

# Preliminary investigation of canine leptospirosis in a rural area of Thailand

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### Summary

Leptospirosis is a major zoonosis throughout the world. The disease can infect several mammal species of farming and domestic animals. The objective of this study was to investigate the prevalence of canine leptospirosis in a rural area of Thailand. One hundred and fifty three non-leptospira vaccinated dogs were selected from the Nakhon Pathom province of Thailand during April 2006 by simple random sampling. The signalment data of age, breed and sex were recorded. All serum samples were reacted with 24 references of leptospira serovars by microscopic agglutination test (MAT). The results showed the highest positivity in dogs aged within 3-6 years ( $P < 0.05$ ) but were not significantly different between the genders. The most prevalent serovar was tarassovi (30.7%) and its titer ranged from 50 to 400. For all 88 MAT positive sera, 62.5% and 37.5% were positive with multiple and single leptospira serovars, respectively. The high disease prevalence in dogs indicates the high leptospira contamination in this area. However, the potential of the disease transmitted source in dogs is indefinite. Consequently, for effective disease eradication strategies, the local prevalence of leptospira serovars should be utilized for further vaccination.

**Keywords:** Canine, leptospirosis, Thailand

Leptospirosis is an important zoonosis in humans and animals, caused by infection with spirochaete bacteria *Leptospira* species. The source of infection is usually either direct or indirect contamination with the urine of an infected animal through abrasions or via the conjunctiva. Many rodent species are primary reservoirs for distinct serovars, which may transfer infection to domestic farm animals, dogs, and humans (12). Diagnostic methods for determination of leptospiral infection included microscopic examination, antigen detection, pathogen isolation, and serological and molecular techniques (9, 12). The epidemiology of leptospirosis in dogs has been reported worldwide. Before the 1970's, dogs are generally maintenance hosts for serovars canicola and icterohaemorrhagiae (11). However the present reports of sero-prevalence associated with canine leptospirosis demonstrate the unusual serovars of *Leptospira interrogans*, including grippotyphosa and pomona (3, 4, 6, 15), bratislava (7, 20), autumnalis (16), copenhageni (14), and *L. kirschneri* serovar grippotyphosa (21), and mankarso (1).

Although the recent studies showed that the sero-prevalence of canine leptospirosis in many areas has changed, a commercial bivalent vaccine against serovars canicola and icterohaemorrhagiae is serovar-specific and does not protect across other serovars (5). Furthermore, farm animals are sometimes maintenance hosts of some

serovars. For this reason, a study of the prevalent serovars is important to update the epidemiology of this disease in any area. Therefore, the purpose of this study was to determine the sero-prevalence of canine leptospirosis in a rural area of Thailand, where agriculture is a usual career of people in this region.

### Material and methods

**Study design and sample collection.** This was a cross-sectional study to determine the seroprevalence and serovars implicated in canine leptospirosis in apparently healthy dogs from Nakhon Pathom province, the most important agricultural and farming area of Thailand, during April 2006. One hundred and fifty three non-leptospira vaccinated dogs were selected from three districts (Amphoe Muang, Amphoe Don Tum and Amphoe Kamphaeng Saen) by simple random sampling. The signalment data of age, breed and sex were recorded. The blood samples of all dogs were collected from cephalic vein by sterile technique with non anticoagulant vacuumed tube set. All blood samples were spun down in a centrifuge, the sera harvested aseptically and frozen at  $-20^{\circ}\text{C}$  until tested.

**Detection of antibody against leptospiras.** The microscopic agglutination test (MAT) was performed on all sera by standard method as briefly explain. The living antigens used were 5-7 days old autoagglutination-free cultures grown in Elinghausen McCullough Johnson Harris (EMJH) medium (Difco, Sparks, MD) with approximately  $1 \times 10^8$  to  $2 \times 10^8$  organisms/mL. The MAT was done at doubling dilutions starting from 1 in 25. The reaction was observed after 3 hrs incubation at

Tab. 1. Reference leptospira antigen using for MAT

Serogroup	Serovar	Strain
Australis	australis	Ballico
	bangkok	BD 92
	bratislava	Jez Bratislava
Autumnalis	autumnalis	Akiyami A
Bataviae	bataviae	Van Tienam
Canicola	canicola	Hond Utrecht IV
Cellidoni	cellidoni	Cellidoni
Djasiman	djasiman	Djasiman
Grippotyphosa	grippotyphosa	Moskva V
Hebdomadis	hebdomadis	Hebdomadis
Icterohaemorrhagiae	copenhageni	Copenhageni
	icterohaemorrhagiae	RGA
Javanica	javanica	Veldrat Bat. 46
Louisiana	saigon	L 79
Pomona	pomona	Pomona
Pyrogenes	pyrogenes	Salinem
	zanoni	Zanoni
Ranarum	ranarum	Ranarum
Sarmin	sarmini	Sarmin
Sejroe	hardjo	Hardjoprajitno
	sejroe	M 84
	wolffi	3705
Semarang	patoc	Patoc I
Tarassovi	tarassovi	Perepelicin

room temperature by dark field microscopy. Positive samples were titrated up to end titers. A single reciprocal titer of 50 or greater was considered seropositive and indicative of exposure to leptospirosis. A battery of 24 reference leptospira serovars was showed in table 1.

**Statistical analysis.** The frequency of detection of *Leptospira agglutinins* and the various serovars and titers were compared for different types of dogs and the various characteristics of dogs and risk factors were compared using Statistical Package for Social Sciences (SPSS), version 10 (SPSS Inc., Chicago, IL, USA) and analyzed by the chi-square tests. All statistical analyses were interpreted at the 5% level of significance.

### Results and discussion

For the leptospira positivity classification by gender, age and location of all dogs were showed in tab. 2. The total positivity of all 153 dogs was 57.5%. There are not significantly different between the positivity of male and female dogs (57.5 and 57.6 respectively). The dogs within the age of 3-6 years had highest positivity (71.4) and significantly different ( $P < 0.05$ ) from the other ages. The dogs in Amphoe Kamphaeng Saen had highest positivity (78.9%) and significantly different ( $P < 0.05$ ) from the other districts.

The MAT titer against leptospira serovars was shown in tab. 3. The most prevalence serovar was tarassovi with

were 30.7% positivity and the titer ranged from 400 to 50. The other 10 positive serovars were ranarum, saigon, bratislava, copenhageni, patoc, bangkok, sejroe, autumnalis, sarmin and canicola (24.8%, 14.4%, 11.1%, 9.2%, 8.5%, 3.9%, 3.9%, 2.6%, 2.0% and 1.3% respectively). For all 88 positive sera, 3.4% (3 cases), 5.7% (5 cases), 11.4% (10 cases), 42% (37 cases) and 37.5% (33 cases) were positive with 5, 4, 3, 2 and single leptospira serovars, respectively.

Leptospirosis is an important zoonotic disease for public health in tropical countries, including Thailand (13). Although leptospirosis was not recognized as an endemic disease in any region of Thailand, the present study demonstrated that dogs which lived in this rural area could be risk for leptospira infection. This is due to suitable climate and humid condition for survival of *Lep-*

Tab. 2. The positivity of leptospirosis in dogs in Nakhon Pathom province

Parameters		% Positivity	Positive ratio
Total		57.5	88/153
Genders	Male	57.5	50/87
	Female	57.6	38/66
Ages	> 1 yr	47.4	9/19
	1-3 yrs	65.7	23/35
	3-6 yrs	71.4	15/21
	< 6 yrs	61.5	8/13
	unknown	50.8	33/65
Location	Amphoe Muang	49.4	44/89
	Amphoe Don Tum	53.8	14/26
	Amphoe Kamphaeng Saen	78.9	30/38

Tab. 3. MAT titer of 153 dogs from Nakhon Pathom against reference leptospira serovar antigens

Leptospira serovars antigen *	Number of positive cases within individual MAT titer				Total positive cases	% positivity
	400	200	100	50		
tarassovi	2	3	17	25	47	30.7
ranarum	2	2	13	21	38	24.8
saigon	0	0	1	21	22	14.4
bratislava	0	3	5	9	17	11.1
copenhageni	0	1	3	10	14	9.2
patoc	1	1	3	8	13	8.5
bangkok	0	0	0	6	6	3.9
sejroe	1	1	0	4	6	3.9
autumnalis	0	0	1	3	4	2.6
sarmin	0	0	2	1	3	2.0
canicola	1	0	0	1	2	1.3

Explanation: \* – the other 13 leptospira serovars (australis, bataviae, cellidoni, djasiman, grippotyphosa, hebdomadis, ictero-haemorrhagiae, javanica, pomona, pyrogenes, hardjo, wolffi and zanoni) were negative with MAT reaction

*toospira* (17). Many rodent species, which are maintenance hosts and usually found in rice fields, farms and domestic farms, is a reason of increasing risk for leptospirosis. Domestic animals are sometimes maintenance hosts. For example, dairy cattle may harbor serovars hardjo, pomona, and grippotyphosa; pigs may harbor pomona, tarassovi, or bratislava; sheep may harbor hardjo and pomona. These farm animals may be reservoirs and shed the organism to the environment. Similarly, dogs may be a reservoir and transfer leptospiral infection to either humans or animals.

In the present study, risk factors, including sex and age were identified for leptospirosis in dogs. Sero-positive dogs were non-specific gender, but another study showed that large-breed male dogs were predisposed to leptospirosis (2) because male dogs seem to be related to the behavior of smelling and licking their genitals after urination. Middle-aged (3-6 years old) dogs were found most in this study. These dogs may be likely to spend more time outside their home than young or old dogs, therefore increasing their exposure to leptospiral organisms in the environment.

To determine the prevalence of leptospirosis among dogs that lived in a rural and agricultural area in Thailand, leptospirosis was diagnosed by serology using MAT method. Many serovars of *Leptospira* selected in this study were important serovars found in humans in Thailand. However, these serovars have not ever been evaluated in dogs. Certain criteria for serologic confirmation of leptospiral infection have not been established, and laboratory variation makes different standardization. However, MAT is considered to be a sensitive and specific serological test for the diagnosis of leptospiral infection (18). A titer > 1 : 100, as determined using MAT, indicates a positive result (10), and a fourfold change in titers of serum samples obtained during the acute and convalescent phases is indicative of a humoral response to an active infection. Furthermore, some retrospective studies determined that a single titer of 1 : 800 (8) and 1 : 3,200 (3) to nonvaccinal serovars was consistent with leptospiral infection. Although vaccination of dogs with the commercially available leptospiral vaccine can induce high titers to serovar icterohaemorrhagiae and canicola, it is ineffective to induce titers and protection to other serovars beyond the vaccinal serovars (19).

A limitation of MAT is cross reactivity that commonly occurs between serogroups (6). It is generally accepted in cases with multiple serovar titers that the higher titer is that induced by the infecting serovar (18).

This study showed positive serum antibody titers to several serovars with MAT titers ranged from 50 to 400. Most dogs (30.7%) were positive to serovar tarassovi. Although there was no history of vaccination, other positive serovars were not serovars containing in commercially available vaccine. Canicola was apparently the least serovar found in this study. These data indicate that the conventional serovars that typically cause disease in dogs has been changed. This finding correlates with previous observations that demonstrate a significant change in the epidemiology of canine leptospirosis (7, 14, 16, 20).

Definitive identification of serovars did not be done by means of bacteriologic culture or polymerase chain reaction (PCR) techniques. So it was not clear that the serovars that were identified in this study caused disease in dogs. Thus, we propose that serologic screening for atypical serovars should be included. Furthermore, because vaccination of dogs with the currently available bivalent vaccine is serovar-specific and cannot protect against infection with other serovars, a multivalent vaccine that induces immunity to other serovars should be developed for dogs.

In conclusion, the most serovars identified in this study were significant serovars for human leptospirosis and non classical in commercially available bivalent vaccine. These data are important to determine of the epidemiology of leptospirosis in rural area in Thailand. They are also valuable database to consider for the future diagnosis and prevention of canine leptospirosis.

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