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Original papers

Molecular detection of *Ehrlichia canis*, *Hepatozoon canis* and *Babesia canis vogeli* in stray dogs in Mahasarakham province, Thailand

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ABSTRACT. Canine tick borne diseases showing distribution worldwide have caused morbidity and mortality in dogs. This study observed the mainly tick borne pathogens described for dogs in Thailand, *Ehrlichia canis*, *Hepatozoon canis* and *Babesia canis vogeli.* From May to July 2014, blood samples were collected from 79 stray dogs from 7 districts of Mahasarakham province to molecular surveyed for 16s rRNA gene of *E. canis* and 18s rRNA gene of *H. canis* and *B. canis vogeli*. Twenty eight (35.44%) of stray dogs showed the infection with tick borne pathogens. The prevalence of *E. canis* infection was the highest with 21.5% (17/79). DNA of *H. canis* and *B. canis vogeli* were detected at the prevalence of 10.1% (8/79) and 6.3% (5/79), respectively. Co-infection between *E. canis* and *B. canis vogeli* were identified in 2 (2.5%) dogs. The results indicated that a wide range of tick borne pathogens are circulation in the canine population in Mahasarakham province. This study is the first report on prevalence of *E. canis*, *H. canis* and *B. canis vogeli* in stray dogs in Mahasarakham, a province in northern part of Thailand. This data providing is important to understand the prevalence of *E. canis, H. canis* and *B. canis vogeli* infection in stray dogs in this region, which will assist in the management of these blood parasite.

Key words: *Ehrlichia canis*, *Hepatozoon canis*, *Babesia canis vogeli*, tick borne pathogens, stray dogs

Introduction

Canine blood parasite transmitted by ticks can cause severe infection in dogs and humans [1]. The mainly tick distributes in the tropical and subtropical country is the brown dog tick, *Rhipicephalus sanguineus* [2–4]. The normal pathogens associated with *R. sanguineus* vector which caused canine vector borne disease are *Ehrlichia canis*, *Hepatozoon canis* and *Babesia canis vogeli* [5–7]. The spreading throughout the year of the infections in dogs is facilitated by distribution of the tick vector, also accumulation of the stray dogs which play importance role as the reservoirs in the transmission cycles [5].

E. canis (Rickettsiales, Anaplasmataceae) is a gram negative intracellular rickettsia which can infect the monocyte of infected dogs worldwide includes Thailand [8]. This pathogen has been reported as the causative agent of canine monocytic ehrlichiosis (CME) and causing various signs from asymptomatic to severe illness associated with pancytopenia, hemorrhage and bone marrow hypoplasia [11]. *H. canis* is an apicomplexan protozoa causes hepatozoonosis which is resulted when dogs eat infected ticks [12]. *H. canis* infects leukocytes and induce severe clinical manifestation such as anemia, emaciation, anorexia and intermittent fever [13]. *B. canis vogeli* is an infected erythrocytes protozoa or piroplasm, which has been described infecting dogs in Thailand [5]. The clinical features of babesiosis caused by *B. canis vogeli* are often mild anemia but can cause severe illness when multiple parasitic infections occurred.

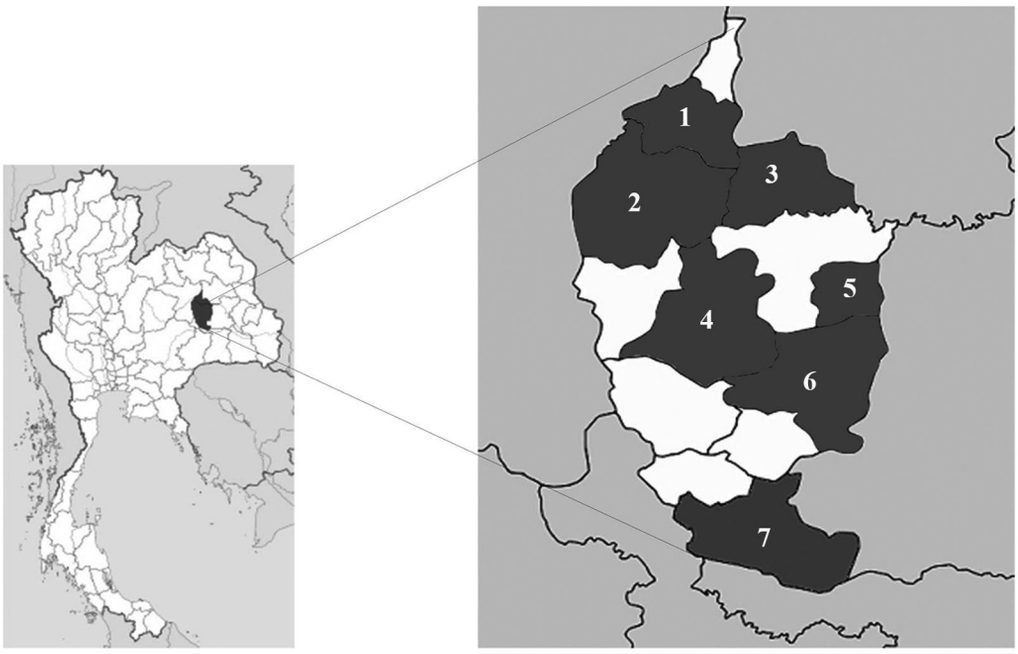

Fig. 1. Map of the study area, showing the Districts of Mahasarakham province where samples were collected. Codes for sampling localities are as follows: 1 = Chiang Yuen; 2 = Kosum Phisai; 3 = Kantharawichai; 4 = Borabue; 5 = Kac Dam; 6 = Wapi Pathum; 7 = Phayakkhaphum Phisai.

In laboratory, microscopic examination of blood smears which time consuming and requires the experience person to identify remains the goal standard for these parasites diagnosis. Although, the serological test kits are available but poor specificity due to cross reaction with other related parasites. Molecular diagnosis based on PCR developed to detect the infection of tick borne parasites on blood was shown to be more sensitivity and specificity for the diagnosis than other methods [14–15]. This study performed PCR methods to evaluate the tick borne pathogens DNA in 79 blood samples of stray dogs in Mahasarakham province where is the endemic areas of the tick vector, *R. sanguineus* [2]. It is important to study the presence of these pathogens infections in order to know what disease can be expected in each region of the country.

Materials and Methods

Study area and blood collection. This cross-sectional study was carried out from May to July 2014 in Mahasarakham province of Thailand. In total, 79 blood samples of stray dogs were collected from 7 districts of Mahasarakham province (Fig. 1). Three ml of blood were collected from the cephalic vein into sterile tubes with anticoagulant (EDTA) and kept on ice during transport to the laboratory

Table 1. Primers used in PCR for detection of DNA of *E. canis*, *H. canis* and *B. c. vogeli* infection in stray dogs in Mahasarakham province, Thailand

Pathogen	Primer	Sequence	Product size [bp]
E. canis	ECC	(5′-AGAACGAACGCTGGCGGCAAGCC-3′)	478
	ECB	(5′-CGTATTACCGCGGCTGCTGGCA-3′)	
	CANIS	(5′-CAATTATTTATAGCCTCTGGCTATAGGA-3′)	389
	HE	(5′-TATAGGTACCGTCATTATCTTCCCTAT-3′)	
H. canis	HepF	(5′-ATACATGAGCAAAATCTCAAC-3′)	666
	HepR	(5′-CTTATTATTCCATGCTGCAG-3′)	
B. c. vogeli	BAB1	(5′-GTGAACCTTATCACTTAAAGG-3′)	590
	BAB4	(5′-CAACTCCTCCACGCAATCG-3′)	

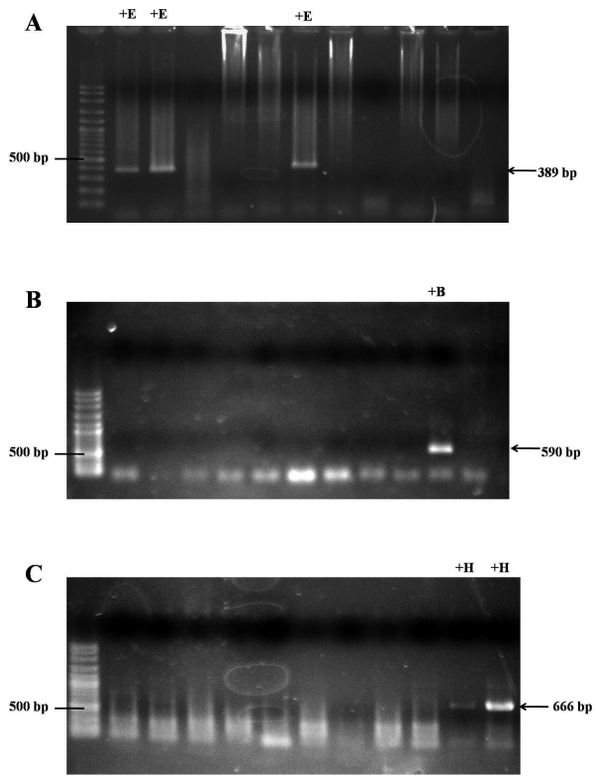

Fig. 2. Sample PCR electrophoresis shows amplicons of *E. canis* (A), *B. canis vogeli* (B) and *H. canis* (C)

and stored at –20°C until DNA extraction. All steps for animal handles and blood collections were conducted by veterinarians.

DNA extraction and PCR amplification of tick borne pathogens. Total DNA was extracted using GF-1 blood DNA extraction kit (Vivantis) according to the manufacturer's instruction. Concentrations of total DNA were determined by exposing the DNA to ultraviolet light at a wavelength of 260 nanometers with UV/Vis spectrophotometer (Mecasys, Korea).

PCR assay were performed to detect 16s rRNA gene of bacteria and 18s rRNA gene of protozoa. The *E. canis*16s rRNA gene was amplified by nested PCR using 2 pairs of specific primers as previously describe [16–18]. Table 1 shows primers used in nested PCR and PCR for detection of DNA of *E. canis*, *H. canis* and *B. canis vogeli* infection in this study. Primers ECC and ECB used in the primary amplification were amplified the 16s rRNA gene of an *Ehrlichia* genus. The primers CANIS and HE3 for the secondary amplification were amplified a 389 bp fragment of the 16s rRNA gene of the *E. canis*.

A single PCR was used for the detection of 18s rRNA gene of *H. canis* and *B. canis vogeli*. *H. canis* was done approximately 666 bp PCR product by HepF and HepR [19]. Specific primers BAB1 and BAB4 were amplified 590 bp PCR product of *B. canis vogeli* [20] (Fig. 2).

The PCR reactions were 10-100 ng of genomic DNA, 0.2 mM dNTP, 1.5 mM $MgCl_2$ performed with 1 unit *Taq* polymerase (Vivantis) with 35 cycles of denaturation at 95°C for 1 min, annealing at 60°C for *E. canis*, 50ºC for *H. canis* and 55°C for *B. canis vogeli* for 1 minute, extension at 72°C for 2 minutes and a final extension at 72°C for 5 minutes. PCR amplification was performed using Biometra GmbH thermocycle (Germany). PCR products were identified by 1% agarose gels stained with ethidium bromide and viewed under ultraviolet light.

Statistical analysis. The sample size was calculated to determine the appropriate number of samples from infinite population by settle on 95% of confidence level, 5% of margin of error and approximately 5% of sample proportion. The formula was as followed [21]:

$$n = \frac{(P)(1-P)(Z)^2}{(e)^2}$$

n: number of animals to be sampled
P: sample proportion at 5%
e: 5% of margin of error
Z: 95% of confidence level

Prevalence of tick borne pathogens infection in stray dogs was calculated by the number of positive samples divided by the number of total samples multiplied by 100.

Results and Discussion

A total of 79 blood samples were collected randomly from stray dogs in difference regions of Mahasarakham province including 7 districts; Kantharawichai (9 samples), Kae Dam (8 samples), Wapi Pathum (6 samples), Borabue (9 samples), Phayakkhaphum Phisai (13 samples), Chiang Yuen (19 samples) and Kosum Phisai (15 samples). Thirty (37.98%) were males and 35 (44.3%) were females. No data on sexes were 14 (17.72%) samples. Positive controls for PCR amplification were obtained from blood of stray dogs that positive diagnosis by microscopic examination of thin blood smear.

The present study represent the first report on tick borne pathogens in stray dogs in Mahasarakham province, northeast Thailand. In this study, 28 (35.44%) stray dogs showed the infection

Table 2. Prevalence of tick borne pathogens infection among stray dogs

Area/District	Samples (n)	*E. canis*	*B. c. vogeli*	*H. canis*	*E. canis* +*B. c. vogeli*
Chiang Yuen	19	2	1	3	–
Kosum Phisai	15	3	1	1	1
Kantharawichai	9	3	–	–	–
Borabue	9	3	–	1	–
Kae Dam	8	–	–	1	–
Wapi Pathum	6	4	3	–	1
Phayakkhaphum Phisai	13	2	–	2	–
Total	79	17 (21.5%)	5 (6.3%)	8 (10.1%)	2 (2.5%)

with tick borne pathogens; 26 (32.91%) dogs were single infection and 2 (2.53%) dogs were co-infection between *E. canis* and *B. canis vogeli*. The result of the tick borne pathogens prevalence is shown in Table 2.

The reports of tick borne pathogens infection in dogs from Southeast Asia country are few. The prevalence of *E. canis* infection in stray dogs in the present study (21.5%) was higher than the prevalence obtained in domestic dogs in Turkey (4.9%) [22], Nigeria (12.7%) [23], Neuva Ecija in Philippines (2.85%) [24] and KhonKaen in Thailand (3%) [15], suggesting that the domestic dogs are more frequently administrated with insecticide to eliminate the tick vector. In fact, the prevalence of parasitic infections in dogs should higher in stray dogs than domestic dogs. However, the higher prevalence of *E. canis* has been reported in domestic dogs in state of Pernambuco Brazil (38.04%) [25], Columbia (40.6%) [14] and Costa Rica (34%) [26], suggesting that the spreading of these pathogens are unpredictable in different region of the world. Moreover, many factors such as susceptibility of host, infection ability of parasite and environment are responsible for succession of tick borne pathogens infection.

The prevalence of *B. canis vogeli* infection in stray dogs in the present study (6.3%) was higher than the prevalence obtained in domestic dogs in Columbia (5.5%) [14] and Nigeria (0.6%) [23]. Nevertheless, the higher prevalence of babesiosis has been reported in domestic dogs in KhonKaen, Thailand (13.2%) [15], Neuva Ecija in Philippines (7.14%) [24], state of Pernambuco in Brazil (7.31%) [25] and Costa Rica (8%) [26].

The prevalence of *H. canis* infection in stray dogs in the present study (10.1%) was higher than the prevalence obtained in domestic dogs in Costa Rica (7.5%) [26] and state of Pernambuco in Brazil (0.49%) [25]. The higher prevalence of *H. canis* has been reported in Nigeria (41.4%) [23].

Conclusions

This study provides the molecular detection on prevalence of *E. canis* by nested PCR, *H. canis* and *B. canis vogeli* by PCR in stray dogs in Mahasarakham, a province in northern part of Thailand. The results show that these pathogens circulate among canine in the north-eastern part of Thailand. The main tick borne pathogens in stray dogs in this region are *E. canis* followed by *H. canis* and *B. canis vogeli*.

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