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Original Article

Prevalence and risk factors associated with *Dirofilaria immitis* infection in dogs and cats in Songkhla and Satun provinces, Thailand

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ABSTRACT

To update the microfilaria infection in companion animals, this study determined the prevalence and risk factors of microfilaria infection in dogs and cats collected from eight districts in Songkhla and Satun provinces, southern Thailand. In total, 482 samples (394 dogs and 88 cats) were subjected to microscopic examination (ME), polymerase chain reaction (PCR) and sequencing analysis. The overall prevalence of microfilaria infection in dogs and cats was 24.1% (95/394) and 36.4% (32/88) using PCR, respectively. Furthermore, the overall results were positive 7.7% (37/482) using ME compared to 26.3% (127/482) using PCR. Sequencing analysis of all positive PCR products identified the microfilaria as *Dirofilaria immitis*. *D. immitis* infection in each sampled district of Songkhla and Satun provinces was in the range 0–48% for dogs and in the range 15.4–75% for cats. Risk factor analysis showed that there was significantly higher *D. immitis* infection in dogs older than 2 yr. The study updated the prevalence of *D. immitis* infection in dogs and cats in two southern provinces of Thailand and there was a high *D. immitis* infection rate in old dogs (aged > 2 yr).

Introduction

Filariasis has been reported as important zoonoses from dogs and cats, which have long been known as reservoirs (Sribhen et al., 1999; Tiawsirisup et al., 2010). The infective stage of the filarid worm is microfilaria with unique taxonomic characters (Guptavanij et al., 1977). It is caused by *Dirofilaria immitis*, *Brugia malayi*, *B. pahangi*, *Onchocerca volvulus* and *Wuchereria bancrofti* and mosquitoes such as *Mansonia*, *Anopheles*, *Culex* and *Aedes* are biological vectors of these worms and elimination and control of filariasis are based on the control of these mosquitoes (Simón et al., 2012; Zielke et al., 1993). Stray animals can also be served as the potential reservoirs (Jittapalapong, 2014). Therefore, the elimination of filariasis relies on controlling the number of stray animals (Thanchomnang et al., 2010, 2013). An update of the situation of filariasis in reservoirs might help with understanding the disease status (Ichimori et al., 2014).

The detection of filarial infections is generally based on microscopic examination (ME), which is cheap and convenient; however, this test has low sensitivity and is not practical for testing a large number of samples (Yen and Mak, 1978; Nuchprayoon et al., 2003). In addition, the differentiation of filarial species under the light microscope is limited (Nuchprayoon et al., 2005). Therefore, polymerase chain reaction (PCR) has become an alternative diagnosis to confirm and differentiate filarid species (Areekit et al., 2009; Thanchomnang et al., 2010). PCR is a useful test to differentiate among filarial parasite species and can detect current infection even under low parasitemia conditions (Nuchprayoon, 2009). A combination of ME and PCR could be used to increase the sensitivity and specificity of identifying microfilaria infections.

Thailand is one of the endemic countries for *Dirofilaria immitis*, *Brugia malayi* and *B. Pahangi* (Nithiuthai, 2003; Thanchomnang et al., 2013). The most frequently filarid worm in dog is *D. immitis*, which causes heartworm disease in animals (Ciucă et al., 2016), while *B. malayi* causes lymphatic filariasis in both animals and humans

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(Guptavanij et al., 1977). Climatic and environmental changes have influenced mosquito's habitats and populations (Ebi and Nealon, 2016; Simón et al., 2017). Lymphatic filariasis has been reported in Thai-Myanmar border, indicating the potential distribution due to migrating foreigners (Triteeraprapab and Songtrus, 1999). Lymphatic filariasis has also been detected in dogs and cats as important reservoirs (Nuchprayoon et al., 2006; Ambily et al., 2011). Lymphatic filariasis in humans has also been reported in southern Thailand, especially in the border areas, where numerous foreign migrants live (Zielke et al., 1993). Moreover, the climate and environment there is suitable for the distribution of mosquitoes as a vector (Genchi et al., 2011; Nithiuthai, 2003). The current study postulated that there should be evidence of microfilaria infection in companion animals which could be potential reservoirs of lymphatic filariasis in this area. In this study, the prevalence and risk factors of microfilaria infection in dogs and cats in Songkhla and Satun provinces were determined using ME, PCR and statistical techniques.

Materials and methods

Study area, blood sampling and data collection

Eight districts in Songkhla and Satun provinces, southern Thailand were sampled during June 2013. Dogs and cats were randomly selected for blood sampling and data collection. In total, 482 blood samples from dogs and cats were collected from the cephalic or saphenous vein or both. Blood samples were preserved in 1.8 mg/ml ethylene diamine tetra acetic acid for microscopic examination and 3.2% sodium citrate for the PCR technique. Data were recorded from interviews with each animal's owner or carer. The sex, age, breed, presence of ectoparasites and the environment and health conditions of animals were also recorded. A consent form was signed by each animal owner or carer before data collection.

Microscopic examination

A thin blood smear was immediately conducted after blood collection, then fixed using methanol and stained using modified Wright Giemsa staining (Eberhard and Lammie, 1991). Examination of microfilaria in a blood smear under a light microscope was performed.

DNA extraction, polymerase chain reaction and sequencing analysis

Blood samples were used for DNA extraction using the phenol-chloroform extraction method (Sambrook and Russell, 2001). DNA was stored at −20 °C prior to use as a template in the PCR technique. Conventional PCR was performed using a DIDR primer set targeting the *5.8S-ITS2-28S* region as previously described (Rishniw et al., 2006). This primer set can be used to differentiate *D. immitis*, *D. repens*, *B. malayi*, *B. pahangi*, *Acanthocheilonema. reconditum* and *A. dracunculoides,* as shown by the PCR products of 542, 484, 615, 664, 578 and 584 base pairs, respectively (Rishniw et al., 2006). Positive DNA products on gel electrophoresis were cut, purified using an UltraClean® DNA purification kit (Mo Bio Laboratories, Inc.; Carlsbad, CA, USA) and sent for direct sequencing. Nucleotide sequences were blasted at the National Center for Biotechnology Information. Similarity and identity were confirmed to the genus and species levels of microfilaria.

Statistical analysis

Risk factors associated with microfilaria infection of dogs and cats were analyzed using $\chi 2$ values and were considered significant at $p \leq 0.05$. The Epi Info software (version 7.0; CDC; Atlanta GA, USA) was used in the statistical analysis.

Results

D. immitis was identified in dogs at 8.6% (34/391) using ME compared to 24.1% (95/394) using PCR and in cats at 3.4% (3/88) by ME compared to 8.6% (34/394) using PCR (Table 1). The PCR product was 542 bp on agarose gel (Fig. 1). Sequencing analysis showed that all positives were 75–91% identities with E (2e-43) when compared to the *D. immitis* 5.8S-ITS2-28S region from GenBank; *D. immitis* infection in dogs in Songkhla and Satun provinces were 24.9% (51/205) and 23.3% (44/189), respectively (Table 2). *D. immitis* infection among dogs in Songkhla was 48% (24/50), 34.2% (14/41), 11.0% (8/73) and 12.2% (5/41) in Khlong Hoi Kong, Sadao, Hat Yai and Rattaphum districts, respectively. *D. immitis* infection among dogs in Satun was 20.2% (20/99), 46.8% (22/47), 7.4% (2/27) and 0% (0/16) in Kuan Kalong, Muaeng, Tha Phae and Khuan Don districts, respectively (Table 2). Based on the PCR method, *D. immitis* infection in male and female dogs was 24.5% (49/200) and 23.7% (46/194), respectively. *D. immitis* infection in cross breed and pure breed dogs was 24.2% (78/322) and 23.6% (17/72), respectively. *D. immitis* infection in dogs with and without the presence of ectoparasites was 22.3% (37/166) and 25.4% (58/228), respectively (Table 3). Risk factor analysis showed that dogs older than 2 yr were significantly more likely to be infected with *D. immitis* than young dogs ($p < 0.007$; Table 3).

D. immitis infection in male and female cats was 48.4% (15/32) and 30.4% (17/56), respectively. *D. immitis* infection in cats in Songkhla and Satun was 34.9% (29/83) and 60.0% (3/5), respectively (Table 2). *D. immitis* of Songkhla cats was 42.5% (17/40), 15.4% (2/13), 33.3% (10/30) in Khlong Hoi Khong, Hat Yai, Rattaphum districts, respectively (Table 2). *D. immitis* of Satun cats was 75% (3/4) in Mueang district. There was variation in the prevalence levels among cats from the different districts. One Persian cat was infected with *D. immitis*, while in Domestic Short Hair cats, the level was 36.9% (31/84). *D. immitis* in cats without ectoparasites was 38.3% (31/81), while it was 14.3% (1/7) in cats with ectoparasites (Table 3).

Discussion

Southern Thailand is known as an endemic area for lymphatic filariasis caused by *B. malayi* in humans with prevalence reported in the range 0–28.6% (Zielke et al., 1993). Cats have been postulated as potential reservoirs of some lymphatic filariasis. However, the current study could not detect *B. malayi* infection in pets from Songkhla and Satun. This result was in agreement with Guptavanij et al. (1977) as they found *B. malayi* infection in humans only in Narathiwat, Nakon Sri Thammarat, and Chumporn provinces but not in Phuket, Phangnga, Yala, Trang, Krabi, Ranong, Songkhla and Satun provinces. Nonetheless, the potential for *B. malayi* infection in pets should be taken into account for future monitoring of the incidence of lymphatic filariasis due to changes in climate, environment and mosquito vectors as well as in human immigration

Table 1
Overall prevalence of *Dirofilaria immitis* infection in dogs and cats from Songkhla and Satun provinces, Thailand tested using microscopic examination (ME) and polymerase chain reaction (PCR).

Animal	Number tested	*Dirofilaria immitis* positives			
		ME	(%)	PCR	(%)
Dog	394	34	8.63%	95	24.11%
Cat	88	3	3.41%	32	36.36%