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RESEARCH NOTE

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Identification of *Brugia malayi*-like Microfilariae in Naturally-infected Cats from Narathiwat Province, Southern Thailand

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Abstract

Brugia malayi-like microfilariae from 21 naturally infected cats were identified by microfilarial morphometry and acid phosphatase activity. The results revealed that the average innenkorper length of 28.56 ± 6.08 µm and intensely positive sites of acid phosphatase activity at the amphids, excretory vesicles, anal vesicles and phasmids were compatible with *B. malayi* microfilariae, thus emphasizing the important role of cats as a reservoir host of *B. malayi* in Narathiwat Province, southern Thailand.

Keywords: cats, *Brugia malayi*-like microfilariae, morphometry, acid phosphatase activity

Human filariasis caused by *Brugia malayi* is still a public health problem in many countries of Asia, especially India, Indonesia, Malaysia, The Philippines, Sri Lanka, and Thailand [1-2]. So far, at least two physiological types of *B. malayi* have been discerned in southern Thailand, *ie*, the nocturnally subperiodic and diurnally subperiodic types [3]. For the nocturnally subperiodic type, endemic areas are located in five provinces of southern Thailand, *ie*, Nakhon Si Thammarat, Phattalung, Pattani, Yala, and Narathiwat. These areas are rural and semi-forested, and *Mansonia uniformis* and *Ma. bonneae* are the primary vectors in open swamps and swamp-forest, respectively, while *Ma. dives*, *Ma. indiana*, *Ma. annulata* and *Ma. annulifera* are considered secondary vectors. For the diurnally subperiodic type, the endemic area is limited to Surat Thani Province, southern Thailand, and *Coquillettidia crassipes* is the important vector [3-4]. When comparing these six provinces, Narathiwat is the most highly endemic area, with approximately 50% of reported brugian filariasis cases. This may be the result of suitable mosquito breeding places or large areas of swamp, and the existence of animal reservoir hosts. Blood examination of both stray and domestic cats in Narathiwat Province revealed that 104 of 2,515 cats (4.13%) were positive for *Brugia* microfilariae [5]. However, no exact species identification of the parasites was carried out, except for one *B. malayi*-

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Table 1 Morphometric measurements and acid phosphatase activity of *B. malayi*-like microfilariae of 21 naturally-infected cats from Narathiwat Province, southern Thailand.

No. cats	Measurements*				Acid phosphatase activity at			
	No. microfilariae examined	Body length	Body width at nerve ring	Innenkorper length	Amphid	Excretory vesicle	Anal vesicle	Phasmid
1	20	169.70 ± 9.45 (144.43-183.32)	5.34 ± 0.68 (4.36-6.54)	25.27 ± 4.93 (11.11-33.33)	+	+	+	+
2	11	204.02 ± 11.68 (183.32-222.20)	5.30 ± 1.01 (3.82-7.63)	30.80 ± 7.18 (22.22-44.44)	+	+	+	+
3	13	207.24 ± 10.73 (188.87-222.20)	4.91 ± 0.70 (4.36-6.54)	23.50 ± 5.63 (16.66-33.33)	+	+	+	+
4	2	202.75 ± 11.79 (194.42-211.09)	4.91 ± 0.77 (4.36-5.45)	27.77 ± 7.86 (22.22-33.33)	+	+	+	+
5	20	196.92 ± 17.70 (172.20-233.31)	4.66 ± 0.62 (3.27-5.45)	30.55 ± 4.60 (22.22-38.88)	+	+	+	+
6	3	214.79 ± 12.83 (199.98-222.20)	4.72 ± 0.63 (4.36-5.45)	35.18 ± 6.41 (27.77-38.88)	+	+	+	+
7	5	211.09 ± 14.16 (194.42-227.75)	5.23 ± 0.49 (4.36-5.45)	31.10 ± 4.97 (27.77-38.88)	+	+	+	+
8	15	197.02 ± 11.85 (172.20-216.64)	5.27 ± 0.67 (4.36-6.54)	28.88 ± 4.30 (22.22-33.33)	+	+	+	+
9	20	195.81 ± 13.95 (172.20-216.64)	4.69 ± 0.57 (3.27-5.45)	25.27 ± 3.36 (22.22-33.33)	+	+	+	+
10	11	194.93 ± 9.44 (183.32-211.09)	4.66 ± 0.51 (3.82-5.45)	27.27 ± 3.89 (22.22-33.33)	+	+	+	+
11	13	207.04 ± 10.49 (183.32-216.64)	5.16 ± 0.61 (4.36-6.54)	31.62 ± 4.17 (27.77-38.88)	+	+	+	+

* Mean in X ± SD (μm), range in parenthesis.

\+ positive for acid phosphatase activity

Table 1 (con't)

No. cats	Measurements*				Acid phosphatase activity at			
	No. microfilariae examined	Body length	Body width at nerve ring	Innenkorper length	Amphid	Excretory vesicle	Anal vesicle	Phasmid
12	20	191.37 ± 10.27 (177.76-211.09)	4.33 ± 0.45 (3.27-4.91)	30.83 ± 4.93 (16.66-38.88)	+	+	+	+
13	20	175.54 ± 10.88 (155.54-194.42)	5.32 ± 0.63 (4.36-6.54)	27.77 ± 4.77 (16.66-38.88)	+	+	+	+
14	20	176.93 ± 11.58 (161.09-199.98)	6.30 ± 0.41 (5.45-7.09)	23.33 ± 4.26 (16.66-27.77)	+	+	+	+
15	20	193.04 ± 23.35 (144.43-227.75)	5.91 ± 0.71 (4.36-7.09)	24.72 ± 5.25 (16.66-38.88)	+	+	+	+
16	20	197.20 ± 11.04 (177.76-216.64)	6.16 ± 0.75 (4.36-7.63)	29.16 ± 6.95 (16.66-44.44)	+	+	+	+
17	20	208.03 ± 16.57 (177.76-249.98)	5.45 ± 0.75 (4.36-6.54)	34.16 ± 6.57 (22.22-44.44)	+	+	+	+
18	13	223.91 ± 17.18 (177.76-249.98)	5.87 ± 0.89 (4.36-7.63)	30.34 ± 5.83 (16.66-38.88)	+	+	+	+
19	14	209.90 ± 20.34 (166.65-227.75)	6.27 ± 0.63 (5.45-7.09)	27.38 ± 7.69 (11.11-38.88)	+	+	+	+
20	20	202.48 ± 12.15 (188.87-233.31)	5.53 ± 0.54 (4.36-6.54)	30.83 ± 4.93 (22.22-38.88)	+	+	+	+
21	13	185.88 ± 12.95 (166.65-211.09)	4.91 ± 0.55 (4.36-5.45)	29.48 ± 6.95 (16.66-44.44)	+	+	+	+
Total	313	195.91 ± 18.92 (144.43-249.98)	5.25 ± 0.85 (3.21-7.49)	28.56 ± 6.08 (11.11-44.44)	+	+	+	+

* Mean in X ± SD (μm), range in parenthesis.
+ positive for acid phosphatase activity

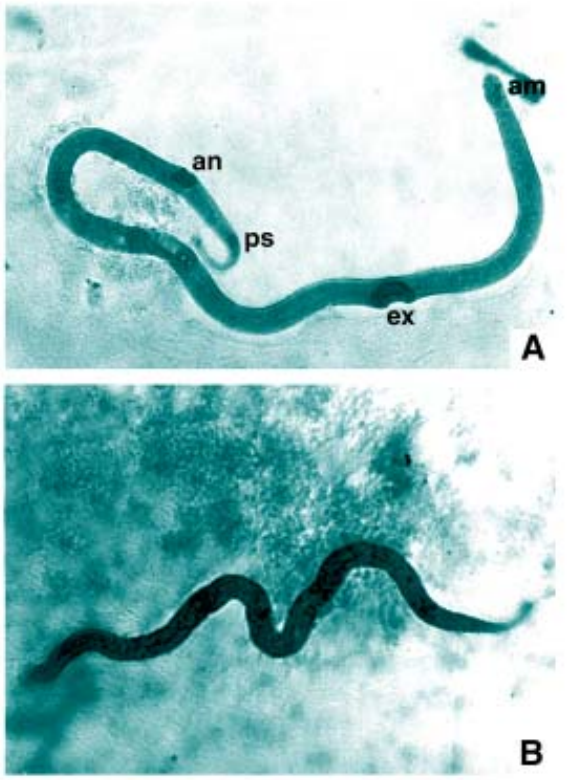

Fig 1 (A): *B. malayi*-like microfilaria from a cat (Narathiwat), showing acid phosphatase activity at the amphids (am), excretory (ex) and anal (an) vesicles, and phasmids (ps). (B): *B. pahangi* microfilaria from a cat (Bangkok), showing acid phosphatase activity along the entire body.

like microfilaria positive cat, which was confirmed to have *B. malayi* [6]. Recently, we carried out a survey of *B. malayi*-like microfilariae in cats from 13 villages comprising 3 districts in Narathiwat Province and found a high microfilaremic rate, *ie*, 8.51% (45/529). The crucial question is whether these *B. malayi*-like microfilariae are true *B. malayi* or *B. pahangi*, a common, natural parasite of cats [7]. This paper confirmed *B. malayi* in twenty-one naturally-infected cats from Narathiwat Province by means of microfilarial morphometry and acid phosphatase activity.

Twenty-one *B. malayi*–like microfilaria positive cats (from our current survey) were selected for investigation. The standard smear method [8] was used to prepare the microfilariae. The Giemsa-stained microfilariae were examined under a compound microscope and the dimensions of the body were assessed using a camera lucida drawing. For microfilarial acid phosphatase activity, the blood films were processed following the method of Barka and Anderson [9]. *B. pahangi* microfilariae obtained from naturally infected cats in Lat Krabang District, an area on the outskirts of eastern Bangkok, outside the endemic area for *B. malayi*, were used as the control.

Details of morphometric measurements and acid phosphatase activity are shown in Table 1. Acid phosphatase activity from *B. malayi*-like microfilaria and *B. pahangi* microfilaria, a control experiment, are illustrated in Fig 1.

Morphometric measurement of a total 313 microfilariae, comprising 21 infected cats revealed that the average and range of body length, body width at the nerve ring and innenkorper length were 195.91 ± 18.92 (144.33-249.98) µm, 5.25 ± 0.85 (3.21-7.49) µm

and 28.56 ± 6.08 (11.11-44.44) µm, respectively. The average innenkorper length of 28.56 µm was compatible to *B. malayi* microfilariae {*B. malayi* = 30.7 (24-34) µm, *B. pahangi* = 53.1 (44-63) µm [10]}. Nonetheless, an innenkorper length of ≥ 44 µm was also found in a total of 6 (1.92%, 6/313) microfilariae, but at very low rates, *ie*, 1/11 in cat No. 1, 1/20 in cat No. 16, 3/20 in cat No. 17, and 1/13 in cat No. 21. For acid phosphatase staining, all *B. malayi*-like microfilariae yielded intense sites at the amphids, excretory vesicles, anal vesicles, and phasmids, and very diffuse activity throughout the remainder of the body (Fig 1A). This was similar to the diagnostic enzyme pattern of *B. malayi*, while in *B. pahangi*, activity was found along the entire body (Fig 1B) [11]. Judging from the above results, it was concluded that the *B. malayi*-like microfilariae in the cats from Narathiwat Province, southern Thailand, were *B. malayi*. The high prevalence of *B. malayi* in the cats in our study emphasized the important role of this animal as natural reservoir hosts of *B. malayi*. Application of the control program in Narathiwat Province, using one-yearly, single-dose, 2-drug treatment [albendazole (400 mg, same dose for all ages) + DEC (6 mg/kg) or albendazole (400 mg) + ivermectin (200 µg/kg)] recommended by the WHO [2], cannot be performed in humans only. The search for suitable drug(s) for mass treatment of cats must be carried out simultaneously.

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