SEROPREVALENCE AND RISK FACTORS ASSOCIATED WITH LEPTOSPIROSIS IN DOGS

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Abstract. This study was done to determine the seroprevalence and risk factors of leptospirosis in dogs. From March to September 2004, a total of 210 dogs were randomly selected from the Small Animal Hospital, Faculty of Veterinary Medicine, Chiang Mai University. Dog sera were collected from the cephalic vein and kept at -20ºC until submitted to the National Institute of Health for a Microscopic Agglutination Test (MAT). Risk factors were analysed using logistic regression modelling. The prevalence of *Leptospira* antibodies was 11% (23/210). The most prevalent *Leptospira* serogroups were Bataviae 5.2% (11/210), Canicola 2.4% (5/210), Australis 1.4% (3/210), Icterohaemorrhagiae 1.4% (3/210), Ballum 0.5% (1/210), Djasiman 0.5% (1/210), Javanica 0.5% (1/210), Mini 0.5% (1/210), and Sejroe 0.5% (1/210). Risk factors, including signalment, environment and health status, were not significantly associated with leptospirosis antibodies. However, playing in sewage, staying outdoors >50% of the time, and consumption raw meat increased the risk of leptospirosis antibodies in dogs.

INTRODUCTION

Leptospirosis is a zoonotic disease with worldwide distribution. The disease is caused by a spirochaete genus *Leptospira*, which is a thin spiral organism 0.1 µm width and 6-20 µm long. This organism moves by spinning or bending both ends. The genus *Leptospira* can be classified according to its pathogenicity into a non-pathogenic group (*Leptospira biflexa*) and a pathogenic group (*Leptospira interrogan*). More than 200 serovars of pathogenic *Leptospira* have been identified. Rodents and mammals, such as cattle, pigs and dogs, are the major reservoir hosts, which can be subclinically infected and bacteria can be shed in the urine for several months to years. *Leptospira* can survive for a long time in an optimal environment with enough humidity, shade, pH 7.2-8.0 and 28-30ºC. Hosts can be infected with *Leptospira* by either direct contact with the bacteria in contaminated urine and other secretions or indirect contact with a contaminated environtment. This organism can be transmitted through open wounds or mucous membranes (Levett, 2001). The clinical picture of human cases of leptospirosis varies from subclinical to severe and fatal. Common clinical signs include fever, severe myalgia, red eyes, nausea, vomiting, diarrhea and intermittent fever. In acute cases, jaundice and hemorrhage in the viscera, eyes and menigitis can be found. Liver or kidney failure are the most common causes of death from this disease (Fain *et al*, 1999). Leptospirosis has a worldwide distribution, but a higher incidence is observed in the tropical areas, particularly in the rainy season. Moreover, activities that increase the risk of *Leptospira* infection, such as walking with bare feet, swimming in a canal, contact with rats or stray dogs, and herding cattle in the field are more prevalent in tropical area.

In Thailand, an outbreak of leptospirosis was first reported in Bangkok after a big flood in 1943, in which 2 of 4 cases were fatal. Since 1996, an annual outbreak of leptospirosis has been reported, which begins in the rainy season in July and lasts until October. The incidence of disease has gradually increased through the years from 358 cases in 38 provinces in 1996 to 5,933 cases in 60 provinces in 1999. Most cases (90%) are clustered in the northeastern part of

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the country. Various animals and environments have had many serovars, which has never been reported in Thailand, such as bratislava, pyrogenes and sejroj. In 2001, 11,155 cases of leptospirosis were reported in Thailand; 182 patients were severe and progressed to death.

The diagnosis of leptospirosis can be achieved using either bacterial identification or serological tests for antibodies. *Leptospira* can be cultured and examined under the dark-field microscope. A polymerase chain reaction assay can also be used to identify the species of *Leptospira* with high sensitivity and specificity (Bal *et al*, 1994; Brown and Levett, 1997; Harkin *et al*, 2003a). *Leptospira* can be isolated from blood (Chandrasekaran and Pankajalakshmi, 1997), serum (Smythe *et al*, 2002), urine (Harkin *et al*, 2003b) and cerebro-spinal fluid (Brown *et al*, 1995). Application of these methods in general laboratories is limited by the nature of the bacteria and the laboratory capacity (Fain *et al*, 1999; Hartskeerl *et al*, 2001). Detection of *Leptospira* antibodies is much more widely used clinically. There are several techniques to detect *Leptospira* antibodies such as ELISA, hemagglutination test, latex agglutination test (Effler *et al*, 2002) and microscopic agglutination test (MAT) (Levett, 2001).

Previous studies in Bangkok, Thonburi, Chiang Mai and Pitsanulok demonstrated that rats were the most important reservoir for leptospirosis, followed by dogs. Nowadays, dogs have become popular pets with a risk for *Leptospira* transmission (Chandrasekaran and Pankajalakshmi, 1995). Leptospirosis in dogs commonly results from serovars canicola and icterohemorrhagiae. Common clinical signs in dogs include fever, vomiting, diarrhea, myalgia, nose bleeding, and jaundice (Nelson, 2003). In acute cases, clinical signs will progress more rapidly, including hypothermia, bleeding and death in 2-3 hours from liver and kidney failure (Nelson, 2003). Initially, dogs develop leptospiremia for the first 1-2 weeks with an elevated serum IgM level. After the first week, the bacteria can be found in the cerebrospinal fluid and urine when the IgG level becomes elevated. IgM levels then decrease rapidly, while IgG levels will slowly decrease over one year. However, infected dogs may shed *Leptospira* in the urine without clinical signs after the IgG level decreases (Hartskeerl *et al*, 2001). A study in Italy found the prevalence of leptospirosis in companion dogs was 3.4%, while the prevalence in stray dogs was 30.3%. The most common serovars were bratislava and grippotyphosa (Scanziani *et al*, 2002).

Although most dogs are vaccinated, the vaccine commonly used protects against serovar canicola and icterohaemorrhagiae, which are less prevalent in Thailand. There is no cross protection with other serovars which may be more prevalent, such as bataviae. Vaccination may reduce clinical disease or severity, but cannot prevent the spread of the bacteria. This study was conducted to determined the risk factors for leptospirosis infection in dogs determined by MAT. The results of this study can be used to provide recommendations for dog owners to prevent leptospirosis in their pets and reduce the risk of infection in the owners.

MATERIALS AND METHODS

A cross-sectional study design was used. Sample dogs were selected from the out-patient department of the small animal hospital, faculty of Veterinary Medicine, Chiang Mai University from March to September 2004. A total of 210 dogs with consent from the owners participated in the study. Three milliliters of blood were collected from the cephalic vein of each dog. After centrifuging at 3,000 rpm for 5 minutes, serum was collected and stored at -20ºC until used.

Leptospira antigens were prepared according to the method provided by the WHO/FAO Collaborating Center of Reference and Research on Leptospirosis (2000). The MAT, which is a standard serological test for serovars of *Leptospira* was performed using the same standard. The MAT was done by the Department of Medical Science, Ministry of Public Health using 23 serogroups of *Leptospira* antigens (Table 1). Serum samples with a titer higher than or equal to 1:20 was classified as positive for the serogroup tested.

Information regarding risk factors associated with *Leptospira* infection in dogs was recorded using a pre-test questionnaire administered to the owner by one of the authors.

The prevalence of leptospirosis was calculated by dividing the number of infected dogs by the total number of dogs tested. Significant associations between risk factors and leptospirosis were determined using multivariable logistic regression modeling.

Table 1
Serogroup of *Leptospira* used in MAT.

Number	Serogroup (Serovar)
1	Australis (bratislava)
2	Autumnalis (autumnalis)
3	Ballum (ballum)
4	Bataviae (bataviae)
5	Canicola (canicola)
6	Celledoni (celledoni)
7	Cynopteri (cynopteri)
8	Djasiman (djasiman)
9	Grippotyphosa (grippotyphosa)
10	Hebdomadis (hebdomadis)
11	Icterohaemorrhagiae (icterohaemorrhagiae)
12	Javanica (javanica)
13	Louisiana (louisiana)
14	Manhao (manhao)
15	Mini (mini)
16	Panama (panama)
17	Pomona (pomona)
18	Pyrogenes (pyrogenes)
19	Ranarum (ranarum)
20	Sarmin (sarmin)
21	Sejroe (sejroe)
22	Shermani (shermani)
23	Tarassovi (tarassovi)
24	Samaranga (patoc I)

RESULTS

The results of the MAT for *Leptospira* antibodies are shown in Table 2. Of 210 serum samples, a total of 23 serum samples were positive for *Leptospira* antibodies yielding a prevalence of 11%. Most serum samples were positive for 1 serogroup of *Leptospira* (87%). Few serum samples were positive for 2 (8.7%) and 3 (4.3%) serogroups. The most common serogroup of *Leptospira* found was Bataviae (5.2%) followed by Canicola (2.4%), Australis (1.4%), Icterohaemorrhagiae (1.4%), Ballum (0.5%), Djasiman (0.5%), Javanica (0.5%), Mini (0.5%), and Sejroe (0.5%).

The highest prevalence of leptospirosis was found in May (19%) as shown in Fig 1. There

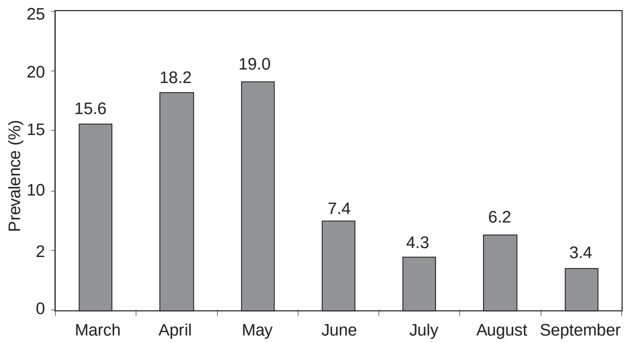

Fig 1–Monthly prevalence of leptospirosis in dogs in Chiang Mai.

Table 2
Leptospira antibody titers in dogs in Chiang Mai.

Serogroup	Seroprevalence	Antibody titer					
		1:20	1:40	1:80	1:16	1:32	1:64
Bataviae	5.2% (11/210)	1	3	1	1	1	4
Canicola	2.4% (5/210)	3	1	1	-	-	-
Australis	1.4% (3/210)	1	-	1	-	1	-
Icterohaemorrhagiae	1.4% (3/210)	1	1	1	-	-	-
Ballum	0.5% (1/210)	-	1	-	-	-	-
Djasiman	0.5% (1/210)	1	-	-	-	-	-
Javanica	0.5% (1/210)	-	-	1	-	-	-
Mini	0.5% (1/210)	1	-	-	-	-	-
Sejroe	0.5% (1/210)	1	-	-	-	-	-

Table 3
Risk factors of *Leptospira* infection in dogs in Chiang Mai.

Factors	Seropositive dogs		Seronegative dogs	
	n	Yes (%)	n	Yes (%)
Gender (Female)	21	7 (33.3)	182	89 (48.9)
Age less than 1 year	19	3 (15.8)	163	32 (19.6)
Stay outside more than 50% of the time	21	11 (52.4)	179	73 (40.8)
Rats infested in household	21	12 (57.1)	180	112 (62.2)
Playing in sewage	21	2 (9.5)	179	3 (1.7)
Consume raw meat	21	2 (9.5)	180	17 (9.4)
Jaundice	21	1 (4.8)	180	5 (2.8)
Dark urine	21	5 (23.8)	180	42 (23.3)

Table 4
Multivariable logistic regression analysis of risk factors for leptospirosis infection in dogs in Chiang Mai.

Factors	OR	95% CI	p-value
Gender (Female)	0.451	0.155, 1.312	0.1439
Age less than 1 year	0.688	0.176, 2.694	0.5914
Stay outside more than 50% of the time	1.828	0.674, 4.958	0.2360
Rats infested in household	0.792	0.285, 2.202	0.6546
Playing in sewage	5.689	0.803, 40.297	0.0818
Consume raw meat	1.217	0.234, 6.342	0.8156
Jaundice	<0.001	<0.001, >999.999	0.9851
Dark urine	1.134	0.362, 3.551	0.8296

seemed to be a seasonal variation with a higher prevalence in the hot season from March to May and a lower prevalence in the wet season from June to September.

Tables 3 and 4 show the risk factors for leptospirosis infection. None of the risk factors investigated were significantly associated with *Leptospira* infection determined by MAT. However, playing in sewage, staying outside more than 50% of the time, and consumption of raw meat may increase the risk of *Leptospira* infection in dogs in Chiang Mai.

DISCUSSION

The prevalence of *Leptospira* antibodies in dogs has varied among different countries: 21.3% in India (Venkataraman and Nedunchelliyan, 1992) and 6.36% in Italy (Cerri *et al*, 2003) while the prevalence was 11% in our study. There was a high level of variation in *Leptospira* serovars found in each area. Our study found that the predominant serovars of *Leptospira* were bataviae (5.24%) and canicola (2.38%), while the most predominant serovars in Illinois, USA were icterohaemorrhagiae (65.4%), canicola (65.4%) and grippotyphosa (72.1%) (Boutilier *et al*, 2003). The *Leptospira* serovars used in the vaccine in Thailand are icterohaemorrhagiae and canicola; which are not the most predominant serovars in Thailand. Climate may be an important factor affecting the prevalence of *Leptospira* in each area. The suitable climate for *Leptospira* is the tropical climate, and the prevalence of *Leptospira* has been found to be the highest in the rainy season (Ward *et al*, 2002).

MAT cannot distinguish between antibodies to *Leptospira* caused by natural infection versus those caused by vaccination. Therefore, paired serum should be used to confirm *Leptospira* infection with MAT (Scanziani *et al*, 2002).

In Thailand, *Leptospira* serogroups found in cattle were Sarmin, Ranarum, Ballum, and Sejroe. While the serogroups found in pigs were Sarmin, Australis, and Pomona. Two of these serogroups are similar to the serogroups observed in dogs in our study, therefore there may be an association between *Leptospira* in dogs, pigs and cattle in Thailand. In other countries, the serogroups of *Leptospira* found in cattle and pigs were different from those found in Thailand. *Leptospira* serogroups Sejroe, Hardjo, Tarrassovi, Bratislava, and Icterohaemorrhagiae were found in cattle in Portugal (Rocha, 1998), Brazil (Guitian *et al*, 2001), and Mexico (Segura-Correa, 2003). *Leptospira* serogroups Australis, Icterohaemorrhagiae, and Pomona were found in pigs in Belize (Everard *et al*, 1988), Japan (Kazami *et al*, 2002) and Malaysia(Bahaman *et al*, 1987). Some of the serogroups found in dogs in this study were similar to serogroups found in other species previously reported to be sources for human infection. In Thailand, the rat (*Bandicota indica*) was an important source of *Leptospira* serovars autumnalis, bataviae, pyrogenes, javanica and australis, some of which were found in this study. Therefore, the rat may be a source of *Leptospira* infection in dogs in Thailand.

In humans, countries in the tropics have similar types of *Leptospira*, since *Leptospira* grows well in this climate. A study in Buri Ram, Thailand in 1999 reported a 41.3% prevalence of leptospirosis in human (Phraisuwan *et al*, 2002), while the prevalence was 37.7% in Mexico (Leal-Castellanos *et al*, 2003) and 50.5% in India (Venkataraman and Nedunchelliyan, 1992). A previous study in Thailand indicates that patients can be found both in the city and rural areas. Incidence in male and female were not different among the age groups. However, occupational infection occurred at a higher rate in male. Risk factors for *Leptospira* infection in human included plowing rice fields, fertilizing, and fishing all of which require a long duration of work in water. A study in Nakhon Ratchasima Pprovince, Thailand also reported that spending more than 6 hours in water is a risk factor for *Leptospira* infection(Tangkanakul, 2000). In Nicaragua, Mexico and North Andaman, *Leptospira* antibody titers in human were associated with keeping dogs in the household (Leal-Castellanos *et al*, 2003). The major serogroup found in human was Icterohaemorrhagiae (Venkataraman and Nedunchelliyan, 1992) which is a similar to that serogroup found in dogs in this study, indicating that dogs may be an important source of leptospirosis in human.

In dogs, Ward *et al* (2000) reported that age, sex and breed were significantly associated with *Leptospira* antibody level. Male dogs age 4-10 years old, herding dogs, hounds, working dogs and mixed breed dogs had a higher risk of infection than female dogs age less than 1 year old and companion dogs. In this study, dogs with outdoor activities or that consumed raw meat were found to have higher risk of leptospirosis, which is similar to what was previously reported.

In conclusion, dogs may be an important source of leptospirosis in humans. Dogs with outdoor activities or that consumed raw meat have a higher risk of infection. In order to reduce the risk of human infection from dogs, companion dogs should be kept in doors and should not be fed raw meat. Improving hygiene and eradication of rodents may also reduce the risk of infection in dogs.

ACKNOWLEDGEMENTS

The authors would like to thank the small animal hospital, Faculty of Veterinary Medicine, Chiang Mai University for providing the sample collection facility and the Department of Medical Science, Ministry of Public Health for Laboratory support. The authors also would like to acknowledge the owners and the dogs that participated in the study.

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