COINFECTION OF *LEPTOSPIRA* SPP AND *TOXOPLASMA GONDII* AMONG STRAY DOGS IN BANGKOK, THAILAND

Sathaporn Jittapalapong[1], Patsima Sittisan[2], Thavajchai Sakpuaram[2], Hidenori Kabeya[3], Soichi Maruyama[3] and Tawin Inpankaew[1]

[1] Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok; [2] Department of Veterinary Public Health and Diagnosis Services, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand; [3] Laboratory of Veterinary Public Health, Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University, Kanagawa, Japan

Abstract. Leptospirosis and toxoplasmosis are zoonotic diseases with global importance. Asymptomatic animals harboring these pathogens may act as carriers to other animals including humans. The objective of this study was to investigate the seroprevalence of *Leptospira* and *Toxoplasma* infections in stray dogs in Bangkok. A total of 230 stray dogs from monasteries in a Bangkok district were examined for specific antibodies to *T. gondii* and *Leptospira*. The seroprevalence of *T. gondii* was determined by a modified latex agglutination test (cut off 1≥64). A microscopic agglutination test was performed to detect antibodies to *Leptospira* (cut off, 1:100). The seroprevalences of *T. gondii* and *Leptospira* were 10.9% (25/ 230) and 83.5% (192/230), respectively. *Leptospira* serovar *bataviae* was the most predominant (20.3%) serovar. Co-infection with *Leptospira* and *Toxoplasma* was found in 22 dogs (9.6%). The prevalence of *Toxoplasma* in females was significantly higher than in males ($p<0.05$), but no significant differences was observed for *Leptospira*. The high seroprevalence of these two diseases in dogs is of public health concern because close contact between dogs and humans may provide a link between a reservoir in the environment and susceptible humans.

INTRODUCTION

Leptospirosis and toxoplasmosis are important zoonoses affecting both animals and human beings. The incidence of human leptospirosis is higher in the tropics than in temperate regions but transmission occurs in both industrialized and developing countries (WHO, 2003). The prevalence of different leptospiral serovars in a human population depends on the reservoir animals, as well as local environmental conditions, occupation, agronomical, and agricultural practices. Southeast Asia is endemic for leptospirosis. It has been reported that dogs play an important role in the transmission of leptospirosis to humans in India (Venkataraman and Nedunchelliyan, 1992). In Thailand, stray dogs have become of public health concern since the numbers increase annually, especially in Bangkok metropolitan areas (Jittapalapong *et al*, 2003). This increases the risk for spreading of leptospirosis to humans since the infection results from

Correspondence: Tawin Inpankaew, Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.
Tel/Fax: 66 (0) 2942 8438
E-mail: fvettwi@ku.ac.th; fvettwi@gmail.com

exposure to urine of carrier mammals, either directly or via contaminated soil or water.

Toxoplasma gondii is an obligate, intracellular, protozoan parasite with a worldwide distribution. Infection with *T. gondii* is wide spread in many species of animals, including humans, and it is estimated to infect 30% of the human population worldwide (Aspinall *et al*, 2002). Although the infection usually does not cause a significant problem for healthy individuals, it can be life-threatening for congenitally-infected and immunosuppressed individuals (Chintana *et al*, 1998).

T. gondii infects human beings either by direct contact with infected animals, ingesting oocysts or by eating raw, undercooked meat or unpasteurised milk containing infective stages of the parasite (Riemann *et al*, 1975; Sacks *et al*, 1982). Dogs play a role in transmission of *T.gondii* to humans without having clinical signs of *Toxoplasma* infection similar to cats (Lindsay *et al*, 1997). Dogs are also capable of mechanically shedding oocysts, contaminating the environment (Dubay, 1996).

These facts indicate the importance to public health of investigating the prevalence of both *Leptospira* and *Toxoplasma* infections in stray dogs. The objective of this study was to determine the seroprevalence of *Leptospira* and *Toxoplasma* infections in stray dogs in Bangkok, Thailand.

MATERIALS AND METHODS

Study areas and animals

The dog samples were collected in a district located in the center of Bangkok. More than 20 Buddhist monasteries exist in this area and many stray dogs inhabit in this area. A total of 230 stray dogs were obtained randomly from monasteries in this district. One hundred fifteen male and 115 female dogs were collected. Dogs were examined for sex, general condition and status of ectoparasites. Blood samples were collected and the sera were separated by centrifugation at 500*g* for 30 minutes at the Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University and stored at -20ºC until analysis.

Detection of antibodies to *Leptospira* spp

Serum samples were tested for the infection due to leptospirosis using the following 21 serovars as antigens: *L. bratislava, L. autumnalis, L. ballum, L. bataviae, L. canicola, L. cellidoni, L. cynopteri, L. djasiman, L. grippotyphosa, L. hebdomadis, L. icterohaemorrhagiae, L. javanica, L. louisiana, L. manhao, L. pomona, L. ranarum, L. sarmin, L. sejroe, L. shermani, L. tarassovi,* and *L. patoc*. A microscopic agglutination test was performed as previously described (Cole *et al*, 1973). In brief, the dog sera were inactivated by heating at 56ºC for 30 minutes and diluted by two fold dilution beginning with a 1:25 solution on a U-shaped micro plate. A volume of 50 µl of each dilution was made for each serum sample with an approximate concentration of 10^8*Leptospira* organisms per sample and incubated at 37ºC for 2-4 hours. Only antigens and an equal volume of PBS were added to the plate as negative antigen controls. Rabbit serum positive for homologous strain antigens were used as a positive control. Fifty percent agglutination at a 1:100 dilution was considered to be positive (WHO, 2003).

Detection of antibodies to *Toxoplasma gondii*

Antibody to *T. gondii* was analyzed using a latex agglutination test kit (TOXOCHECK-MT 'Eiken' Chemical, Tokyo, Japan). Positive and negative controls were used for each test. The procedure was carried out as previously described (Jittapalapong *et al*, 2005). The cut-off titer for a positive test was 1:64, as set by the manufacturer of the

Table 1
Results of *Leptospira* and *Toxoplasma* in stray dogs in Bangkok, Thailand.

Seropositive	Overall		Sex			
			Males		Females	
	Number	%	Number	%	Number	%
Leptospira only	183	79.6	101	87.8	82	71.3
Toxoplasma only	25	10.9	7	6.1	18	15.7
Co-infection	22	9.6	7	6.1	15	13
Leptospira overall	205	89.1	108	93.9	97	84.3
Toxoplasma overall	47	20.4	14	12.2	33	28.7
Total	230		115		115	

test kit (Tsubota *et al*, 1977a).

Statistical analysis

A chi-square test (χ^2) was used to examine for significant differences between findings. A probability less than 0.05 were considered to be statistically significant.

RESULTS

Seroprevalence of *Leptospira* in stray dogs

Of 230 dog sera samples, 205 (89.1%) were seropositive for *Leptospira* infection, 183 were infected with *Leptospira* only (Table 1).

The positive rates in males and females were 87.8% and 71.3%, respectively, the difference was not significant. Fifteen different serovars of leptospira had titers ranging from 1:100 to 1:800. *Leptospira* serovar *bataviae* was the most predominant serovars (20%) (Table 2). There were co-infections with more than 1 serovar of *Leptospira* in some dogs. There were no significant differences showed in *Leptospira* infections between the sexes.

Seroprevalence of *T. gondii* infections

The overall seropositive rate of *T. gondii* was 20.4% (47/230); 25 dogs (10.9%) were infected with only this parasite. The *T. gondii* seropositive rate in males (15.7%) was significantly higher than that in females (6.1%) ($p<0.05$). The highest titer was 1:512.

Table 2
Leptospira serovars found in stray dogs, Bangkok, Thailand.

Serovars	% Positive	Titer
bratislava	1	1:100
autumnalis	3	1:100-1:200
bataviae	20	1:100-1:800
cynopteri	1	1:100
grippotyphosa	2	1:100
hebdomadis	2	1:100
louisiana	1	1:100
manhao	1	1:200
pomona	1	1:100
ranarum	3	1:100-1:200
sarmin	3	1:100-1:200
sejroe	6	1:100-1:200
shermani	4	1:100-1:200
tarassovi	7	1:100-1:200
patoc	8	1:100-1:200

Co-infection with *Leptospira* and *Toxoplasma*

Co-infection with *Leptospira* and *T. gondii* was found in 22 dogs (9.6%). The posi-

tive rate of co-infection in males was significantly higher than in females ($p<0.05$).

DISCUSSION

The importance of stray dogs as a source of human infection is well recognized (Thorten *et al*, 1971; Aslantas *et al*, 2005). The large number of stray dogs in the monastery increases the possibility of acquiring an infection. In many tropical countries, dogs are significant reservoir of zoonoses and may be an important source for outbreaks (Levett, 2001).

Stray dogs are easily infected by *Leptospira* via rodents living in monasteries in Bangkok. *Leptospira* serovars *canicola* and *icterohaemorrhagiae* are common serogroups infecting dogs, but the relative importance of these two serogroups varies from country to country (Rentko *et al*, 1992; Faine, 1994; Weekes *et al*, 1997). In Thailand, a proposed shift in predominant infecting serovars is thought to be responsible for the spread of leptospirosis in the northeastern provinces of Thailand between 1996 and 2003 (Tangkanakul *et al*, 2005). The changing epidemiology of canine leptospirosis in Thailand reflects the variable nature of leptospirosis within animal populations.

In our study, *Leptospira* serovar *bataviae* was found to be the predominant serovar in stray dogs. In Thailand, leptospirosis in pet dogs is normally controlled by a bivalent vaccine using the serovars, *canicola* and *icterohaemorrhagiae*. Humoral immune responses induced by the bivalent vaccine appear to be serovar-specific (Faine *et al*, 1999). These results indicate animals that receive the bivalent vaccine may not be protected from leptospirosis due to other serovars, such as *bataviae, tarassovi, patoc,* and *sejroe*. Urines of infected dogs are a risk to the dog owner and their family.

Climate and ecological conditions influence some endemic diseases in Thailand and may be factors that trigger outbreaks (Tangkanakul *et al*, 2005). Flooding during the rainy season favors the transmission of disease (Fuortes and Nettleman, 1994; Levett, 2001). Ecological and epidemiological variations in domestic and wild animals are also factors that may influence disease transmission. The role of rats has been reported to play in increasing role in the transmission of leptospirosis (Hartskeerl and Tepstra, 1996; Levett, 2001; Bhati *et al*, 2003). Analysis of contributing factors, such as animal reservoirs, methods of prevention and control and animal vaccination and treatment, should be in included in the evaluation and control of disease in these areas (Levett, 2001; Tangkanakul *et al*, 2005).

The seroprevalence of *Toxoplasma* infection in stray dogs in Bangkok was 20.4%. Our previous study found a prevalence of 9.4% in stray dogs from 38 districts in 2003 (Jittapalapong *et al*, 2007). The data suggest the prevalence of *Toxoplasma* in dogs differs by location. The rate in this district is considerably higher than in previous studies in Bangkok. The route of transmission to stray dogs is thought to be by ingestion of rodents that have infective cysts. Another route suggested is the ingestion of food or water contaminated with oocysts shed by stray cats in the environment. *Toxoplasma* infection is prevalent among rodents (Holliman, 1997). Rats with *T. gondii* infection appear to be less neophobic than uninfected rats (Webster *et al*, 1994). Consequently, increased activity and movement in *Toxoplasma*-infected rodents may increase the chance of predation by cats or dogs. Dogs have been reported to be a mechanical vector of toxoplasmosis (Lindsay *et al*, 1997).

Our findings regarding *Leptospira* and *T. gondii* infections in stray dogs in Bangkok, Thailand signify a significant potential public health problem.

ACKNOWLEDGEMENTS

We thank the Faculty of Veterinary Medicine and Kasetsart University Research Development Institute (KURDI), Kasetsart University for their essential supports. We would also like to acknowledge the excellent field assistance of the sixth year veterinatian students: Walaiporn Sacholsawatwong, Sanchai Phruksakorn, Siripan Sujiputto, who volunteer to carry out the study on the weekends.

REFERENCES

Aslantas O, Ozdemir V, Kilic S, Babur C. Seroepidemiology of leptospirosis, toxoplasmosis, and leishmaniosis among dogs in Ankara, Turkey. *Vet Parasitol* 2005; 129: 187-91.

Aspinall TV, Marlee D, Hyde JE, Sims PFG. Prevalence of *Toxoplasma gondii* in commercial meat products as monitored by polymerase chain reaction–food for thought? *Int J Parasitol* 2002; 32: 1193-9.

Bharti AR, Nally JE, Ricaldi JN, *et al*. Leptospirosis: a zoonotic disease of global importance. *Lancet Infect Dis* 2003; 3: 757-71.

Chintana T, Sukthana Y, Bunyakai B, Lekkla A. *Toxoplasma gondii* antibody in pregnant woman with and without HIV infection. *Southeast Asian J Trop Med Public Health* 1998;29: 383-6.

Cole JR, Sulzer CR, Pursell AR. Improved microtechnique for the leptospiral microscopic agglutination test. *Appl Microbiol* 1973; 25: 976-80.

Dubay JP. Strategies to reduce transmission of *Toxoplasma gondii* to animals and humans. *Vet Parasitol* 1996; 64: 65-70.

Faine S. Leptospira and leptospirosis. Boca Raton, Fl: CRC Press, 1994: 215-28.

Faine S, Adler B, Bolin CA, Perolat P. *Leptospira* and leptospirosis. 2nded. Melbourne: Medical Science, 1999: 272.

Fuortes L, Nettleman M. Leptospirosis: a consequence of the Iowa flood. *Iowa Med* 1994; 84: 449-50.

Hartskeerl RA, Teprtra WJ. Leptospirosis in wild animals. *Vet Q* 1996; 18: 149-50.

Holliman RE. Toxoplasmosis, behaviour, and personality. *J Infect* 1997; 35: 105-10.

Jittapalapong S, Pinyopanuwat N, Boonchob S, Chimnoi W, Jansawan W. Impact on animal rearing or yielding in the public land in Thailand, A manual of regulation of animal rearing or yielding in the public land of Thailand. Bangkok: Kasetsart University Press, 2003: 250 pp.

Jittapalapong S, Sangvaranond A, Pinyopanuwat N, *et al*. Seroprevalence of *Toxoplasma gondii* infection in goats in Satun Province, Thailand. *Vet Parasitol* 2005; 127: 17-22.

Jittapalapong S, Nimsupan B, Pinyopanuwat N, Chimnoi W, Kabeya H, Maruyama S. Seroprevalence of *Toxoplasma gondii* antibodies in stray cats and dogs in the Bangkok metropolitan area, Thailand. *Vet Parasitol* 2007; 145: 138-41.

Lindsay DS, Dubey JP, Blangburn BL, Mechanical transmission of *Toxoplasma gondii* oocysts by dogs. *Vet Parasitol* 1997; 73: 27-33.

Rentko VT, Clark N, Ross L, Schelling SH. Canine leptospirosis: a retrospective study of 17 cases. *J Vet Int Med* 1992; 6: 235-44.

Riemann HP, Meyer MG, Theis JH, Kelso G, Behymer DE. Toxoplasmosis in an infant fed unpasteurized goat milk. *J Pediatr* 1975; 87: 1728-32.

Sacks JJ, Roberts RR, Brooks NF. Toxoplasmosis infection associated with raw goat milk. *J Am Vet Med Assoc* 1982; 148: 1728-32.

Tangkanakul W, Smits HL, Jatanasen S. Leptospirosis: an emerging health problem in Thailand. *Southeast Asian J Trop Med Public Health* 2005; 36: 281-7.

Thorten M, Kaplun A, Shenberg E. A survey of leptospirosis in stray dogs. *Refuah Vet* 1971; 28: 98-101.

Tsubota N, Hiraoka K, Sawada Y, Watanabe T, Ohshima S. Studies on latex agglutination test for Toxoplasmosis. *Jpn J Parasitol* 1977a; 26: 286-90.

Tsubota N, Hiraoka K, Sawada Y, Oshino M.

Studies on latex agglutination test for toxoplasmosis. III. Evaluation of the microtiter test as a serologic test for toxoplasmosis in some animals. *Jpn J Vet Sci* 1977b; 26: 291-8.

Venkataraman KS, Nedunchelliyan S. Epidemiology of an outbreak of leptospirosis in man and dog. *Comp Immun Microbiol Infect Dis* 1992; 15: 243-7.

Webster JP, Brunton CF, MacDonald DW. Effect of *Toxoplasma gondii* upon neophobic behaviour in wild brown rats, *Rattus norvegicus*. *Parasitology* 1994; 109: 37-43.

Weekes CC, Everard COD, Levett PN. Seroepidemiology of canine leptospirosis on the island of Barbados. *Vet Microbiol* 1997; 51: 215-22.

WHO. Human leptospirosis: guidance for diagnosis, surveillance and control. Malta: WHO, 2003.