

TOXOPLASMA GONDII ANTIBODY IN THAI CATS AND THEIR OWNERS

Yaowalark Sukthana¹, Jaranit Kaewkungwal², Chun Jantanavivat¹, Amorn Lekkla¹,
Rachatawan Chiabchalard¹ and Waraporn Aumarm³

¹Department of Protozoology, Faculty of Tropical Medicine, Mahidol University; ²Department of Social and Environmental Medicine, Faculty of Tropical Medicine, Mahidol University; ³Department of Medicine, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand

Abstract. Humans are thought to acquire *Toxoplasma* infection by three major routes: ingesting food and water contaminated with oocysts from cat excreta, consumption of under-cooked infected meat, and transplacental transfer. Congenital clinical toxoplasmosis in the newborn indicating definite transplacental transmission had been reported in Thailand, whilst studies concerning infection due to the other two routes were inconclusive. Since the way domestic cats live and eat and also the eating behavior of Thai people differ from those in the West, we conducted a sero-epidemiological study of *T. gondii* in cats and their owners in Bangkok metropolitan area. Among 327 humans, the prevalence of *Toxoplasma* antibody was 6.4% and in 315 cats it was 7.3%. These relatively low prevalence rates may result from the predominantly well-cooked fish and rice diet of stray cats, which congregate in temples where they are fed. *Toxoplasma* antibody seropositive was associated with living in close proximity to seropositivity cats [OR (95% CI) = 5.43 (1.28-23.04); p=0.01]. Risks were increased in and around temples, particularly if courtyards were of earth or grass, suggesting ground temperature was an important determinant of oocyst survival.

INTRODUCTION

Though *Toxoplasma gondii* was discovered since 1908, its life cycle was not known until 1969. The obligate intracellular protozoan, *T. gondii*, causes worldwide infection not only in humans, but also in warm-blooded domestic and wild animals, birds and rodents. Cats are the definitive host of *Toxoplasma gondii*. It is commonly believed that humans acquire the infection by three major routes namely: ingesting food and water contaminated with oocysts from cat excreta, consuming under-cooked infected meat, and transplacental transfer.

High prevalences of *Toxoplasma* infection have been reported in France and Central America. The prevalence in France is 85% and is

believed to result from consumption of infected meat, whilst in Central America the weather favors the survival of the cysts from stray cats (Solari, 1998).

Cats are a major risk factor for *Toxoplasma* transmission. Diet, especially raw meat, and free access to the outdoor environment are equally important as predisposing factors to the risk of *Toxoplasma* infection in cats (Nogami *et al*, 1998; Galvan *et al*, 1999; Lucas *et al*, 1999; Sumner and Ackland, 1999). A study of residents and workers on swine farms in Illinois, USA showed that canine infection with *T. gondii* increased the risk of human infection and contact with soil was a likely mechanism for transmission. The increased risk of seropositivity in males is attributed to less attention paid to cleanliness in food preparation and consumption (Weigel *et al*, 1999).

There are less data on the epidemiology of toxoplasmosis in Asia. Congenital clinical toxoplasmosis in the newborn has been reported in Thailand (Chokephaibulkit, personal communication) with 0.8% of neonates being seropositive for *T. gondii* antibody, indicating definite

Correspondence: Dr Yaowalark Sukthana, Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand.

Tel: +66 (0) 2354 9100-19 ext 1830; Fax: +66 (0) 2643-5601

E-mail: tmymv@mahidol.ac.th

transplacental transmission (Saweangthamchai, 1999). In our earlier studies there was a statistically significant difference between pregnant women with and without *T. gondii* antibody in terms of a history of eating under-cooked meat and cat ownership of dogs, whilst in kidney transplant recipients only the former factor was significant (Chintana *et al*, 1998; Sukthana *et al*, 2001). These findings raise the question of which of the two risk factors predominates in Thailand.

Since the way cats are kept as pets, and the eating behavior of Thai people differs from those in the West, we conducted an epidemiological study of *T. gondii* infection in humans and cats in Bangkok.

MATERIALS AND METHODS

Study sites and questionnaire interview

The present study involves community households and those in Buddhist temples in the north and outer north, the south, the west and the inner east of the Bangkok metropolitan area (Fig 1). Bangkok is located at latitude 13° 45' north and longitude 100° 28' east, in the central part of the country on the low-flat plain of the Chao Phraya River. It is a tropical land with long hours of sunshine, high temperature and humidity. Bangkok has a total area of 1,568.7 km² with a population of approximately seven million. The studied sites covered an area of 106.6 km² with 494,931 inhabitants.

After informed consent, questionnaires about owners' personal data, number of cats, the way they were kept, their defecation places including cats' excreta cleaning and cats' feed were completed in the interviews. Serum samples were collected from every household member as well as from monks, novices and nuns who lived in the temples visited. Venous blood samples were also taken from household and stray cats in the temple boundary. Rectal swabs were taken from cats in both settings; households and temples and simple smears for *Toxoplasma* oocysts were done.

Laboratory procedures

All serum samples taken from cats and owners were kept in 4°C until used. IgG *Toxoplasma* antibody was determined from each serum sample

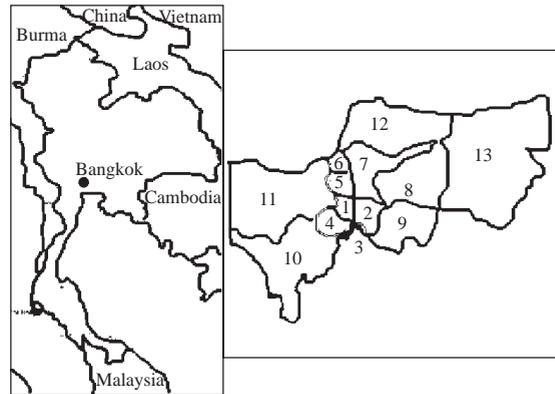


Fig 1—Map of Thailand and Bangkok. The studied areas are the north and outer north (5,6 and 12), the south (4), the west (11) and the inner east (8) of Bangkok. 1& 2 central area, 3 riverside, 4 south of city fringe, 5 north of city fringe, 6 government area, 7 inner north, 8 inner northeast, 9 east, 10 south, 11 west, 12 outer north, 13 outer east.

using the Sabin-Feldman dye test (Sabin and Feldman, 1948) which is highly specific and sensitive, and is regarded as the reference method for the serodiagnosis of toxoplasmosis. A value of > 4 IU or a titer of at least 1:16 was taken as the threshold for positivity as recommended by Reiter-Owona *et al* (1999). Cat rectal swabs were examined by simple smear in normal saline and iodine solution. One hundred of microscopic fields were examined before reporting the sample as negative.

Data entry and statistical methods

Laboratory data and cat owners' personal data were double entered and analyzed. The IgG *Toxoplasma* prevalence rates of the cats and their owners were determined using the serological cut-off criteria described above. The proportion of cats excreting oocysts was also determined. Crude odds ratios (OR) were reported for the association of *Toxoplasma* antibody between cats and their owners. In addition, adjusted OR was calculated by controlling for the number of reported cats and the number of infected cats in each study area.

RESULTS

Among 327 humans, 104 cat owners from 30 households in four communities and 223 per-

sons from 14 temples including monks, novices and nuns were enrolled in this study. There were altogether 315 cats, 116 from households and 199 from temples. About 65% of cat owners were aged between 20-59 years (range: 2-75 years) whilst the average age of cats was 1.2 years (range from 4 months to 4 years). Male to female ratio was 1.8:1 in humans and 1.1:1 in cats.

Descriptive data concerning the number of own cats, cats' living and defecation areas, clean up cat's excreta and feeding are shown in Table 1. Of the studied cases, 80% were cat owners whilst the remainder were people who lived in the area where the cats were roaming around. The mean number of cats living in the residential area as reported by the respondents was 10.4 (range

Table 1
Descriptive statistics of respondents (N=327).

Characteristics	Negative cases (%)	Positive cases (%)	Total (%)
Sex			
Male	199 (60.9)	13 (4.0)	212 (64.8)
Female	103 (31.5)	11 (3.4)	114 (34.9)
No answer	1 (0.3)	-	1 (0.3)
Age(year)			
>12	12 (3.7)	2 (0.6)	14 (4.3)
13-19	22 (6.7)	-	22 (6.7)
20-39	109 (33.4)	10 (3.1)	119 (36.5)
40-59	100 (30.7)	9 (2.8)	109 (33.4)
>60	59 (18.1)	3 (0.9)	62 (19.0)
No answer	1 (0.3)	-	1 (0.3)
Residential area			
Community	97 (29.7)	7 (2.1)	104 (31.8)
Temple	206 (63.0)	17 (5.2)	223 (68.2)
Own cat (s)			
No	60 (18.3)	3 (0.9)	63 (19.3)
Yes	243 (74.3)	21 (6.4)	264 (80.7)
No answer	-	-	-
Cat's living area			
With subject	129 (39.4)	13 (4.0)	142 (43.4)
Within residential area	92 (28.1)	7 (2.1)	99 (30.3)
No answer	82 (25.1)	4 (1.2)	86 (26.3)
Cat's defecation area			
Fixed location	85 (26.0)	8 (2.4)	93 (28.4)
Anywhere/other location	158 (48.4)	13 (4.0)	171 (52.4)
No answer	60 (18.3)	3 (0.9)	63 (19.3)
Cat's excreta cleaning			
By owner	70 (21.4)	8 (2.4)	78 (23.9)
None	173 (52.9)	13 (4.0)	186 (56.8)
No answer	60 (18.3)	3 (0.9)	63 (19.3)
Cat's feeding			
Rat catching	27 (8.3)	2 (0.6)	29 (8.9)
Commercial food	19 (5.8)	3 (0.9)	22 (6.7)
Home-cooked food	156 (47.7)	14 (4.3)	170 (52.0)
Others	41 (12.5)	2 (0.6)	43 (13.1)
No answer	60 (18.3)	3 (0.9)	63 (19.3)

Table 2
Association between infected cats and infected subjects.

	Negative cases	Positive cases	Crude OR	Adjusted OR (95% CI) ^a
Infected cats in the residential area				
Yes	203 (90.6%)	21 (9.4%)	3.45	5.43 (1.28-23.04)
No	100 (97.1%)	3 (2.9%)		

^aAdjusted for number of reported cats in the area and number of infected cats within the area.
Note: No other risk factors were found significantly associated with infected cases.

1-20); however, the mean number of cats that were captured within the area was 17.5 (range 1-41). Of the studied cats, 28% had a fixed place for defecation, others defecated anywhere, and in most cases (up to 75%) nobody buried or removed the cat's excreta. More than half of Thai cats (52.0%) were fed by home-cooked feed.

Antibody prevalence

In total, 7.3% of studied cats and 6.4% of the studied humans were sero-positive for *Toxoplasma* antibody. The association between exposure to infected cats and the serological status of the respondents was calculated. The number of human cases exposed to this risk factor (cat owner) was 224 of whom 21 were sero-positive whilst only 3 cases out of 100 who were not exposed were positive for *T. gondii*, the relative risk (95% CI) was 3.5 (0.95-10.2). As shown in Table 2, after adjustment for the population density of cats the odds ratio was approximately 5 times greater among the exposed group compared with that of the non-exposed group [OR (95% CI) = 5.43 (1.28-23.04); p=0.01]. However, it should be noted that there were four studied sites with infected cats but no sero-positive humans or vice versa (Table 3). No oocysts of *T. gondii* were found in rectal swabs from 315 cats.

DISCUSSION

In Thailand, cats are kept as pets but they are allowed to roam freely outdoors. They are very rarely trained to defecate in litter boxes, so most of them defecate anywhere that is convenient ranging from the backyard to the house roof. In the present study, fewer than 30% of the studied

Table 3
Number of infected cats and infected human cases by study sites.

Study sites	Number of infected cats/total captured cats	Number of infected human cases/total cases
WK ^b	2/20	0/16
WD ^a	0/19	1/14
WP	2/21	2/29
WJN	0/6	1/4
WIB ^b	3/4	0/8
WBK ^a	0/8	1/20
WYS	1/18	1/8
WDM	1/22	2/8
WA ^b	2/13	0/11
WC	1/12	2/25
WRY ^b	1/15	0/14
WS	1/12	4/23
WMK	0/9	0/12
WTL	2/20	3/31
Community A	6/39	4/32
Community B	0/41	0/38
Community C	1/20	3/19
Community D	0/16	0/15

^aStudy sites with infected subjects but no infected cat;
^bStudy sites with infected cat but no infected subject;
W=Wat or temple

cats had a fixed place for defecation and in approximately 75% of cases their excreta were not removed or buried. Thus exposure to cat feces in urban environment is considerable in areas with high cat population densities. After infection, especially by the tissue cyst of *T. gondii*, almost 100% of cats shed oocysts in a period of 3-10

days (Frenkel and Ruiz, 1981). Thus, infected cats with unrestricted defecation will contaminate their immediate environment and provide a potential source of human infection.

Ninety-five percent of Thai people are Buddhist, who adhere to the five normal precepts, one of which is not to destroy life. To kill any pet is, therefore, considered sinful. Moreover, in Thailand, birth control in cats is expensive and thus is usually ignored by the owners. When too many kittens are born to be looked after by the home, they are taken to the temples and left there. Buddhist temples are, therefore, not only places monks live, people prey, and religious events take place, but also centers for unwanted dogs and cats from the community. The animals are fed not only by both monks and nuns, but also by the general population. There are nearly 500 temples in Bangkok, and it is estimated that there are at least 30 stray cats in each temple, so altogether ~15,000 cats live there. The situation in the temples is therefore worse than that in the houses. The ownerless cats roam freely, breed unrestrictedly and defecate anywhere in the area. Temples therefore provide a significant human exposure risk.

In the present study, the risk of *Toxoplasma* seropositivity in the exposed human group was five times more than that of non-exposed group. Thus, it seems that cat ownership is a risk factor for *toxoplasma* infection in Thailand. Earlier studies revealed that infection of cats with *T. gondii* increased the risk of human infection and contact with soil was a likely mechanism for transmission (Bobic *et al.*, 1998; Nogami *et al.*, 1998; Sumner and Ackland, 1999; Weigel *et al.*, 1999). Free access of cats to the outdoor environment, and feeding cats with leftovers or raw viscera and raw meat were the risk factor for humans to become infected by *T. gondii* in Mexico and Brazil (Galvan *et al.*, 1999; Lucas *et al.*, 1999). The situation is different in Thailand. Thai cats have strong teeth and very well developed powerful claws, but they are usually fed with rice and well-cooked fish and not raw meat. So they catch rodents in response to hunting instincts but generally not for food. This may explain why the prevalences of *Toxoplasma* infections in cats and humans are low in Thailand even though many factors seem to promote the transmission.

There were four temple areas with abundant cats but without any human seropositive cases. This may be due to the environment of those temples which had concrete or stone and no soil courtyards. *T. gondii* oocysts are much less infective when exposed to higher temperatures. Dubey (1998) demonstrated that oocysts remained infective for only one minute at 60°C compared with 54 months at 4°C. Thus oocysts from cat excreta deposited on hot roofs and stone or concrete courtyards are less likely to be successful in disease transmission. At the other remaining 8 temples and 2 communities (A and C), cat's excreta were left on the ground and grassy shaded areas where it was much cooler. Oocysts deposited in these areas are thus more likely to be successful in disease transmission. Moreover, food preparation for monks and meals also took place there. The chance of viable oocyst contamination was probably higher and may well account for the significant association between human positives cases and infected cats.

In communities B and D where household owners prepared litter boxes for their pets or removed the cat's excreta more than other communities, there were no positive human cases nor infected cats.

In conclusion, in Thailand close association with infected cats is a risk for acquiring *Toxoplasma* infection in adults particularly in places where animals are kept in cool surroundings. The relatively low prevalence of disease transmission from cats to humans compared with that reported from the West probably results from a diet, predominantly of rice and well-cooked fish, and the hot environment.

ACKNOWLEDGEMENTS

We would like to thank Mrs Vipa Prariyanupharb, Miss Sunee Chittamas, Miss Krisana Kabkaew and their nursing team who helped in human blood collection and to Mr Saiyood Incheang and his team for cat blood collection. We are grateful to leaders of the 14 Buddhist temples and four communities and all cats. Without them our study could not be accomplished. Comments from Professor Nicholas White are gratefully acknowledged.

This study was financially supported by Thanat-Molee Khoman Foundation.

REFERENCES

- Bobic B, Jevremovic I, Marinkovic J, Sibalic D, Djurkovic-Djakovic O. Risk factors for Toxoplasma infection in a reproductive age female population in the area of Belgrade, Yugoslavia. *Eur J Epidemiol* 1998; 14: 605-10.
- Chintana T, Sukthana Y, Buyakai B, Lekkla A. *Toxoplasma gondii* in pregnant women with and without HIV infection. *Southeast Asian J Trop Med Public Health* 1998; 29: 383-6.
- Dubey JP. *Toxoplasma gondii* oocyst survival under defined temperatures. *J Parasitol* 1998; 84: 862-5.
- Frenkel JK, Ruiz A. Endemicity of toxoplasmosis in Costa Rica. Transmission between cats, soil, intermediate host and humans. *Am J Epidemiol* 1981; 113: 254.
- Galvan RML, Sanchez VG, Vielma SM, Soto MJL. Presence of anti-Toxoplasma antibodies in humans and their cats in the urban zone of Guadalajara. *Rev Soc Bras Med Trop* 1999; 32: 483-8.
- Lucas SR, Hagiwara MK, Loureiro VD, Ikesaki JY, Birgel EH. *Toxoplasma gondii* infection in Brazilian domestic outpatient cats. *Rev Inst Med Trop Sao Paulo* 1999; 41: 221-4.
- Nogami S, Moritomo T, Kamata H, *et al.* Seroprevalence against *Toxoplasma gondii* in domiciled cats in Japan. *J Vet Med Sci* 1998; 60: 1001-4.
- Reiter-Owona I, Petersen E, Joynson D, *et al.* The past and present role of the Sabin-Feldman dye test in the serodiagnosis of toxoplasmosis. *Bull WHO* 1999; 77: 929-35.
- Sabin AB, Feldman A. Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite (*Toxoplasma*). *Science* 1948; 108: 660-3.
- Saweangthamchai K. Serological study on congenital toxoplasmosis in Chonburi Province. Bangkok: Chulalongkorn Univeristy. 1999. MSc Thesis.
- Solari M. Toxoplasmosis. Available from: URL: <http://www2.austin.cc.tx.us/microbio/2704c/toxo.htm> 1998.
- Sukthana Y, Chintana T, Damrongkitchaiporn S, Lekkla A. *Toxoplasma gondii* antibody in kidney transplanted recipients. *J Med Assoc Thai* 2001; 84: 1137-41.
- Sumner B, Ackland ML. *Toxoplasma gondii* antibody in domestic cats in Melbourne. *Aust Vet J* 1999; 77: 447-9.
- Weigel RM, Dubey JP, Dyer D, Siegel AM. Risk factors for infection with *Toxoplasma gondii* for residents and workers on swine farms in Illinois. *Am J Trop Med Hyg* 1999; 60: 793-8.