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Seroprevalence of *Leishmania* infection in domestic animals in Songkhla and Satun provinces, southern Thailand

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Abstract. Leishmaniasis is a zoonotic disease that is a major public health problem in tropical countries caused by flagellate protozoa of the genus *Leishmania*. Domestic animals and wild rodents have been reported as natural reservoir hosts. Thailand is a non-endemic area for leishmaniasis but human cases have been reported sporadically, particularly in southern Thailand. The objective of the present study was to determine the seroprevalence of *Leishmania* infection in domestic animals in southern Thailand. Blood samples from 519 dogs, 250 cats, 113 rats, 19 shrews, 125 cattle, 95 buffaloes and spleen samples from 45 rats and 19 shrews were collected in Songkhla and Satun provinces. Seroreactivity to the Direct Agglutination Test (DAT) was found in 2.7% (14/519) of the dogs while 5.6% (14/250) of the cats were positive using ELISA. There were significant differences between positive samples based on adult age group in dogs and cats (χ^2= 5.396, P= 0.020, χ^2= 8.304, P = 0.004, respectively) and for the outdoor group in cats (χ^2= 6.956, P= 0.008). The results found low seroprevalence of *Leishmania* infection in both dogs and cats, but they could be natural reservoir hosts for leishmaniasis in southern Thailand.

INTRODUCTION

Visceral leishmaniasis (VL) is caused by *Leishmania donovani* complex (*L. donovani* and *L. infantum*) and is transmitted by phlebotomine sandflies (Alexander *et al*., 1999; Courtenay *et al*., 2002). The study of VL in foreign countries determined that the disease was primarily a zoonosis with large reservoirs in animals such as rodents, dogs, cats, cattle and goats (Handman, 1999; Poli *et al*., 2002; Savani *et al*., 2004; Oliveira *et al*., 2005; Hassan *et al*., 2009; Bhattarai *et al*., 2010; Singh *et al*., 2013). The prevalence of VL in dogs was lower than 10% and was 1-60% in cats in endemic areas (Cardoso *et al*., 2010; Sousa *et al*., 2011).

The most common diagnosis is serological diagnosis because this method has less effect on patients and is suitable for screening for VL (Sundar and Rai, 2002). Serological diagnoses can utilize several methods such as the direct agglutination test (DAT) and ELISA (Singh *et al*., 2006; Srivastava *et al*., 2011a), but these methods have been reported to produce a cross-reaction with *Trypanosoma* infection (Sukmee *et al*., 2008) due to *Leishmania* and *Trypanosoma* being categorized as haemoflagellate protozoa of the family

Trypanosomatidae. Natural cases of co-infection by *Leishmania* and *Trypanosoma* have been reported in dogs endemic in several countries such as Brazil and Colombia (Savani *et al.*, 2005; Rosypal *et al.*, 2007). PCR is also a useful diagnostic technique which targets DNA for amplification usually using two genes such as minicircle kinetoplast DNA in the mini-exon gene region and 18s SSU-rRNA gene in the ITS1 region (Schõnian *et al.*, 2003).

Thailand is a non-endemic area for VL. Sporadic cases in humans were reported from 1996 to 2010, including in Suratthani, Phangnga, Nakhon si thammarat, Songkhla, Satun, Trang, Nan, Chanthaburi, Chiangrai provinces and in Bangkok (Kongkaew *et al.*, 2007; Maharom *et al.*, 2008; Sukmee *et al.*, 2008; Suankratay *et al.*, 2010; Wiwanitkit, 2011; Bualert *et al.*, 2012; Chusri *et al.*, 2012; Leelayoova *et al.*, 2013), with six of these cases being autochthonous. Most infected human lived in the southern part of Thailand (Kongkaew *et al.*, 2007; Maharom *et al.*, 2008; Sukmee *et al.*, 2008; Suankratay *et al.*, 2010). However, there is limited information on *Leishmania* infection in animals in Thailand. The previous studies reported seropositive animals being checked using DAT in southern Thailand but the study area was restricted to only around the patients' houses. Cattle and cats were reported to be serologically positive for VL and were considered as the major reservoirs in Thailand (Kongkaew *et al.*, 2007; Sukmee *et al.*, 2008). There was only one study that reported a low sero-prevalence of VL in cats in Surathani and Phangnga provinces (Nimsuphan *et al.*, 2014). Moreover, infected humans have been reported annually, especially in the past decade and a new species (*L. siamensis*) was also found in Thailand. Therefore, the objective of this study was to study the seroprevalence of *Leishmania* infection in domestic animals which can possibly be natural reservoir hosts of leishmaniasis in Thailand.

MATERIALS AND METHODS

Selection of study area

The present study was conducted in the two provinces of southern Thailand where human cases of VL were reported (Kongkaew *et al.*, 2007; Maharom *et al.*, 2008; Sukmee *et al.*, 2008; Suankratay *et al.*, 2010). Sample collections were performed in 13 districts – including Khuan Kalong, Khuan Don, Tha Phae, Mueang Satun and Lipe Island in Satun province and Rattaphum, Hat Yai, Khlong Hoi Khong, Sadao, Ranot, Krasae Sin, Sathing Phra, Singhanakhon and Khuan Niang in Songkhla province (Figure 1).

Samples size, data collection and animal grouping

In total, 1 121 blood samples from 519 dogs, 250 cats, 113 rats, 19 shrews, 125 cattle and 95 buffaloes were collected randomly using probability proportional to size (PPS) sampling (Srivastava *et al.*, 2011b) in June 2013. All dogs and cats were thoroughly examined to develop their health profiles. Information regarding age (juvenile (<2y) or adult (>2y)), sex (male or female), breed (purebred or crossbred) and living area (outdoors or indoors) was recorded. Most of the animals lived with their owners in rubber or palm or bamboo plantations and the rest were stray dogs and cats living in temples. The buffaloes and cattle lived nearby Songkhla Lake. Rats and shrews were caught in traps. The species of rodents were identified on the basis of their morphology (Chaval *et al.*, 2010). This study was approved by the Animal Experimental Committee of Kasetsart University, Bangkok (ACKU 03656).

Sample collection

Three-mL blood samples were collected from the cephalic or jugular vein depending on the type of animal. After collection, half of the blood volume was transferred to a tube containing EDTA (Fushino®, Japan) for DNA

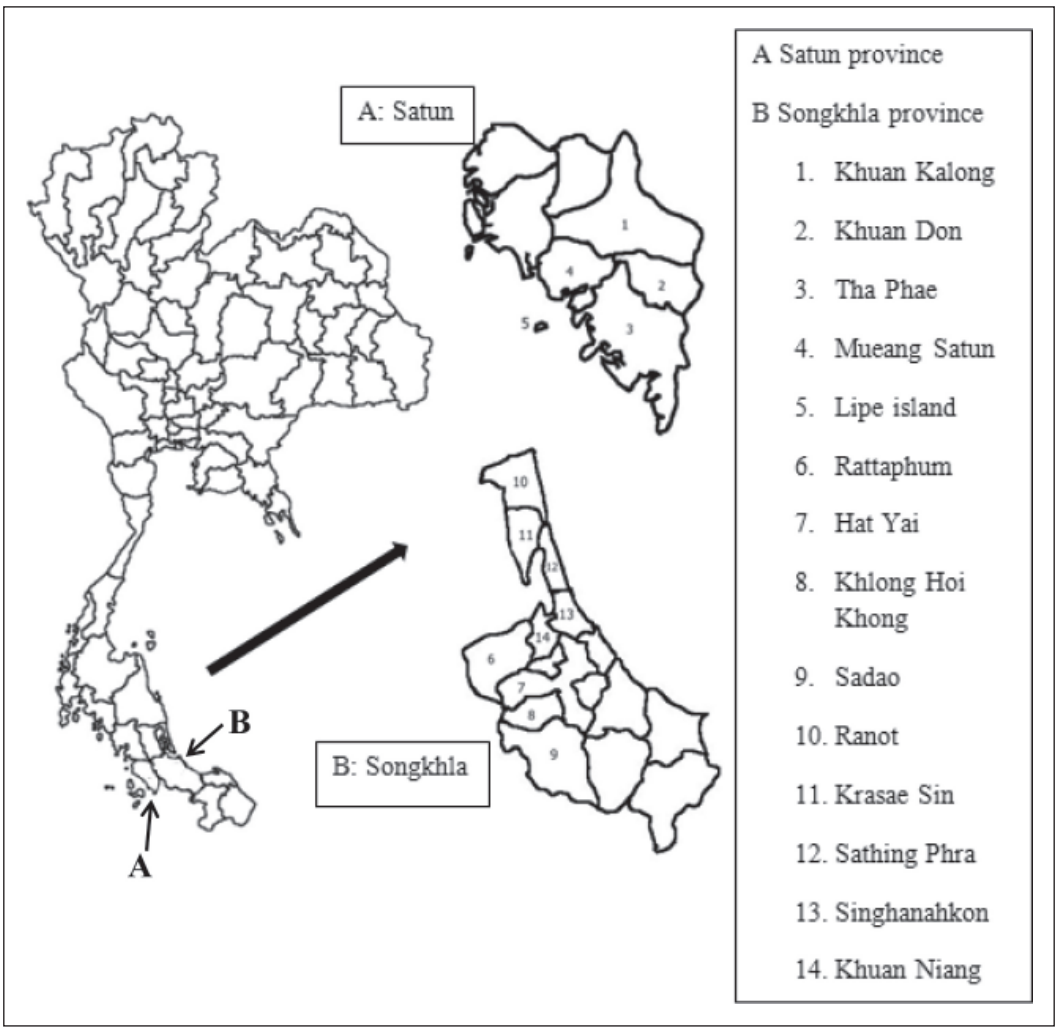

Figure 1. Map of Thailand showing the 13 districts and one island in Satun and Songkhla provinces where sampling occurred.

extraction. The remaining volume was transferred to clotted blood tubes (Fushino®, Japan) and centrifuged at 978 ×*g* for 10 min and then the serum supernatant was harvested. All blood, serum and spleen samples were kept at -20ºC until use. Rats and shrews were anesthetized with diethyl ether and the spleens were collected after euthanasia.

Soluble *Leishmania* antigen preparation

L. siamensis was kindly provided by Prof. Saovanee Leelayoova, Department of Parasitology, Phramongkutklao College of Medicine, Bangkok. Promastigotes were cultured with Schneider's insect medium (Sigma®, USA) supplemented with 20% FBS, multivit (Sigma®, USA), 1 gm gentamicin. Parasites were harvested and crude soluble antigen (CSA) was prepared using 10 cycles of freeze-thaw. CSA was centrifuged at 3 913 ×*g* for 30 min and the supernatant was harvested. The protein concentration of CSA in supernatant was measured using the Bradford assay.

Serological diagnoses

1. Direct Agglutination Test (DAT)

The DAT for titration of dog antibodies specific to *L. donovani* complex followed the manufacturer's procedures using a

standard freeze-dried antigen at a concentration of 5×10^7 parasites per mL (Royal Tropical Institute, KIT Biomedical Research, the Netherlands). Briefly, the serum samples were diluted in DAT-diluent (0.9% w/v NaCl solution and 0.2 M β-mercaptoethanol). Two times serial dilutions were performed ranging from 1:100 to 1:6 400 on a V-shaped microtiter plate (Nunc™, Denmark). Positive and negative controls were also run at the same time. Plates were incubated at room temperature (~30°C) for 1 hr. An amount of 50 mL of DAT antigen subsequently was added into each well containing 50 µL of diluted serum samples, shaken gently and incubated overnight (18 hr) at room temperature. The results were expressed as the end-titer, ascribed to the last serum dilution in which the agglutination (large diffuse blue mat) was still clearly visible compared to the negative control. Repeated examination was performed for positive samples to confirm the positive reactions and the serial serum dilutions were run starting from 1:100 to 1:25 600.

2. Enzyme linked immunosorbent assay (ELISA)

Sera of cat, rat, cattle and buffalo were evaluated for anti-*Leishmania* antibodies using ELISA. The ELISA was performed following the method described by Cardoso *et al.* (2010) with some modifications. Briefly, ELISA plates were coated with 2 µg/well of CSA in 0.1M carbonate buffer, pH 9.5 and incubated overnight at 4°C. The plates were washed five times with washing buffer (0.1% Tween-20 in PBS, pH 7.0) and blocked with 3% skim milk at 37°C for 1 hr. An amount of 100 mL of 1:100 serum dilution was added into each well, incubated at 37°C for 1 hr. The rabbit anti-cat IgG (Fc specific)-peroxidase antibody (Sigma®, USA) or rabbit anti-bovine IgG (whole molecule)-peroxidase antibody (Sigma®, USA) or goat anti-rat IgG (whole molecule)-peroxidase antibody (Sigma®, USA) was added into each well and the plates were incubated at 37°C for 1 hr. Finally, 3, 3'5, 5'-tetramethyl benzidine, TMB substrate (Invitrogen™, USA) was added and the plates were incubated at room temperature (~30°C) for 30 min in the dark. The reaction was stopped by adding 20 µL of 6N H_2SO_4 into each well. The absorbance was read at 490 nm using an ELISA plate reader (BioTekELx800™, USA). The positive and negative controls were also checked for every ELISA plate at the same time.

The cut-off point was analyzed from the mean optical density (OD) obtained from the sera of 32 negative samples. Three standard deviations of these measurements were added to the mean OD of negative samples.

DNA extraction

Blood samples (100 µL each) were incubated with 500 µL of denaturing solution (4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7, 0.1 M 2-mercaptoethanol, and 0.5% (w/v) N-lauroylsarcosine) with shaking for 5 min. DNA samples were extracted using the phenol-chloroform method, precipitated using ethanol (Sambrook and Russell, 2001), dissolved in a TE buffer (50 mM Tris, pH 8, 1 mM EDTA), and then stored at -20°C until use. Spleen samples were extracted for genomic DNA using a Tissue DNA kit (Omega bio-tek, USA).

ITS-1 PCR amplification for *Leishmania* infection

Leishmania DNA analysis followed the protocol by Schõnian *et al.* (2003) using the specific primers LITSR: 5'-CTGGATCA TTTTCCGATG-3' and L5.8S: 5'-TGATACCAC TTATCGCACTT-3'with an expected product size of 300-379 bp. Amplifying the reaction performed in a total volume of 20 µL, 2 µL of DNA template was added into a PCR mixture containing 1× buffer (10 mM Tris-HCl, pH 8.8, 50 mM KCl and 0.1% (v/v) TritonX-100), 1.5 mM $MgCl_2$, 0.2 µM of each dNTP, 0.5 nM primers and 2 U of *Taq* DNA polymerase (Invitrogen™, Brazil). The thermal cycling conditions were set at 95°C for 2 min, followed by 35 cycles of 95°C for 20 sec, 53°C for 30 sec, and 72°C for 60 sec, and then a final 72°C for 6 min. The positive control consisted of DNA of *L. siamensis*. The negative control used water in place of the DNA template.

Trypanosoma DNA was also checked using PCR to confirm the infection, particularly in the seropositive samples in which cross-reactivity between *Leishmania*

and *Trypanosoma* infection can occur. *Trypanosoma* DNA analysis was performed following the protocol by Desquesnes *et al.* (2002) using the specific primers TRYP1S: 5'-CGTCCCTGCCATTTGTACACAC-3' and TRYP1R: 5'-GGAAGCCAAGTCATCCATCG-3'with an expected product size of 545 bp. The cycling conditions were 94ºC for 1 min, followed by 30 cycles of 94ºC for 45 sec, 55ºC for 45 sec, and 72ºC for 45 sec, and then a final 72ºC for1 min. Amplification products were detected using 1.5% (w/v) agarose-TAE gel electrophoresis and visualized using ultraviolet trans-illumination after being stained with nucleic acid gel stain (GelStar®, USA).

Statistical analysis

The seroprevalence of *Leishmania* infection was determined and analyzed from the ratio of positive results and total numbers of animals. Data was analyzed using the Chi-Square test or Fisher's Exact test, according to age, sex, breed and living area using the Number Cruncher Statistical System software (NCSS) version 2000 (Kaysville, UT), with a P value < 0.05 considered as statistically significant.

RESULTS

Serological test for *Leishmania* infection

Seroprevalence of *Leishmania* infections checked by DAT in dogs was 2.7% (14/519) (Table 1). The highest dilution titer was 1:12 800 (Table 2). The cut-off OD values for ELISA were 0.2 for cats, 0.1 for rats and 0.7 for cattle and buffaloes. A sample was considered as a positive result when its OD was greater than the cut-off point. Anti-*Leishmania* antibodies checked using ELISA were found in 14 cats (5.6%) and 3 buffaloes (3.16%) (Table 1). The ELISA results in cattle and rats were negative. The end-point titers varied, ranging from 1:800 to 1:3 200 in cats (Table 2) and from 1:100 to 1:400 in buffaloes. Anti-*Leishmania* antibodies were detected in both dogs and cats in two provinces. Only

Table 1. Number of seropositive animals with *Leishmania* infection in two provinces from southern Thailand

District	Infected animals (positive sample/ total; (%))				
	Dogs	Cats	Rats	Cattle	Buffaloes
Satun					
Khuan Kalong	0/122 (0%)	1/28 (3.57%)	0/16 (0%)	0	0
Khuan Don	0/27 (0%)	0/14 (0%)	0	0	0
Mueang Satun	2/76 (2.63%)	0/30 (0%)	0/3 (0%)	0	0
Tha Phae	0/32 (0%)	1/22 (4.55%)	0/7 (0%)	0	0
Lipe	4/48 (8.33%)	0/31 (0%)	0	0	0
Songkhla					
Rattaphum	2/43 (4.65%)	0/30 (0%)	0	0	0
Hat Yai	0/74 (0%)	1/45 (2.22%)	0/72 (0%)	0	0
Khlong Hoi Khong	3/53 (5.66%)	10/40 (25.0%)	0/12 (0%)	0	0
Sadao	3/44 (6.82%)	1/10 (10.0%)	0/3 (0%)	0	0
Ranot	0	0	0	0/25 (0%)	3/19 (15.79%)
Krasae Sin	0	0	0	0/25 (0%)	0/20 (0%)
Sathing Phra	0	0	0	0/25 (0%)	0/29 (0%)
Singhanakhon	0	0	0	0/25 (0%)	0/27 (0%)
Khuan Niang	0	0	0	0/25 (0%)	0
Total	14/519 (2.7%)	14/250 (5.60%)	0/113 (0%)	0/125 (0%)	3/95 (3.16%)

Table 2. Anti-*Leishmania* antibody titers of dogs and cats in southern Thailand checked by DAT and ELISA, respectively

Dog code	DAT result	Cat code	ELISA results
KKD 21	1:12,800	KKC 20	1:800
KKD 22	1:3,200	KKC 24	1:1,600
KKD 43	1:6,400	KKC 25	1:800
KRD 10	1:3,200	KKC 31	1:800
KRD 16	1:3,200	KKC 32	1:800
KSD 13	1:200	KKC 33	1:800
KSD 16	1:200	KKC 36	1:800
KSD 29	1:6,400	KKC 38	1:800
ST1 D14	1:3,200	KKC 39	1:3,200
TMD11	1:200	KKC 40	1:3,200
D16	1:3,200	KSC 3	1:800
D19	1:800	KHC 42	1:800
D38	1:800	TKC 9	1:1,600
D42	1:800	TTC 11	1:1,600

Abbreviations: KKD = Khlong Hoi Khong district Dog, KRD = Rattaphum district Dog, KSD = Sadao district Dog, ST = Mueang Satun district Dog, TMD = Mueang Satun district Dog, D = Lipe island Dog, KKC = Khlong Hoi Khong district Cat, KSC = Sadao district Cat, KHC = Hat Yai district Cat, TKC = Khuan Kalong district Cat, TTC = Tha Phae district Cat.

three buffaloes were detected positive – all in the Ranot district of Songkhla province (Table 1).

In addition, most of rats were identified as *Rattus tanezumi* and all shrews were identified as *Suncus murinus*. However, all rats tested negative for *Leishmania* infection. Furthermore, the statistical analysis of the age factor of the seropositive animals showed a significant difference in dogs and cats (χ^2= 5.396, P = 0.020, χ^2=8.304, P= 0.004, respectively) and cats also showed a significant difference for ELISA-positive samples in the outdoor group (χ^2= 6.956, P = 0.008) (Table 3 and 4). There were no significant differences between seropositive animals based on sex, breed and living area for dogs and buffaloes.

PCR amplification for detection of *Leishmania* infection

Only one dog tested positive using PCR where the size of the PCR product was 370 bp. However, *Leishmania* DNA was not found in the blood samples of other dogs, cats, rats, shrews, cattle, and buffaloes, including the spleen samples from the rats and shrews.

Cross-reaction of animals infected with *Leishmania* and *Trypanosoma*

All blood samples of dogs, cats, rats, shrews, cattle, buffaloes and sample from the spleens of rats and shrews were also checked for *Trypanosoma* infection using PCR but all samples produced a negative result.

DISCUSSION

This study was conducted in Songkhla and Satun provinces, southern Thailand because these areas are considered at risk of exposure to leishmaniasis (Sukmee *et al.*, 2008). In Thailand, previous studies of *Leishmania* infection on domestic animals were limited to animals living around a patient's house, where three cows and one cat tested positive using DAT in Nan province (Kongkaew *et al.*, 2007), and 60% (9/15) cats in Phangnga province (Sukmee *et al.*, 2008). However,

Table 3. Seroprevalence of *Leishmania* infection in dogs checked using DAT in relation to age, sex, breed and living area

Parameter	Chi-squared test/ Fisher's Exact Test	Number of dogs (n)	DAT positive (n) (%)
Age	$\chi^2 = 5.396$; df =1, $P = 0.020$	519	14 (2.70%)
Juvenile		270	3 (1.11%)
Adult		249	11 (4.42%)
Sex	$\chi^2 = 0.665$; df =1, $P = 0.414$	519	14 (2.70%)
Male		241	5 (2.07%)
Female		278	9 (3.24%)
Breed	$P = 0.143$	519	14 (2.70%)
Purebred		139	6 (4.32%)
Crossbred		380	8 (2.11%)
Living area	$P = 0.243$	519	14 (2.70%)
Outdoors		343	11 (3.21%)
Indoors		176	3 (1.70%)

Table 4. Seroprevalence of *Leishmania* infection in cats checked using ELISA in relation to age, sex, breed and living area

Parameter	Chi-squared test (df = 1)	Number of cats (n)	ELISA positive (n) (%)
Age	$\chi^2 = 8.304$, $P = 0.004$	250	14 (5.60%)
Juvenile		161	4 (2.48%)
Adult		89	10 (11.24%)
Sex	$\chi^2 = 1.036$, $P = 0.309$	250	14 (5.60%)
Male		104	4 (3.85%)
Female		146	10 (6.85%)
Breed	$\chi^2 = 1.118$, $P = 0.290$	250	14 (5.60%)
Purebred		44	1 (2.27%)
Crossbred		206	13 (6.31%)
Living area	$\chi^2 = 6.956$, $P = 0.008$	250	14 (5.60%)
Outdoors		102	1 (0.98%)
Indoors		148	13 (8.78%)

there was one study of the seroprevalence that found anti-*L. donovani* complex antibodies in cats at 0.84% (2/237) but no results were seropositive in 407 dogs in Surathani, Nakhon si Thammarat and Phangnga provinces, southern Thailand (Nimsuphan *et al.*, 2014). The seroprevalence of infected animals with asymptomatic infection in non-endemic areas showed low seroprevalence which was similar to a study of seroprevalence in dogs in Vietnam which also found no infection (Rosypal *et al.*, 2009). In the present study, the seroprevalent levels of anti-*Leishmania* antibodies were 2.7% in dogs and 5.6% in cats which were lower than in previous studies in endemic areas; for example, the seroprevalence of dogs ranged from 1.6 to 34.6% in Spain

(Ballart *et al*., 2013), and cats had a range from 6.5 to 20% in Portugal (Cardoso *et al*., 2010). Two possible reasons for the low seroprevalence in the present study are, firstly, that the autochthonous and imported human cases are sporadic and no animal case has been reported in Thailand, and secondly, there is a low density of sandflies as a potential vector in southern Thailand (Chusri *et al*., 2014). However, a significant difference was detected from the comparison between the age of the seropositive sample in dogs and cats and also was observed between seropositive samples and outdoor group activity in cats but not in other animals. The positive samples were found in the adult group (older than 24 months) of both dogs and cats which was similar to the study in Portugal which reported seropositive adult cats with an age range from 31 to 84 months (Cardoso *et al*., 2010) and seropositive adult dogs in the age group older than 12 months (Sousa *et al*., 2011). Our results show that adult cats are at risk of infection with leishmaniasis in Thailand because they are likely to be living outdoors where they may be bitten by an infected sandfly. Therefore, it is possible that cats could be a potential reservoir in Thailand because a previous report found that cats were infected with leishmaniasis more than dogs and most of the Islamic residents living in southern Thailand like to rear cats in their houses which places these people at risk of infection with leishmaniasis from asymptomatic cats.

The seroprevalence of anti-*Leishmania* antibodies from buffaloes checked using ELISA in this study was 3.16% but all cattle were negative. Our result was lower than previously reported from endemic areas where 9.4% cattle were found to be positive when checked using ELISA in Bangladesh (Alam *et al*., 2011) and the prevalences in cattle and buffaloes in Nepal checked using PCR were 5% and 4%, respectively (Bhattarai *et al*., 2010). The seroprevalence of buffaloes in the present study showed low antibody titers (1:200-1:400); false seroreactivity can possibly occur from the cross-reaction of other parasitic infections because of the use of crude antigen for serological tests presents problems of cross-reactivity with antibodies from other diseases (Badaró *et al*., 1996; Goto *et al*., 2011; Spada *et al*., 2013) which were found in buffaloes associated with other parasites such as liver fluke and intestinal nematodes. However, further evaluation such as investigation using a specific-*Leishmania* antigen for research is needed. These results indicate that dogs and cats can play a role as potential reservoir hosts for leishmaniasis in southern Thailand.

Moreover, all DAT-positive samples were not positive with *Trypanosoma* infection when checked using PCR. This confirms the seropositive results of anti-*Leishmania* antibodies checked by DAT in which these antibodies were not produced from *Trypanosoma* infection. The cross reactivity was checked because *Trypanosoma* infection could possibly react with DAT (Kongkaew *et al*., 2007).

In addition, a previous study in the Nathawi district of Songkhla province reported *L. siamensis* DNA from the liver and spleen of two black rats which showed an amplicon size of 379 bp (Chusri *et al*., 2014). Our study found *Leishmania* DNA in a dog in the Meuang Satun district, Satun province although there is no report of leishmaniasis in this area and this dog also showed antibody titer against *Leishmania* infection at 1:200. Unfortunately, DNA was not extracted for sequencing because the DNA band was thin, possibly because of a low number of infected macrophages in the peripheral blood of the asymptomatic dog. PCR assay with blood samples has been reported with a sensitivity of 55% and could often detect parasitemia a few weeks before the appearance of any clinical signs or symptoms (Lachaud *et al*., 2000) while the sensitivity was highest (near 100%) in the spleen and bone marrow samples. In the present study, peripheral blood sampling was used because its collection was non-invasive, easily performed in field and spleen aspiration is associated with a risk of fatal hemorrhage (Elmahallawy *et al*., 2014).

There are several serological tests used for the detection of anti-*Leishmania* antibody and the sensitivity and specificity vary for each test. The best method is IFAT (sensitivity 96% and specificity 98%) but the

disadvantages of this method are that it is unable to detect antibody in patients with VL in the initial phase and the testing process is intricate (Sukmee *et al*., 2008). The sensitivity and specificity of DAT for diagnosis in humans has been shown to be 94.8% and 85.9%, respectively (Srivastava *et al*., 2011). This method is suitable for screening VL in dogs for which the sensitivity and specificity are 100% and 98.8%, respectively (Schalling *et al*., 2001). DAT is frequently used to detect anti-*Leishmania* antibody from humans and dogs; the manufacturer of the test kit recommends using DAT to detect the antibody in humans and dogs only but there have been a few reports of its use in cats (Cardoso *et al*., 2010).

The present study is the first report of the seroprevalence of *Leishmania* infection in domestic animals in the southern most part of Thailand. From the results, cats and dogs might be potential reservoir hosts of leishmaniasis in southern Thailand. Therefore, further study is required on the role of cats and dogs in the transmission cycle via insect vectors.

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