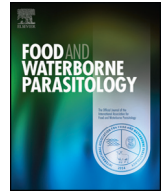


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Detection of antibodies to *Toxoplasma gondii* among owned dogs in Cambodia

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ABSTRACT

Toxoplasma gondii is a protozoan parasite belonging to the phylum Apicomplexa, which has a two-host life cycle and an extensive global distribution. The presence of antibodies to *T. gondii* was examined in owned dogs in Cambodia. In total, 103 dog serum samples from 37 households in northern Cambodia were collected and examined for evidence of *T. gondii* infection using an indirect immunofluorescent antibody test. In total, 52 of 103 (50.5%) samples were serologically positive for *T. gondii* in this study. No significant risk factor associated with *T. gondii* infection was found. To the best of our knowledge, this is the first report of the seroprevalence of *T. gondii* in dogs from Cambodia, which revealed a considerable risk of infection for humans. Therefore, consuming undercooked dog meat or contacting feces with contaminated cat feces should be restricted to avoid the possibility of zoonosis. Further studies are needed to determine the epidemiology of *T. gondii* in populations of larger dogs and other animals to improve our understanding of the situation of the pathogen in this country.

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1. Introduction

Toxoplasma gondii is an obligate intracellular protozoan that has a complex life cycle involving transmission and diversification among a vast array of hosts. Birds and warm-blooded animals, including humans, are considered to be the intermediate hosts of *T. gondii*. These hosts get infected with this parasite when consuming oocysts shed by felid definitive hosts, or tissue cysts of intermediate hosts (Taylor et al., 2015). Despite the worldwide distribution of the parasite, immunocompetent patients infected with *T. gondii* are usually asymptomatic. Nevertheless, toxoplasmosis can cause abortion in congenital infection or encephalitis and ocular disease in immunocompromised individuals (Halonen and Weiss, 2013; Park and Nam, 2013).

Dogs are often regarded as devoted and intimate companions for humans. However, due to their coprophagous habit, dogs can transmit and mechanically disseminate oocysts of *T. gondii* from cat feces, allowing contamination of the environment with the infective form (Frenkel and Parker, 1996; Lindsay et al., 1997). In addition, it was previously demonstrated that oocysts of *T. gondii* can remain intact even after they have been ingested by dogs and excreted in their feces (Schaes et al., 2005). Although governments have actively campaigned against the dog meat trade, the animals are still used for human consumption in some Southeast Asian countries (ACPA, 2013). Handling dog meat in unsanitary conditions or the consumption of undercooked meat can pose an additional health risk of life-threatening diseases, such as rabies and toxoplasmosis (ACPA, 2013; El Behairy et al., 2013).

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In Cambodia, antibodies to *T. gondii* were found in 5.8% of women aged 15–39 years (Priest et al., 2016). However, to date, there has been no known report on toxoplasmosis in dogs or other animals in this country. Therefore, the objective of this study was to examine the presence of antibodies to *T. gondii* in dogs owned by residents in 37 households in Cambodia using the indirect immunofluorescent antibody test (IFAT).

2. Materials and methods

2.1. Animal ethics

This study adhered to strict guidelines outlined by the European Convention for the “Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes”. In addition, permission was granted by the Ministry of Agriculture, Forestry and Fisheries, Cambodia whose representatives were present during sampling and personally oversaw that animals were handled with respect according to the laws on experimental animal care in Cambodia. Written informed consent was also obtained from each dog owner prior to sample collection.

2.2. Sample collection

The local Cambodian Veterinary Services reported a population of 350 dogs (N) in Dong village, a rural area in Rovieng district, Preah Vihear province, Cambodia (Fig. 1). Using statistical theory, the representative sample size was calculated as $n = [1 - (1 - CL)^{1/e}] \times (N - (e - 1)/2]$ (CL: Confidence Level (95%), e: number of detectable individuals with the event in the population ($e = N \times p \times Se$), where p is— expected prevalence at 15% (Lopes et al., 2014), Se is sensitivity of IFAT at 80% (Liu et al., 2015)); thus, a sample size (n) of 22 was determined. However, it was decided to collect 103 samples for better representation and to compensate for rejected samples. Convenience sampling was used to obtain blood from 103 dogs owned by the residents in 37 households (15.5%) from a total of 238 households in Dong Village. For sera separation, blood was collected in sterile tubes without anticoagulant and centrifuged at 1448 ×g for 10 min and the serum fractions were removed and stored at -20 °C until analyzed. The animals sampled were divided into two age groups: 41 juveniles (1–12 months) and 62 adults (> 1 year).

2.3. Toxoplasma serology

Antibodies to *T. gondii* in the dog serum samples were detected using IFAT as previously described (Kengradomkij et al., 2018). Tachyzoites of RH strain *T. gondii* were maintained using Vero cells in MEM (minimum essential medium, Sigma, USA). They were harvested using a #27 needle and a 5.0 µm syringe filter (Millipore, USA) after scrapping infected cells, washed in cold PBS three times (2000 rpm/10 min), and then diluted to 10⁶ tachyzoites/ml. Teflon-coated slides (Cel-Line Associates, Newfield, New Jersey, USA) were coated with 10 µl of tachyzoites/well, air-dried at room temperature, and fixed with cold acetone before storing at -20 °C until used. Each serum sample was tested at dilutions of 1:100, 1:200, 1:400, 1:800, and 1:1600 using anti-canine IgG conjugated to fluorescein isothiocyanate (VMRD, Inc. Pullman, Washington, USA), respectively. Incubations were performed at 37 °C for 30 min and washed three times with PBS before coverslips were applied. A titer of 1:100 was used as a positive cut-off titer. Negative and positive controls (field dog samples) that were confirmed positive based on the latex agglutination test (LAT) and IFAT at high titers were included in each test run. Additionally, all the 103 samples were screened for cross-reactivity with *Neospora caninum* using IFAT as previously described (Inpankaew et al., 2014a, 2014b). In addition, all samples were confirmed for the detection of *T. gondii* antibodies using the LAT kit (Toxocheck-MT; Eiken Chemical Company, Tanabe, Tokyo, Japan) as the reference test.

2.4. Statistical analysis

Statistical analysis was conducted using the R software package version 3.6.3 (R Core Team, 2020). The association between individual *T. gondii* seropositive dogs and risk factors (age and sex) was evaluated using the χ^2 test. Statistical significance was considered at $p < 0.05$ with the 95% confident interval (CI).

3. Results

The data in Table 1 provide the *T. gondii* seroprevalence of dogs in Dong village, Cambodia. In general, antibodies to *T. gondii* were found in 52 (50.5%) of the 103 serum samples examined using IFAT. There was an overall prevalence of 54.8% in adults and 43.9% of the juveniles were infected with this parasite but the values were not significantly different ($p = 0.37$; Table 1). The majority of infected dog samples (42/52) yielded low to moderate IFAT titers; high titers were only observed in adult dogs. Of the 18 young dogs, 11 were positive at 1:100, with 2 and 5 animals at 1:200 and 1:400 titers, respectively. No sample was positive at the high titers of 1:800 and 1:1600. Conversely, high titers of antibodies to *T. gondii* were observed in samples of all adult dogs. The most common titer was the moderate 1:400 in samples from 11 adults, followed by titers of 1:200 or 1:800 from 7 animals. The number of positive dogs at the 1:100 titer was 6, which was twice as high as that of 1:1600 (3 animals) (Table 2).

The seroprevalence rate for males was 50.0% (21/42) which was slightly lower than for females at 50.8% (31/61). No significant variation was found between males and females ($p = 1$) (Table 1). Most households (30/37, 81.1%) had currently or

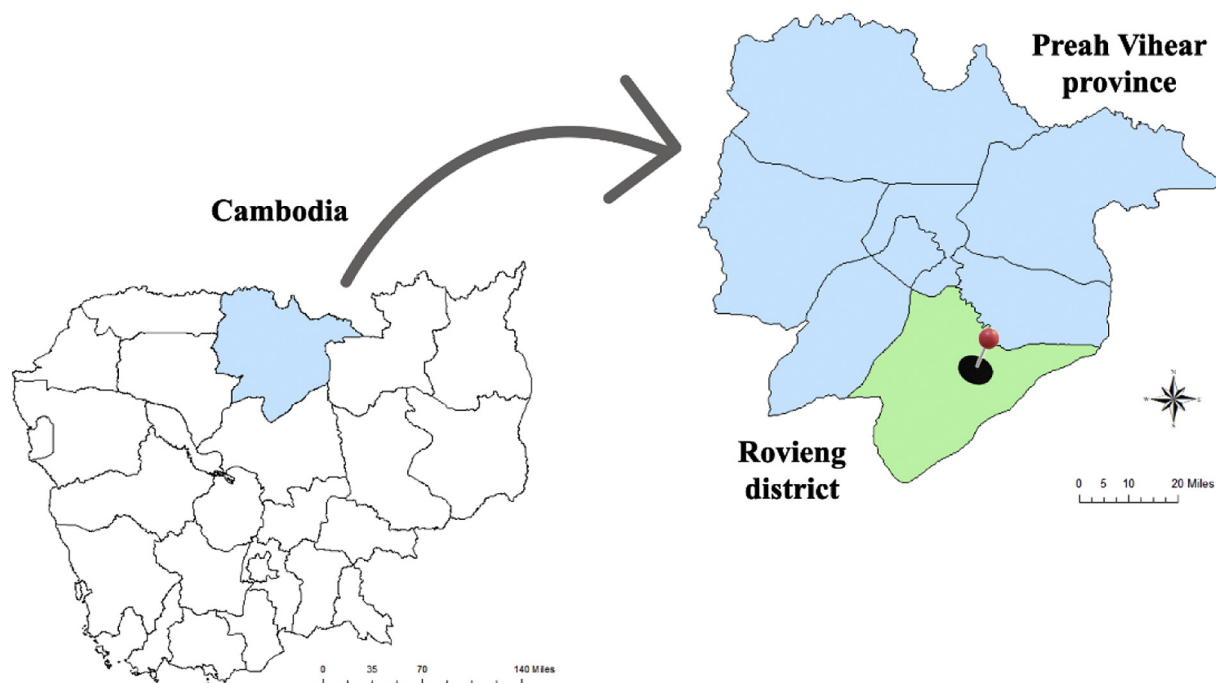


Fig. 1. Map showing the study area in Dong village, Rik Reay commune, Preah Vihear province, Cambodia.

Table 1

Seroprevalence of *T. gondii* infection in dogs by age and sex in Dong village, Cambodia.

Variable	Category	No. of tested dog	No. of positive dog	Seroprevalence (%)	95% CI	<i>p</i> -value
Age	Juveniles	41	18	43.90	28.80–60.10	0.37
	Adults	62	34	54.84	41.80–67.30	
Gender	Male	42	21	50.00	35.50–64.50	1
	Female	61	31	50.82	37.80–63.70	
Total		103	52	50.48	40.5–60.4	–

Table 2

Titer distribution by age group of *T. gondii* antibodies in dogs.

Age group	No. of sera with IFAT titer of				
	1:100	1:200	1:400	1:800	1:1600
Juvenile	11	2	5	0	0
Adult	6	7	11	7	3
Total (<i>n</i> = 52)	17	9	16	7	3

previously infected dogs with antibodies to *T. gondii*. All the dogs in eight households tested positive; interestingly, a large proportion of them were juveniles with low antibody titer (1:100).

Even though five samples (4.9%) were positive with *N. caninum* based on IFAT, no cross-reactivity between this parasite and *T. gondii* was found in this population of dogs.

4. Discussion

The overall seroprevalence of *T. gondii* infection was 50.5% for owned dogs from the sampled households in northern Cambodia (Table 1). This level of seroprevalence was noticeably lower than in other studies, such as 98.0% in Egypt (El Behairy et al., 2013) and 83.5% in Thailand (Jittapalapong et al., 2009). Nevertheless, it was substantially higher than those observed in pet dogs from other regions, such as 9.4% in Thailand (Jittapalapong et al., 2007), 15.5% in Angola (Lopes et al., 2014), and 10.8% in northeast China (Wu et al., 2011). The differences in seroprevalence rates among these countries may have been due

to the different serological techniques used and the distinct environmental and management conditions in various parts of the world. It can be inferred that stray dogs are more likely to be infected with toxoplasmosis than the other groups consisting of pet dogs and dogs raised in households. This might be due to the fact that stray animals are more likely to eat dead birds or rodents and share habitats with cats and *T. gondii*. In contrast, the other groups are raised with better sanitary conditions and a balanced food diet (Jadoon et al., 2009). In addition, the water in Dong village was supplied by wells, well pumps, and rainwater tanks. These water sources are easily contaminated with waterborne parasitic pathogens from semi-domesticated animals mainly due to the lack of treated water. Free-roaming dogs may have negative impacts upon human health and the environment, if they are reservoirs of parasites. This important transmission role could also include the transmission of other parasites, such as hookworms, as demonstrated by Inpankaew et al. (2014a, 2014b). Another key point is the unhygienic conditions in slaughterhouses and kitchens where equipment can be infected with tissue cysts from dog meat during processing. Under these circumstances, the public health concern lies not only with toxoplasmosis but also with other life-threatening diseases (FP, 2019). Therefore, authorities in rural areas of Cambodia should pay attention to the public health risks posed by contaminated water and food.

Although the difference between the two age groups was not statistically significant ($p = 0.37$), there was a general trend for older animals to be more heavily infected (54.8%) than younger ones (43.9%). Low and moderate levels of *T. gondii* antibodies were found in all infected juveniles and 12/16 of infected adults aged 2 years. In contrast, older dogs tended to have high titers of antibodies (1:800, 1:1600), indicating that the cumulative likelihood of exposure to *T. gondii* increases with age and the antibodies have a lifelong persistence (Robert-Gangneux and Darde, 2012). Indeed, one study suggested that the acquisition of disease may be due to a longer exposure period with age rather than congenital transmission in this population, as adults have more opportunities for exposure to contaminated water, foods, or the environment (Wu et al., 2011). Nonetheless, it has been shown that dogs can vertically transmit *T. gondii* to their offspring via semen (Arantes et al., 2009).

The percentages of the infection in males and females were similar at 50.0% and 50.8%, respectively and not significantly different. Therefore, sex is not a critical factor for *T. gondii* in dogs. Since the male and female dogs were raised together in the same habitat, a difference in the degree of exposure risks between two genders was unlikely. This was in agreement with studies elsewhere indicating that the levels of infection based on sex were not substantially different at 12.5% for males and 9.5% for females in China (Wu et al., 2011) and at 40.0% for males and 53.0% for females in Pakistan (Jadoon et al., 2009).

Serological tests for *T. gondii* may show some levels of cross-reactivity with related Apicomplexa, particularly *H. hammondi* and *N. caninum* (Gondim et al., 2017). In the present study, over half of the dogs were seropositive with *T. gondii* while the antibodies to *N. caninum* were found in an insignificant portion of the population. However, no serological cross-reactivity between *T. gondii* and *N. caninum* was observed, which was in agreement with Dubey et al. (1988) who reported that dogs that were naturally or experimentally infected with *N. caninum* did not show any cross-reactivity with *T. gondii* at a titer of 1:50 in IFAT. Therefore, the same or higher cut-off values have been tailored to avoid the cross-reactivity between *T. gondii* and *N. caninum* in dogs and other species (Benetti et al., 2009; Lobato et al., 2006; Silva et al., 2007). Due to the moderate specificity found when using LAT to validate IFAT for detecting *T. gondii* in dog sera, a titer of 1:100 was implemented as the cut-off value in the present study. Nonetheless, the cross-reactivity between these parasites has been established in other hosts, such as mice and cats based on enzyme-linked immunosorbent assay (ELISA) using soluble antigen (Nishikawa et al., 2002).

In another experiment on serological cross-reactivity between *T. gondii* and *Hammondia*, IFAT was considered the most specific method for detecting *T. gondii* antibodies in mice, dogs, rabbits and pigs, where the sera of animals experimentally inoculated with *H. hammondi* did not cross-react with *T. gondii* in IFAT. In addition, cross-reactivity could be established by using other techniques, such as dye test, ELISA, and complement fixation test (Gondim et al., 2017; Weiland et al., 1979).

In conclusion, this study is the first known survey of *T. gondii* in dogs from Cambodia. The results revealed the potential for human infection which could be prevented by applying hygienic conditions and by not consuming undercooked food which may have been exposed to the pathogen. A *T. gondii*-contaminated environment, such as water and food, may be responsible for disease in dogs. The present study has provided preliminary information on the presence of *T. gondii* in this community. Hence, further investigations into larger populations of dogs and other animals should be carried out nationally to determine the epidemiology of toxoplasmosis and to assess the public health risk factors of this disease.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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