

Prevalence of canine vector-borne blood parasites in the plain regions of Cambodia

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Abstract

Canine vector-borne blood parasites (CVBP) cause various diseases in dogs. The aim of this study was to survey canine blood parasites in the plain regions of Cambodia. The study was conducted from November 2014 to September 2015. A total of 455 blood samples were collected from rural and urban areas in the plain regions of Cambodia. Six infected dogs were found (1.32%), with the following parasites: *Dirofilaria immitis* (2/6), *Brugia pahangi* (1/6), *Babesia vogeli* (1/6) and two co-infections of *Ehrlichia canis* with *Anaplasma platys* (1/6) and *Babesia vogeli* with *Anaplasma platys* (1/6). However, the rate of detection of blood parasitic infections depended on the diagnostic technique used. The prevalence of parasites indicated low levels of infection among dogs in the plain regions of Cambodia. However, *Brugia* infection and filariasis can cause zoonotic disease, so their detection is a cause for concern. Further research on vector borne transmission and control in the plain regions of Cambodia is required to understand these diseases, not only in dogs but also in other animals.

Keywords: dogs, *Brugia pahangi*, blood parasites, plain regions, Cambodia

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Introduction

Canine vector-borne blood parasites (CVBP) are a group of infectious pathogens transmitted by blood sucking arthropod vectors or blood-feeding ectoparasites including mosquitoes, tabanids, ticks, fleas and lice. CVBP include protozoa, filariae, viruses and bacteria (Dantas, 2008). Dogs are considered as reservoir hosts of these pathogens and serve as a source of transmission via blood-feeding arthropods. Therefore, dogs are able to spread the diseases via vectors to humans (Otranto *et al.*, 2009b; Menn *et al.*, 2010).

As well as being pet animals that live with humans, dogs are eaten in parts of China, Vietnam, Cambodia. In addition, with the increasing movement of people, environmental change, and increasing pet animal travel and the movement of animal goods/material, CVBP have become a global issue. CVBP may play a potential role in (re)emerging diseases and the establishment of novel vector species and pathogens (Otranto *et al.*, 2009a).

Cambodia, a country in Southeast Asia, is surrounded by uplands and low mountains and includes the Tonle Sap Lake and the upper reaches of the Mekong River delta. The climate is controlled by tropical monsoons and has two different seasons: the rainy season from May to October and the dry season from November to April (http://en.wikipedia.org/wiki/Geography_of_Cambodia#Climate). The temperature ranges from 21 to 35 °C (69.8 to 95.0 °F) and is similar throughout the Tonlé Sap Basin, with an average annual temperature of around 25 °C (77.0 °F); the maximum mean is about 28.0 °C (82.4 °F) and the minimum mean is about 22.98 °C (73.36 °F). Cambodia is divided into four regions including the plain regions or the lower area, located to the east and southeast of the border with Vietnam.

Canine vector-borne organisms include *Babesia vogeli*, *Hepatozoon canis*, *Ehrlichia canis*, *Anaplasma platys*, *Mycoplasma haemocanis*, and *Dirofilaria immitis*, all of which are mainly found in dogs. Moreover, *Babesia vogeli* is the species that can be found in Cambodia and also Southeast Asia (Inpankaew *et al.*, 2016). Importantly, some of these organisms can cause life threatening diseases in dogs and zoonotic diseases in many countries (Menn *et al.*, 2010; Chungpivat and Taweethavonsawat, 2008; Dantas, 2008; Rani *et al.*, 2011). However, information about CVBP in the plain regions of Cambodia is limited. Therefore, this study aimed to survey CVBP in the plain regions of Cambodia.

Materials and Methods

Sample size: A total of 455 blood samples were collected from rural and urban areas in the plain regions of Cambodia. All blood samples were randomly selected by walking from house to house and asking permission from the dogs' owners. The village authorities, villagers and veterinarian surveillance services were also asked for their permission.

Regions of study: The samples were collected in the plain regions including Takeo, Kompong Spue,

Kompong Chhnang, Prey Veng, and Svay Rieng and from a private clinic, the Veterinary Service Center, in Phnom Penh City, Cambodia. This study was carried out from November 2014 to September 2015.

Criteria for sample collection: Information about the dogs was collected via a questionnaire completed by the dog owners and through observation. Information recorded included breed, sex, and age of each dog, grouped as puppy (< 6 months old), juvenile (6 months - 1 year old), adult (1-7 years), old (>7 years). Clinical signs including anemia, fever, depression, anorexia, weight loss and skin problems were recorded.

Blood collection and blood parasite examination: Blood samples (4 ml) were collected from the cephalic vein of dogs and immediately put into two tubes of ethylenediaminetetraacetic acid (EDTA) and stored in an ice box. Two ml of blood was stored at 4 °C for 12-48 hours for laboratory examination using conventional techniques including fresh blood smear, Buffy coat thin blood smear with Wright-Giemsa staining, and the Modified Knott's test. The presence of microfilaria species was confirmed by Acid Phosphatase Activity (APA) (Chungpivat and Taweethavonsawat, 2008) for extracellular parasites, and another 2 ml of blood was kept at -20 °C for molecular identification. Fifty suspected samples were examined by using the PCR technique (Malheiros *et al.*, 2016) for intracellular parasitic identification.

Statistical analysis: The results were interpreted by descriptive statistical analysis. The percentage of positive results was calculated as the total positive number multiplied by 100 and divided by the total number of tested dogs.

Results

In this study, the age distribution of the dogs was as follows: puppy 14.51%; juvenile 16.70%; adult 53.85%; and old 12.53%. Regarding the gender, 52.52% were male and 47.47% were female. The 95% of dogs in rural areas were of mixed breed.

CVBP were found in only six samples (1.32%) (Table 1), and included *B. vogeli*, which was found in one sample from a private clinic in Phnom Penh city. Two cases of co-infection were found: *E. canis* with *A. platys* and *B. vogeli* with *A. platys* in Phnom Penh city. *E. canis* was present in the morulae form of monocyte, as indicated by Wright-Giemsa staining and confirmed by PCR. *A. platys* was not detected using the conventional technique but the PCR technique gave a positive result in suspected samples. The other three positive samples were found in rural areas using a modification of Knott's test. Microfilarial identification was carried out using Wright-Giemsa staining and was confirmed by APA. There were two positive samples of *D. immitis* in Prey Veng province and another positive sample of *B. pahangi* was found in Kompong Chhnang province. In this study, six CVBP-positive samples were found in four areas including Prey Veng province (0.22%), Takeo province (0.22%), Kompong Chhnang province (0.22%) and a private clinic (0.66%). No parasitic infection was found in samples from

Kompong Speu and Svay Rieng province (Table 1). Clinical signs were not correlated with the infected cases in this study.

Table 1 The number of positive cases of canine vector borne blood parasites infection in each location from the plain regions of Cambodia

Pathogens	Takeo (n = 97)	Kompong Speu (n=100)	Kompong Chhnang (n= 77)	Prey Veng (n= 87)	Svay Rieng (n= 70)	Phnom Penh city (n= 24)
<i>B. vogeli</i>	NF	NF	NF	NF	NF	1
B + A*	NF	NF	NF	NF	NF	1
E+ A**	NF	NF	NF	NF	NF	1
<i>D. immitis</i>	1	NF	NF	1	NF	NF
<i>B. pahangi</i>	NF	NF	1	NF	NF	NF

NF= not found

B + A* = *B. vogeli* + *A. platys*

E+ A** = *E. canis* + *A. platys*

Discussion

This study was the first investigation of CVBP using conventional methods and molecular confirmation in the plain regions of Cambodia. The rate of CVBP infection among dogs in this study was low, with only 1.32% of positive blood samples (N=455). Many countries have reported a similar prevalence of CVBP infection, including Malaysia, where the rate of infection by *D. immitis* was 9.6%, *H. canis* 1.2%, *B. canis* 1.1%, and *E. canis* 0.2% (Rajamanickam et al., 1985). In Thailand, rates of infection in a previous study were as follows: *B. canis* 3.07%, *H. canis* 4.54%, and *E. canis* 2.32% (Salakij et al., 1999). In India, PCR detected *H. canis* in 30% of samples, *E. canis* in 20.6%, and *A. platys* in 6.5%. *H. canis* gamonts were found in 2.3% (N=525) by examination of blood smears (Rani et al., 2011). The prevalence of infection with *E. canis* (27%; 46/170), *Babesia* spp. (24%; 90/372), *B. canis vogeli* (12%; 43/372), *B. gibsoni* (10%; 36/372), *A. platys* (11%; 17/157) and *H. canis* (6%; 15/266) based on serological and PCR evidence has also been published (Kelly et al., 2013). However, the prevalence of CVBP may change if other diagnostic methods with a higher sensitivity, specificity and accuracy, e.g. serological and molecular methods, are used. Surprisingly, *H. canis* infection was not demonstrated in this study. The absence of *H. canis* may be due to the low incidence of ticks or the lack of this parasite in the plain regions. However, Inpankaew et al. (2016) reported a high prevalence of *H. canis* in northern Cambodia using a molecular detection method. *Babesia* spp. are mainly found in young dogs, although dogs of all ages can be affected this pathogen (Schoeman, 2009); in the current study, the infected dog was a 3-year-old male mixed breed dog. A previous serological study of *B. canis* showed no difference in its prevalence between age groups (Imre et al., 2013). Due to very low prevalence, an impact of age on CVBP could not be demonstrated in the present study.

In the current study, the fresh blood smear method did not detect any parasitic infection. This method uses only one drop of blood to search for moving extracellular parasites, so if the dog has low levels of

parasitemia, the sample may not be recorded as positive. The modified Knott's test is the gold standard method for microfilarial detection in blood. One sample with microfilaria was found with low parasitemia in this study. However, these two methods are inexpensive and rapid compared to molecular methods. Antigen detection for canine heartworm disease may reveal more positive cases in occult dirofilariasis.

Canine dirofilariosis is a parasitic infectious disease that has been found worldwide. In this study, microscopic examination was used to identify species via the APA technique and parasitic morphology. Only 0.43% of the 455 samples were positive for this parasite, with very low levels of parasitemia in Takeo and Prey Veng province. In Thailand, a prevalence of 18.2% (N= 589) revealed a high risk for dirofilariasis in dogs in the Chiang Mai province (Boonyapakorn et al., 2008). In China, the prevalence of *D. immitis* in the 886 specimens was indicated by two methods, microscopic examination (16.6%) and PCR (24.0%), with a high risk of infection in that region (Hou et al., 2011) and also in wild animals in Chengdu zoo, China [2.26% (N=177)] (Bo et al., 2009). In South Korea, a prevalence of 20.9% (N= 81) was determined by ELISA (Song et al., 2010). *D. immitis* infection was associated with dogs aged up to 7 months, depending on the life cycle and endemic area of infection. However, older dogs have a higher risk of infection due to their longer exposure to mosquito bites (Fan et al., 2001; Boonyapakorn et al., 2008; Hou et al., 2011).

Interestingly, this is the first report of *B. pahangi* infection in a dog in Cambodia. The low infection rate could be due to the lack of the mosquito vector or low parasitemia in the study regions. This prevalence differs from that of *B. pahangi* in Thailand (Thanchomnang et al., 2010). The mosquito *Armigeres subalbatus* was recently reported as the vector of this zoonotic infectious disease (Muslim et al., 2013a). Although *B. pahangi* has been reported in humans (Palmieri et al., 1985; Muslim et al., 2013b), little is known about this parasite; a study in Thailand reported *B. malayi* in a 2-year-old boy living in Surat Thani province (Yokmek et al., 2013). In Malaysia, five patients were infected with *B. pahangi* based on clinical

findings, serology results and PCR confirmation (Tan *et al.*, 2011).

The results of this study show the presence of CVBP in the plains of Cambodia, but the prevalence of parasites indicated low rates of infection among dogs. Detecting pathogens by morphological observation and conventional methods alone might lead to misinterpretation or misdiagnosis due to a lack of sensitivity or specificity, particularly for parasitic species. The epidemiology of CVBP is complex and the pattern of transmission from vectors to hosts is unclear. Thus, further research on the prevalence of these diseases is required not only in dogs but also in other animals.

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