Agriculture and Natural Resources 51 (2017) 299–302

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

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

Ketsarin Kamyingkird,[a] Witchuta Junsiri,[a] Witsanuwat Chimnoi,[a] Chanya Kengradomkij,[a] Sinsamut Saengow,[a] Keplee Sangchuto,[a] Wicha Kajeerum,[b] Decha Pangjai,[c] Burin Nimsuphan,[a] Tawin Inpankeaw,[a] Sathaporn Jittapalapong[a,*]

[a] *Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand*
[b] *Satun Provincial Livestock Office, Satun 91000, Thailand*
[c] *National Institute of Health (NIH), Department of Medical Sciences, Ministry of Public Health, Nonthaburi 11000, Thailand*

ARTICLE INFO

Article history:
Received 22 January 2017
Accepted 24 May 2017
Available online 16 October 2017

Keywords:
Pets
Dirofilaria immitis
5.8S-ITS2-28S
Songkhla
Satun

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

* Corresponding author.
E-mail address: fvetspj@ku.ac.th (S. Jittapalapong).

https://doi.org/10.1016/j.anres.2017.05.003

(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%

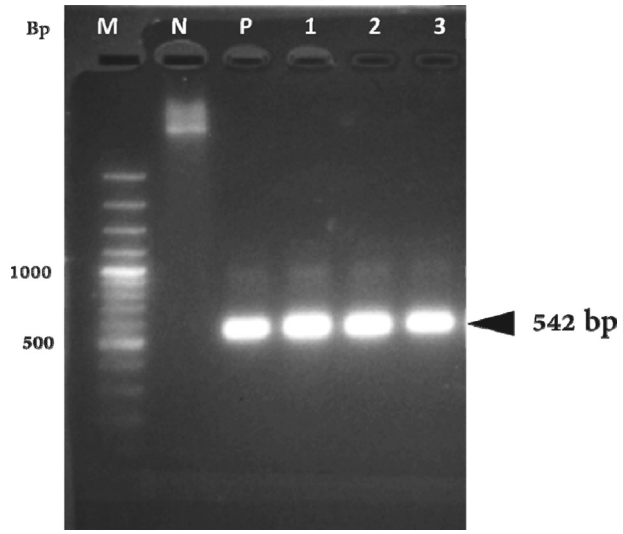

Fig. 1. Polymerase chain reaction results showing stained positive products of *Dirofilaria immitis* on 1.5% agarose gel: Lane M: molecular marker; N: *D. immitis* negative DNA sample; P: *D. immitis* positive DNA sample; lane 1–3: *D. immitis* positive dog sample with microfilaria.

Table 2
Prevalence (positives/total sample) of *Dirofilaria immitis* infection in dogs and cats from Songkhla and Satun provinces, Thailand.

Location		Dogs	Cats
		Positives (%)	Positives (%)
Province	Songkhla	51/205 (24.88)	29/83 (34.94)
District	Khlong Hoi Khong	24/50 (48.00)	17/40 (42.50)
	Sadao	14/41 (34.15)	0/0 (0.00)
	Hat Yai	8/73 (10.96)	2/13 (15.38)
	Rattaphum	5/41 (12.20)	10/30 (33.33)
Province	Satun	44/189 (23.28)	3/5 (60.00)
District	Khuan Kalong	20/99 (20.20)	NA*
	Mueang	22/47 (46.81)	3/4 (75.00)
	Tha Phae	2/27 (7.41)	0/1 (0.00)
	Khuan Don	0/16 (0.00)	NA
Total	PCR	95/394 (24.11)	32/88 (36.36)

*NA = not applicable as no sample available.

Table 3
Risk factors associated with microfilaria infection of *Dirofilaria immitis* in dogs and cats from Songkhla and Satun provinces, Thailand based on positives/total sample.

Factor	Category	Dogs	Cats	*p*-value Dogs, Cats
		Positives (%)	Positives (%)	
Age group	<2yr	24/158 (15.19)	17/42 (40.48)	0.0007, 0.4461
	≥2yr	71/236 (30.08)*	15/46 (32.61)	
Sex	Male	49/200 (24.50)	15/32 (46.88)	0.8550, 0.1234
	Female	46/194 (23.71)	17/56 (30.36)	
Breed	Pure breed	17/72 (23.61)	1/4 (25.00)	0.9127, 0.6306
	Cross/Domestic Short Hair	78/322 (24.22)	31/84 (36.90)	
Ectoparasite	Yes	37/166 (22.29)	1/7 (14.29)	0.4711, 0.2082
	No	58/228 (25.44)	31/81 (38.27)	
Living status	Indoor	63/252 (25.00)	16/42 (38.10)	0.5834, 0.7483
	Outdoor	32/142 (22.54)	16/46 (34.78)	
Health condition	Healthy	84/345 (24.35)	31/81 (38.27)	0.7715, 0.2082
	Clinical signs	11/49 (22.45)	1/7 (14.29)	

* = statistically significant at $p \leq 0.05$.

routes. The prevalence of *D. immitis* infections in dogs has been studied in some areas of Thailand and reported to be in the range 0.36–25.2% using microscopic examination (Jittapalapong, 1990; Boonyapakorn et al., 2008; Sanisuriwong et al., 2012). Most of the tests used one of the haematocrit technique, the modified knot technique or a thin blood smear. The advantages of microscopic tests are that they are fast and cheap. However, differentiation of filarial species is limited and its low sensitivity might occur due to the nocturnal behavior of the worm. The current study used the advantage of the high sensitivity of the PCR technique to identify filarial species and to measure the actual prevalence of microfilaria in pets and found a high prevalence of *D. immitis* infection in both dogs (24.1%) and cats (36.4%) Therefore, it is suggested that better prevention and control of *D. immitis* infection in pets is urgently required in these areas. In addition, dogs older than 2 yr had significantly greater levels of infection with *D. immitis* than young dogs. This was in accord with Boonyapakorn et al. (2008) who considered it was due to the increased exposure to mosquitoes in older dogs (age > 2 yr). Furthermore, the prevalence of *D. immitis* varied in dogs from different districts, perhaps due to geographical differences and the differences in the sample sizes of animals in this study.

Stray dogs and cats might serve as a source of microfilaria infection for other animals including humans (Tiawsirisup et al., 2010). To reveal the role of dogs and cats as microfilaria reservoirs, the current study used ME and PCR for detection and confirmation of microfilaria infections. PCR has the advantage of characterizing filarial species and confirmed the existence of the infection in negative animals determined using ME. The current study found a high infection rate of microfilaria (*D. immitis*) infection in both dogs and cats which suggests that better protection by using microfilaria prophylactic drugs, or repellent or an insect net and using regular health checks for pets should be recommended to reduce the prevalence of *D. immitis* infection. Moreover, this information could be useful for provincial veterinarians to help establish a *D. immitis* elimination program in these two provinces. Lymphatic filariasis in pets should be regularly monitored as the disease can be harmful to human health (Nuchprayoon et al., 2006).

In conclusion, *D. immitis*, infection is endemic in Songkhla and Satun provinces. The prevalence of *D. immitis* in dogs and cats in each districts was in the range 0–75%. The results from this study suggested that better control of microfilaria infection in companion animals is required to reduce the microfilaria infection rate in this region.

Conflicts of interest

The authors declare that there is no conflict of interest.

Acknowledgements

The authors greatly appreciate support from the Songkhla and Satun Provincial Livestock Offices and the kind cooperation of the animals' owners for assistance with data and blood sample collection. This study was funded by a grant from the Kasetsart University Research and Development Institute (KURDI), Bangkok, Thailand and a grant from the Parasitology Department, Faculty of Veterinary Medicine, Kasetsart University.

References

Ambily, V.R., Usha, N.P., Arun, R., Pramod, S., Jayakumar, K.M., 2011. Detection of human filarial parasite *Brugia malayi* in dogs by histochemical staining and molecular techniques. Vet. Parasitol. 181, 210–214.

Areekit, S., Singhaphan, P., Khuchareontaworn, S., Kanjanavas, P., Sriyaphai, T., Pakpitchareon, A., Khawsak, P., Chansiri, K., 2009. Intraspecies variation of *Brugia* spp. in cat reservoirs using complete ITS sequences. Parasitol. Res. 104, 1465–1469.

Boonyapakorn, C., Srikitjakarn, L., Morakote, N., Hoerchner, F., 2008. The epidemiology of *Dirofilaria immitis* infection in outpatient dogs at Chiang Mai university small animal hospital, Thailand. Southeast Asian J. Trop. Med. 39, 33–38.

Ciucă, L., Musella, V., Miron, L.D., Maurelli, M.P., Cringoli, G., Bosco, A., Rinaldi, L., 2016. Geographic distribution of canine heartworm (*Dirofilaria immitis*) infection in stray dogs of eastern Romania. Geospatial Health 11, 318–323.

Eberhard, M.L., Lammie, P.J., 1991. Laboratory diagnosis of filariasis. Clin. Lab. Med. 11, 977–1010.

Ebi, K.L., Nealon, J., 2016. Dengue in a changing climate. Environ. Res. 151, 115–123.

Genchi, C., Kramer, L.H., Rivasi, F., 2011. Dirofilaria infections in Europe. Vector Borne Zoonotic Dis. 11, 1307–1317.

Guptavanij, P., Harinasuta, C., Surathin, K., Vutikes, S., Deesin, T., 1977. Studies on the prevalence of Malayan filariasis in South Thailand. Southeast Asian J. Trop. Med. 8, 42–52.

Ichimori, K., King, J.D., Engels, D., Yajima, A., Mikhailov, A., Lammie, P., Ottesen, E.A., 2014. Global programme to eliminate lymphatic filariasis: the processes underlying programme success. PLoS. Negl. Trop. Dis. 8, e3328. https://doi.org/10.1371/journal.pntd.0003328.

Jittapalapong, S., 1990. Studies of prevalence of canine heartworm infestation of pet dogs in Samut-Prakarn province. J. Thai Vet. Prac. 4, 265–277.

Jittapalapong, S., 2014. Applied Parasitology: Parasitism, Immunity to Parasites, Anti-parasite Vaccine, and Update Situation of Parasitic Disease in Pet and Food Animals in Thailand, first ed. Bangkok. Thailand.

Nithiuthai, S., 2003. Risk of canine heartworm infection in Thailand. In: Proceedings of 28th World Small Animal Veterinary Association World Congress. Bangkok, Thailand.

Nuchprayoon, S., 2009. DNA-based diagnosis of lymphatic filariasis. Southeast Asian J. Trop. Med. 40, 904–913.

Nuchprayoon, S., Junpee, A., Nithiuthai, S., Chungpivat, S., Suvannadabba, S., Poovorawan, Y., 2006. Detection of filarial parasites in domestic cats by PCR-RFLP of ITS1. Vet. Parasitol. 140, 366–372.

Nuchprayoon, S., Junpee, A., Poovorawan, Y., Scott, A.L., 2005. Detection and differentiation of filarial parasites by universal primers and polymerase chain reaction-restriction fragment length polymorphism analysis. Am. J. Trop. Med. Hyg. 73, 895–900.

Nuchprayoon, S., Sangprakarn, S., Junpee, A., Nithiuthai, S., Chungpivat, S., Poovorawan, Y., 2003. Differentiation of *Brugia malayi* and *Brugia pahangi* by PCR-RFLP of ITS-1 and ITS-2. Asian Pac. J. Allergy 34, 67–73.

Rishniw, M., Barr, S.C., Simpson, K.W., Frongillo, M.F., Franz, M., Dominguez Alpizar, J.L., 2006. Discrimination between six species of canine microfilariae by a single polymerase chain reaction. Vet. Parasitol. 135, 303–314.

Sanisuriwong, J., Juntuck, N., Pavasutthipaisit, S., Boonsriroj, H., Wiengcharoen, J., 2012. Prevalence of hemoparasite in dogs in Nongchok, Bangkok, Thailand. J. Mahanakorn Vet. Med. 7, 25–35.

Sambrook, J., Russell, D.W., 2001. Molecular Cloning: a Laboratory Manual, third ed. Cold Spring Harbor, New York.

Simón, F., González-Miguel, J., Diosdado, A., Gómez, P.J., Morchón, R., Kartashev, V., 2017. The complexity of zoonotic filariasis episystem and its consequences: a multidisciplinary view. Biomed. Res. Int. https://doi.org/10.1155/2017/6436130.

Simón, F., Siles-Lucas, M., Morchón, R., González-Miguel, J., Mellado, I., Carretón, E., Montoya-Alonso, J.A., 2012. Human and animal dirofilariasis: the emergence of a zoonotic mosaic. Clin. Microbiol. Rev. 25, 507–544.

Sribhen, C., Kasemsant, N., Kaewmokul, S., Sribhen, K., 1999. Blood chemistry profile and cardiac troponin T concentration in Thai stray dogs infected with heartworms. Kasetsart J. (Nat. Sci.) 33, 251–257.

Thanchomnang, T., Intapan, P.M., Lulitanond, V., Sangmaneedet, S., Chungpivat, S., Taweethavonsawat, P., Choochote, W., Maleewong, W., 2010. Rapid detection of *Dirofilaria immitis* in mosquito vectors and dogs using a real-time fluorescence resonance energy transfer PCR and melting curve analysis. Vet. Parasitol. 168, 255–260.

Thanchomnang, T., Intapan, P.M., Tantrawatpan, C., Lulitanond, V., Chungpivat, S., Taweethavonsawat, P., Kaewkong, W., Sanpool, O., et al., 2013. Rapid detection and identification of *Wuchereria bancrofti, Brugia malayi, B. pahangi*, and *Dirofilaria immitis* in mosquito vectors and blood samples by high resolution melting real-time PCR. Korean J. Parasitol. 51, 645–650.

Tiawsirisup, S., Thanapaisarnkit, T., Varatorn, E., Apichonpongsa, T., Bumpenkiattikun, N., Rattanapuchpong, S., Chungpiwat, S., Sanprasert, V., et al., 2010. Canine heartworm (*Dirofilaria immitis*) infection and immunoglobulin G antibodies against *Wolbachia* (Rickettsiales: *Rickettsiaceae*) in stray dogs in Bangkok, Thailand. Thai J. Vet. Med. 40, 165–170.

Triteeraprapab, S., Songtrus, J., 1999. High prevalence of *Bancroftian* filariasis in Myanmar-migrant workers: a study in Mae Sot district, Tak province, Thailand. J. Med. Assoc. Thail. 82, 734–739.

Yen, P.K.F., Mak, J.W., 1978. Histochemical differentiation of *Brugia, Wuchereria, Dirofilaria* and *Breinlia* microfilariae. Ann. Trop. Med. Parasitol. 72, 157–162.

Zielke, E., Hinz, E., Sujarit, S., 1993. Lymphatic filariasis in Thailand: a review on distribution and transmission. Trop. Med. Parasitol. 15, 141–148.