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

Sathaporn Jittapalapong¹, Patsima Sittisan², Thavajchai Sakpuaram², Hidenori Kabeya³, Soichi Maruyama³ and Tawin Inpankaew¹

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
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 widespread 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 500g for 30 minutes at the Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University and stored at -20°C 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. bataovae, L. canicola, L. celdoni, L. cynopteri, L. djasiman, L. grippotyphosa, L. hebdomadis, L. icterohaemorrhagiae, L. javanica, L. louisiana, L. manhao, L. pomona, L. rana-rum, 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 56°C 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 10⁸ *Leptospira* organisms per sample and incubated at 37°C 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
COINFECTION OF *LEPTOSPIRA* AND *T. GONDII* AMONG STRAY DOGS

**Table 1**

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

<table>
<thead>
<tr>
<th>Seropositive</th>
<th>Overall</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td><em>Leptospira</em> only</td>
<td>183</td>
<td>79.6</td>
</tr>
<tr>
<td><em>Toxoplasma</em> only</td>
<td>25</td>
<td>10.9</td>
</tr>
<tr>
<td>Co-infection</td>
<td>22</td>
<td>9.6</td>
</tr>
<tr>
<td><em>Leptospira</em> overall</td>
<td>205</td>
<td>89.1</td>
</tr>
<tr>
<td><em>Toxoplasma</em> overall</td>
<td>47</td>
<td>20.4</td>
</tr>
<tr>
<td>Total</td>
<td>230</td>
<td>115</td>
</tr>
</tbody>
</table>

**Table 2**

*Leptospira* serovars found in stray dogs, Bangkok, Thailand.

<table>
<thead>
<tr>
<th>Serovars</th>
<th>% Positive</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>bratislava</td>
<td>1</td>
<td>1:100</td>
</tr>
<tr>
<td>autumnalis</td>
<td>3</td>
<td>1:100-1:200</td>
</tr>
<tr>
<td>bataviae</td>
<td>20</td>
<td>1:100-1:800</td>
</tr>
<tr>
<td>cynopteri</td>
<td>1</td>
<td>1:100</td>
</tr>
<tr>
<td>grippotyphosa</td>
<td>2</td>
<td>1:100</td>
</tr>
<tr>
<td>hebdomadis</td>
<td>2</td>
<td>1:100</td>
</tr>
<tr>
<td>louisiana</td>
<td>1</td>
<td>1:100</td>
</tr>
<tr>
<td>manhao</td>
<td>1</td>
<td>1:200</td>
</tr>
<tr>
<td>pomona</td>
<td>1</td>
<td>1:100</td>
</tr>
<tr>
<td>ranarum</td>
<td>3</td>
<td>1:100-1:200</td>
</tr>
<tr>
<td>sarmin</td>
<td>3</td>
<td>1:100-1:200</td>
</tr>
<tr>
<td>sejroe</td>
<td>6</td>
<td>1:100-1:200</td>
</tr>
<tr>
<td>shermani</td>
<td>4</td>
<td>1:100-1:200</td>
</tr>
<tr>
<td>tarassovi</td>
<td>7</td>
<td>1:100-1:200</td>
</tr>
<tr>
<td>patoc</td>
<td>8</td>
<td>1:100-1:200</td>
</tr>
</tbody>
</table>

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

**Statistical analysis**

A chi-square test ($\chi^2$) 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.

**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 reservoirs 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 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 veterinarian students: Walaiporn Sacholsawatwong, Sanchai Phruksakorn, Siripan Suiputto, who volunteered to carry out the study on the weekends.

REFERENCES


