population in Thailand (OIE, 2019), the particularly close contact between these species and humans in the study area (Savvides, 2013; Toukhkati et al., 2015) and the seroprevalence detected in this study, outdoor cats and dogs in Bangkok should be considered as hosts with a possibly significant epidemiological role in the maintenance of *T. gondii*. Regarding other Asian countries, a study carried out in China showed higher seroprevalences (8.2–30.9%) in dogs using the same technique (MAT) on a similar sample size (Dubey et al., 2020b), in cats, slightly higher seroprevalences have also been described using MAT on a similar sample size in China (19.3–24.4%), and much higher seroprevalence has been reported in Qatar (82%) (Dubey et al., 2020a).

Our results indicate that mixed-breed dogs and those with permanent outdoor access are more likely to be seropositive and, therefore, these characteristics are risk factors associated with *T. gondii* exposure. Previous studies have determined that, in general, outdoor dogs have a greater risk of *T. gondii* infection than those which are indoors (Cano-Terriza et al., 2016; Ding et al., 2017; Wang et al., 2017; Zarra-Nezhad et al., 2017). Outdoor only dogs could have an increased probability of exposition to oocysts-contaminated environments and hunting prey infected by *T. gondii*, which can explain their higher seroprevalence. Regarding breed, several authors have detected higher
To Bangkok was 7.8 and 18.6%, respectively, indicating the exposure populations in Bangkok.

are required to assess the parasite circulation within dog and cat ever, provide supportive evidence for the results obtained by MAT state. Consistency of antibody titres over a period of time, does how-
suffered from mammary gland cancer with lung metastasis, so that had a negative result after approximately 1 month. This cat seropositive individuals, except in one cat with a low titre (1:25) On the other hand, MAT titres were maintained over time in all longer sampling intervals (11 years and 4

et al (Cano-Terriza 2016; Rengifo-Herrera et al. 2017). Considering cats, age was the only risk factor identified, with T. gondii seroprevalence rising significantly as the cat’s age increased. This finding is consistent with those previously reported in this host species (reviewed in Dubey et al., 2020a). Age-related seropositivity could reflect the long persistence of anti-T. gondii antibodies in the animal and/or the highest probability of having contacted with the parasite along its lifetime (Cano-Terriza et al., 2016; Rengifo-Herrera et al., 2017).

Seroconversion was not detected in the animals longitudinally assessed for 6 weeks. However, these results should be interpreted with caution due to the short duration of the present study. Other studies detected changes in serological status in 9.1% (Afonso et al., 2006) and 18% (Pereira et al., 2018) of cats, but they had longer sampling intervals (11 years and 4–8 months, respectively). On the other hand, MAT titres were maintained over time in all seropositive individuals, except in one cat with a low titre (1:25) that had a negative result after approximately 1 month. This cat suffered from mammary gland cancer with lung metastasis, so this finding could be associated with its immunodeficiency state. Consistency of antibody titres over a period of time, do however, provide supportive evidence for the results obtained by MAT testing. Further longitudinal studies with a longer testing period are required to assess the parasite circulation within dog and cat populations in Bangkok.

In conclusion, T. gondii seroprevalence in dogs and cats from Bangkok was 7.8 and 18.6%, respectively, indicating the exposure to T. gondii of these domestic species in urban and peri-urban areas. Considering these results, Bangkok citizens should take preventive measures to avoid T. gondii infection when they interact mainly with outdoor cats, their habitat and their co-habitants. The presence of T. gondii was associated with mixed-breed and permanent outdoor contact in dogs and old age in cats.

Data

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Conflict of interest. None.

Ethical standards. Serum samples were opportunistically collected from animals subjected to health programmes, medical check-ups or surgical interventions during the study period; therefore, no ethical approval was necessary for this study.

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Table 3. Potential risk factors associated with Toxoplasma gondii seropositivity in outdoor dogs and cats in Bangkok, Thailand

| Species | Variables | Categories | β    | P value | d.f. | Exp (β) | 95% CI |
|---------|-----------|------------|------|---------|------|---------|-------|
| Dog     | Breed     | Mixed-breed | 1.246 | 0.005   | 1    | 3.478   | 1.460–8.286 |
|         |           | Purebred   | 1    | 1       |      | 1       |       |
|         | Outdoor contact | Total     | 1.297 | 0.011   | 1    | 3.657   | 1.344–9.952 |
|         |           | Partial    | 1    | 1       |      | 1       |       |
| Cat     | Age       | Kitten     | 0.549 | 0.280   | 1    | 1.731   | 0.639–4.685 |
|         |           | Sub-adult  | 0.839 | 0.066   | 1    | 2.314   | 0.945–5.666 |
|         |           | Old        | 1.946 | 0.002   | 1    | 7.000   | 2.049–23.192 |

*Reference category.
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