J Trop Med Parasitol 2004;27:15-20.

ORIGINAL ARTICLE

Available online at www.ptat.thaigov.net

Periodicity of *Brugia malayi* Appearance in Blood of Domestic Thai Cats in Surat Thani Province

Usa Lek-Uthai[1], Kosum Chansiri[2], Supit Yodmek[3]

[1]Department of Parasitology, Faculty of Public Health, Mahidol University, Bangkok, Thailand
[2]Department of Biochemistry, Faculty of Medicine, Srinakarinviroj University, Bangkok, Thailand
[3]Center for Vector-Borne Diseases Control 45, Surat Thani Province,Thailand

Abstract

This study aimed to determine the periodicity of appearance in the blood circulation of a cat sample. Sixty-five domestic Thai cats from Tha Chana District, Surat Thani Province were recruited and their blood was collected, stained with Giemsa and examined microscopically for the parasite. Ten cats (15.4%) were infected with *Brugia* spp, but the remainder were not. The average age of the infected cats was $5\frac{1}{2}$ years. All isolates of *Brugia* spp from infected cats were identified as *B. malayi,* using PCR technique with primer Bm-1/Bm-2 (specific to *B. malayi*) and using DNA (at 10 ng/50 ml) extract from feline WBC and *W. bancrofti* as negative control. The sensitivity of *B. malayi* DNA detection was 0.0001 ng, and the amplified DNA was shown to represent the same specific type as infects man. The appearance of *B. malayi* was monitored every two hours over 24 hours' observation. A modified harmonic equation showed the highest parasitemia peak at 00.54 hr. All five domestic cats showed the appearance of microfilariae at almost all observed intervals with the maximal peaks at nighttime, the highest peaks expressed being 59.3, 74.0, 618.0, 210.3 and 32.0 microfilariae at 20.00, 18.00, 24.00, 06.00 and 06.00 hr, respectively, These findings indicated the nocturnal subperiodic type of microfilariae presented in the cat sample. They also indicated that the mode of transmission was transient and restricted, considerably at nighttime, and would be helpful for epidemiological study of the parasite reservoir.

Keywords: periodicity, *Brugia malayi*, domestic Thai cat

Introduction

Lymphatic filariasis has a wide geographic distribution. *Wuchereria bancrofti* and *Brugia malayi* infect some 128 million people, and about 43 million have symptoms, 10.7 million with *W. bancrofti,* and 13 million with *B. malayi* and *B. timori* [1]. *B. malayi*, a zoonotic infection, is endemic in Asia and is transmitted by mosquitoes of the genera *Mansonia, Anopheles,* and *Aedes*. Wild monkeys and felines are reservoirs for *B. malayi* [2]. In Thailand, a survey of human filariasis first revealed infection in Nakhon Si Thammarat, Phatthalung, Pattani, and Surat Thani provinces. Twenty-one percent of individuals were infected with *B. malayi*, of whom 5.2% were symptomatic [3]. The study by Harinasuta *et al* [2] suggested a nocturnal subperiodic character of *B. malayi*

Correspondence:
Dr Usa Lek-Uthai
Tel & Fax: 66-2644-5130
e-mail: phulu@mahidol.ac.th

microfilariae in Chumporn Province, where two cases presented peak counts of microfilaria periodicity at 22.00 hr and 24.00 hr, but the highest number of microfilariae in the peripheral blood was found in the daytime, at 12.00 hr. Study of the prevalence of sub-periodic *B. malayi* in areas near the Thai-Malaysian border found that *Mansonia* mosquitoes were important vectors [4]. Phantana *et al* [5] reported 104 (4.1%) of 2,515 cats in 5 districts of Narathiwat, a southern province of Thailand, were infected with *Brugia* spp, 76 cases (3.0%) with *Dirofilaria repens*, and 2 cases (0.2%) with *D. immitis*. The peak of periodicity for *Brugia* spp in 6 infected cats was 20.04 hr. The *Brugia* species were not identified.

This experimental field research was designed to study the periodicity of microfilariae for 24 hours and to identify *Brugia* spp in domestic cats. The study was performed among microfilaria-infected people at Tha Chana District, Surat Thani Province, where *M. bonneae* is the primary vector of *B. malayi*. The objective of this study was to describe the periodicity of microfilariae in cats over a 24-hour period.

Materials and methods

Study area

The research was carried out at Tha Chana District, Surat Thani Province, where *Brugia* spp are endemic.

Assessment of microfilariae

Sixty-five adult male and female domestic Thai cats were screened for *Brugia* infection by blood smear. The *Brugia*-positive cats were then investigated and diagnosed for type of infection. *B. malayi* infection per 20 µl of fresh blood from the ear veins was identified, using morphological characteristics [6], and PCR-based methods. Three duplicate lines of thick-blood samples were immediately made on a clean glass slide by Sahli's pipette. The microfilariae (mf) were counted microscopically after Giemsa staining of blood films. Cats that showed > 5 mf/60 µl of blood were maintained in the laboratory for 15 days, after which they were determined for microfilaria periodicity by collecting blood at 2-hour intervals for 24 hours (12 records) and analysis by modified harmonic equation method [7-11].

Extraction of microfilaria DNA from blood samples

Microfilaremia was determined by filtration of 5 ml venous cat blood with 15 ml phosphate buffer saline (PBS), through a polycarbonate membrane millipore (0.5 µm pore size). One microliter of blood from each microfilaria-positive cat was then thoroughly mixed with 800 µl PBS and centrifuged to pellet red cells and microfilariae. The pellets were centrifuged for 10 min at 5,000 rpm. After centrifugation, the pellet was washed again in 800 ml PBS and then carefully resuspended in 200 µl DSP buffer (20 mM Tris-HCl, pH 7.6, 2.5 mM $MgCl_2$, 50 mM KCl, 0.01% proteinase K, 0.5% Tween 20®). Incubation in DSP buffer was performed at 42 ˚C for 14 hours to lyse the microfilaria and release the DNA. The proteinase K was then inactivated by incubating the samples at 90˚C for 10 min. Following brief centrifugation to pellet debris, the supernatant was kept for PCR analysis. DNA extracts were kept frozen at -20˚C in 0.1 M EDTA until processed for PCR analysis. In addition, DNA of *W. bancrofti*, *D. immitis*, and *D. repens*, were used to confirm the species-specificity of the PCR assay. Blood was also obtained from human volunteers living in a non-endemic area. These additional blood samples were processed and amplified by PCR, exactly as described for the cat blood samples.

Polymerase chain reaction (PCR)

Specific identification of *Brugia* from cats was done by PCR. This technique can detect as little as one femtogram (10^{-15}g) of purified *B. malayi* DNA, and has been used to identify microfilaremia 'day blood' in areas where periodic or nocturnal subperiodic microfilaria are endemic [9]. Blood samples from microfilaria-positive cats were collected in EDTA tubes for identification by PCR. Forward and reverse PCR primers, designated Bm-1 and Bm-2, were designed. The Bm-1/Bm-2 primer set designed for PCR amplification of a 280 bp DNA fragment from *B. malayi* are 5´-GCG CAT AAA TTC ATC AGC AA-3´ and 5´-ATG ACA ACA CAA TAC ACG AC-3´ [13]. The PCR was performed using 50 µl of the DNA extracts prepared from

blood samples, and 10 mM Tris-HCl, pH 9.2, 2 µl 50 mM $MgCl_2$, 8 µl 10 mM deoxynucleotide triphosphate (dNTPs), 5 µl of each primer, and 2 units of *Taq* polymerase were used as reagents. The mixture was denatured for 1 min at 95˚C and chilled on ice. *Taq* polymerase (2 units) was added, and the mixture was overlaid with mineral oil. Thirty amplification cycles were completed with denaturation for 1 min at 95˚C, annealing for 1 min at 72˚C and a final extension of 10 min at 72˚C. Twenty microliters of the PCR product was loaded onto 1.5% agarose gel and a unique band of 280 bp was visualized by ethidium bromide staining, and amplified DNA bands were visualized by ultraviolet (UV) light illumination.

Results

Domestic cat blood survey

Ten of 65 (15.4%) domestic cats were positive for *Brugia* spp by blood smear (3-68 mf/60 µl). Six (17.1%) cats were male, and four (13.3%) were female, aged between 1-12 years, with an average age of $5\frac{1}{2}$ years. Sheathed microfilariae of *B. malayi* in infected domestic cats were identified with Giemsa staining under a microscope (10 x 100).

Microfilaria periodicity study

Of the ten positive cats, five had shown more than 5 mf/60 µl of blood and they were studied for microfilaria periodicity.

The periodicity found in this study was between 24.00-06.00 hr. The periodicity index was 63.02. The highest peak of *Brugia* microfilariae was at 00.54 hr (Fig 1). Table 1 shows the microfilaria count for *B. malayi* from positive cats over the 24-hour period. All five cats showed the appearance of microfilariae at almost all observed intervals. Cat no. 3 showed a maximal peak count, 618.0 mf, at 24.00 hr, cats nos. 1 and 2 showed the highest peaks, 59.3 and 74.0 mf, at 20.00 hr and 18.00 hr, respectively, while cats nos. 4 and 5 showed the highest peaks, 210.3 and 32.0 mf, at 06.00 hr. These findings indicated the nocturnal subperiodic type of microfilariae presented in the cat sample.

PCR screening for *Brugia malayi*

Ten *Brugia*-positive cats were venipunctured with 100 µl in EDTA tubes for *B. malayi* screening. The sensitivity of primers Bm-1/Bm-2, which was used for *B. malayi* DNA detection, were concentrations of 10, 1.0, 0.1, 0.01, 0.001, and

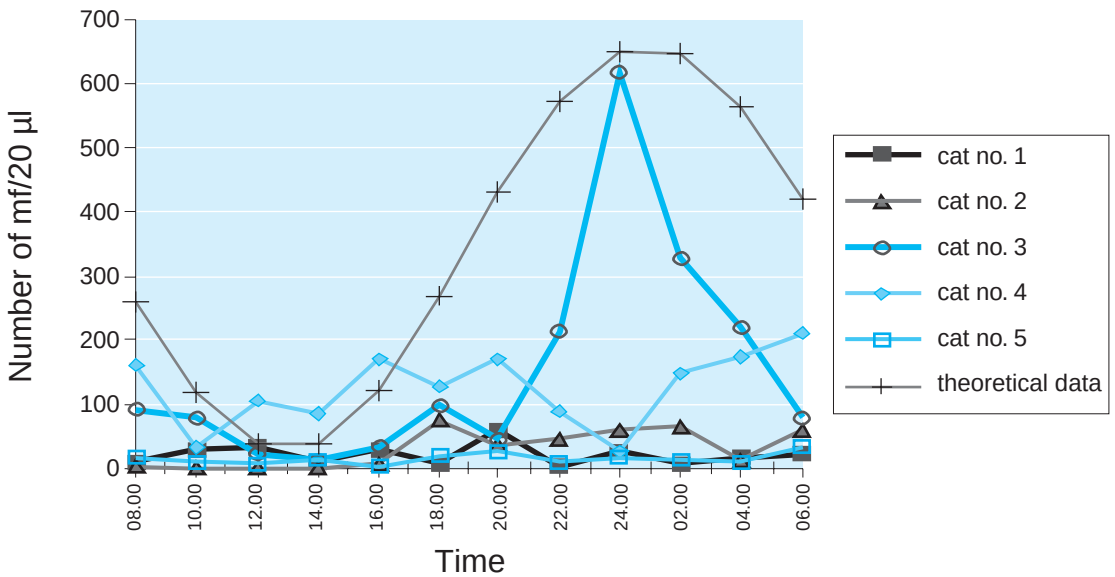

Fig 1 The appearance of microfilariae of *Brugia* sp in the blood of five domestic cats (fitted harmonic wave after theoretical data plot).

Table 1 The microfilaria count for *B. malayi* from five domestic cats at two hour-intervals for 24 hours.

Cat no.	Number of microfilariae in every 2 hours											
	08.00	10.00	12.00	14.00	16.00	18.00	20.00	22.00	24.00	02.00	04.00	06.00
1	9.0	28.0	31.3	10.0	30.0	7.7	59.3	3.7	27.0	7.7	16.3	23.3
2	4.7	0	0.3	0	6.3	74.0	37.0	44.7	57.7	66.3	13.7	58.3
3	92.0	79.7	22.3	12.7	32.3	99.7	47.0	213.7	618.0	327.0	219.7	78.3
4	160.3	32.7	106.3	84.3	170.3	127.7	170.3	87.7	26.0	146.7	174.0	210.3
5	15.0	10.7	7.3	12.3	2.3	18.3	25.7	11.0	16.3	13.7	8.3	32.0

0.0001 ng. These confirmed the effectiveness of the PCR method, which could be used to detect less than 1 microfilaria density, according to a concentration of 0.0001 ng (Fig 2a). The specificity of Bm-1/Bm-2 primers, which was specific to detecting only *B. malayi* (Fig 2b), was shown.

Discussion

This outcome agrees with Phantana *et al* [5], who found maximal numbers of *B. malayi* microfilariae in domestic cats at midnight. Nevertheless, this finding contrasts with those of the Institute for Medical Research in Malaysia [7], who indicated the maximal peak counts at 20.00 hr in Wiang Sa District, Surat Thani Province. Hawking [13] also reported that transmissions of nocturnal subperiodic type *B. malayi* from man to animals developed peak periodicity shifting from subperiodic type to periodic type, or periodic type to subperiodic type, in cats and langurs, respectively. Moreover, Guptavanij *et al* [14] found the periodicity of *B. malayi* in cats had changed its natural peak of nocturnal periodic type to nocturnal subperiodic type. Chunhasawadikul *et al* [15] reported that there was a relationship between the infection rate in the transmission area

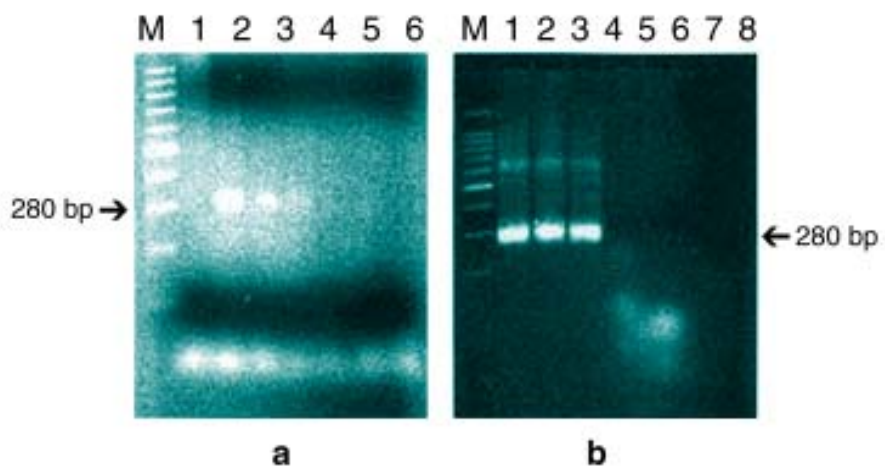

Fig 2 PCR screening for *Brugia malayi*; 1.5% agarose gel electrophoresis of PCR products with 280 bp from Bm-1/Bm-2 primers in cats; (a) sensitivity of less than 1 mf, DNA concentrations in Lanes 1-6 were 10, 1.0, 0.1, 0.01, 0.001, and 0.0001 ng, respectively; and (b) the specificity to only *B. malayi*. (Lane 1-3), *W. bancrofti* (Lane 4), *D. repens* (Lane 5), *D. immitis* (Lane 6), human DNA (Lane 7), negative control (Lane 8), M, molecular weight standards (280-bp ladder).

and the breeding places. They observed and also compared the relation between the cats' circadian temperature cycle and the microfilaria cycle. A daytime body temperature < 35˚C caused the microfilaria count in the blood to decline according to body activity. Cats normally sleep during the day and are alert at nighttime; the periodicity of the microfilariae could be controlled by minor cyclical changes in the body temperature of the host. Hawking [16] revealed that host factors played an essential role in the removal of microfilariae, which was emphasized by the fact that DEC was not microfilaricidal (*in vitro*) without host factors, but induced a rapid decrease in microfilaria levels *in vivo*. The periodicity of microfilariae is also linked to the feeding habits of the vectors. Moreover, this study found that 7 from 10 infected cats had been living in houses that had reported microfilaria-infected cases. The other three cats were resting at nearby houses where non-infected cases were found. This study demonstrated the presence of *B. malayi* by PCR reactions in domestic cats. Although the number of subjects examined was very limited, variations in peaks were found among nocturnal subperiodic type in all cases [17]. Obviously, a nocturnal subperiodic type of *B. malayi* was present individually in five domestic cats in Tha Chana District, Surat Thani Province. These findings indicated that the mode of transmission is transient and restricted, considerable at nighttime and would be helpful for epidemiological study of the parasite reservoir. Therefore night-blood-screening campaigns and vector control are important links to consolidate filariasis control. In addition, personal protection from bites of vector mosquitoes is a practical way of preventing filariasis infection.

Acknowledgements

We gratefully acknowledge the help provided by Mr S Phantana, Office of Communicable Disease Control, Ministry of Public Health, and all staff at the Department of Biochemistry, Faculty of Medicine, Srinakarinviroj University, and Mr R Choavalit, Head of the Center for Vector-borne Disease Control 45, Surat Thani Province, Ministry of Public Health, Bangkok, Thailand.

References

1. Harinasuta C, Charoenlarp P, Sucharit S, Deesin T, Surathin K, Vutikes S. Studies on filariasis in Thailand. *Southeast Asian J Trop Med Public Health* 1970;1:20-39.
2. Harinasuta C, Sucharit S, Guptavanij P. Sub-periodic *Brugia malayi* in the South of Thailand. *Southeast Asian J Trop Med Public Health* 1970;1:162-3.
3. Guptavanij P, Harinasuta C, Sucharit S, Vutikes S. Studies on sub-periodic *Brugia malayi* in southern Thailand. *Southeast Asian J Trop Med Public Health* 1971;2:44-50.
4. Guptavanij P, Harinasuta C, Sucharit S, Vutikes S. Prevalence of sub-periodic *Brugia malayi* in areas near the Thai-Malaysian border. *Southeast Asian J Trop Med Public Health* 1971;2:100-1.
5. ศิริชัย พรรธนะ, สาลินี เซ็นเสถียร, สุรพงศ์ ชุมพล. การสำรวจพยาธิไมโครฟิลาเรียในแมวในจังหวัดนราธิวาส. *วารสารโรคติดต่อ* 2538;21:10-3.
6. Aoki Y, Nakajima Y, Kanamina D. Studies on Malayan filariasis in Che-ju Is, Korea. 3. Microfilarial surface architecture of *Brugia malayi* (Che-ju strain) in comparison with that of *Brugia pahangi*. *Jpn J Trop Med Hyg* 1976;4:129-37.
7. Redington BG, Montgomery CA, Jervis HR, Hockmeyer WT. Histochemical differentiation of the microfilariae of *Brugia pahangi* and sub-periodic *Brugia malayi*. *Am J Trop Med Parasitol* 1975;69:489-92.
8. Mak JW. Filariasis: epidemiology and control of filariasis. *Bull Inst Med Res Press* 1983;19: 83-94.
9. Sasa M. Human filariasis: analysis of the microfilarial periodicity. Tokyo: University of Tokyo Press;1976. pp. 594, 701-17, 819.
10. Aikat TK, Das M. A modified statistical method for analysis of periodicity of microfilariae. *Indian J Med Res* 1977;65:58-64.
11. Control of lymphatic filariasis: a manual for health personnel. Geneva: WHO; 1987.
12. Michelle RJ, taniavati S, Felix P, Steven AW. A polymerase chain reaction assay for the detection of *Brugia malayi* in blood. *Am J Trop Med Hyg* 1994;5:314-21.

13. Hawking F. The periodicity of microfilariae. Geneva: WHO; 1967.
14. Guptavanij P, Harinasuta C, Vutikes S, Deesin T. A trial on the transmission of periodic *Brugia malayi* from man to cats. *Southeast Asian J Trop Med Public Health* 1971;2:98-9.
15. บัณฑิต ชุนหสุวัสดิกุล, จิระพัฒน์ เกตุแก้ว, พิษณุวัฒน์ พานารถ. พื้นที่พรุกับการเกิดโรคและการแพร่ระบาดของโรคฟิลาเรีย. *วารสารโรคติดต่อ* 2532;15:23-7.
16. Hawking F. Diethylcarbamazine and new compounds for the treatment of filariasis. *Adv Pharmacol Chemother* 1979;16:129-94.
17. Purnomo PF. Periodicity studies of *Brugia malayi* in Indonesia: recent findings and a modified classification of the parasite. *Trans R Soc Trop Med Hyg* 1987;81:657-62.