

THE OBSERVATION OF MICROFILARIAL RATE AND DENSITY IN CATS INOCULATED WITH INCREASING NUMBERS OF *BRUGIA PAHANGI* INFECTIVE LARVAE

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Abstract. Having close kinship to *Brugia malayi*, *B. pahangi* is a member of the family Filariidae, which causes lymphatic filariasis in dogs and cats. Although this nematode is unlikely to cause a zoonotic disease in humans, study of the *B. pahangi* life cycle may help control human filariasis. The objective of this study was to examine microfilarial rates and densities of *B. pahangi* in experimentally induced infections in cats as a relative measurement. Cats were infected with 3 different amounts of 3rd-stage larvae (L3); 100, 300 and 500. Cats infected with 100 L3 became patent for microfilariae longer than the other groups (mean₁₀₀ = 99±44 days). In comparison, the pre-patent period of *B. pahangi* was somewhat shorter in cats with 300 and 500 L3 infections (mean₃₀₀ = 76±13 and mean₅₀₀ = 63±5 days). The microfilarial densities of these cats were also determined; the density of microfilariae (mf/ml blood) increased relative to the duration of infection. One-way ANOVA tests were used to compare the microfilarial densities of the cats with varying numbers of L3. We found that the microfilarial density of cats with 500 L3 exhibited significant differences ($p < 0.05$) from cats with 300 and 100 L3. However, we concluded that the amount of microfilariae produced in the blood circulation of these cats were not increasing relative to the numbers of L3 taken by the host.

INTRODUCTION

The major causes of human lymphatic filariasis are *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. Although there has been one human case caused by *B. pahangi*, a number of animal species are more susceptible to this nematode. The microfilarial rate of *B. pahangi* in cats living near Bangkok was determined as 25.30% (Chungpivat and Sucharit, 1993) and the microfilarial rate in dogs was 4.17% (Nithiuthai and Chungpivat, 1992). The life cycle of *B. pahangi* is similar to *B. malayi* by mode of mosquito transmission, such as *Aedes* spp, *Armigeres* spp and *Mansonia* spp (Deesin *et al*, 1988). Delivered by mosquitoes, 3rd developmental stage larvae, L3, migrate via the lymphatic vessels to the lymph nodes where the adult worm develops and produces microfilariae. The pathogenesis of filariasis progresses when the lymphatic vessels are obstructed. Severity may increase when the host lymphatic system is invaded. With the aim of finding the biological distribution of *B. pahangi*, we examined the microfilarial rate and density of experimentally infected cats when administered varying numbers of L3. Both the rates and densities of

microfilariae were determined 24 weeks post-infection. Particular attention was given to relationship between L3 concentration and the pre-patent period of this nematode in cats.

MATERIALS AND METHODS

Parasites

Infective-stage larvae (L3) of *B. pahangi* were selected by mass dissection (Mak, 1983). Laboratory-reared *Aedes aegypti* (Liverpool strain) were fed on microfilaremic cats 2 weeks before dissection. Engorged mosquitoes were kept at -20°C for 5 minutes. They were then crushed frozen by roller technique and poured with cold PBS onto a finely meshed sieve to retain mosquito debris. The filtrate containing L3 was collected and washed in several changes of cold PBS to remove insect debris. The L3 were then transferred individually with a fine tipped dissecting needle to a glass cavity block containing 0.85% normal saline and checked for motility.

Animals

Eighteen healthy cats weighing 3-5 kg with neither intestinal parasitic disease nor blood parasites were used in these experiments. All cats were kept in the Parasitology Unit. They were arranged into 3 experimental groups (n=6), which were infected with varying numbers of L3 (100, 300 and 500) by subcutaneous inoculation in the lower medial aspect of the left hind limb. The cats were kept as experimental

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animals following the standard protocol accepted by the Laboratory Animal Ethics Committee of the Faculty of Veterinary Science, Chulalongkorn University.

Eight weeks post-infection, 60 µl blood smears were examined weekly from ear picks. Blood samples were stained with 3% Giemsa, determined for the presence of microfilariae and counted for microfilarial density.

RESULTS

The *B. pahangi* microfilarial rate was determined on week 24 by the number of cats with microfilariae in the blood circulation; the microfilarial rate of cats (n=6) in

the group receiving 500 L3 was 100%, with 88.3% in the groups of 300 and 100 L3 (Table 1).

Microfilarial density was also determined by the concentration of microfilariae in the bloodstream. The microfilarial density of cats receiving 500 L3 was as high as 8,083 mf/1ml blood (week 24, Fig 1). One-way ANOVA tests were used to compare the microfilarial density of cats with varying numbers of L3, and the microfilarial density of cats with 500 L3 exhibited significant differences (p < 0.05) from cats with 300 and 100 L3, whereas the microfilarial density of cats with 300 and 100 L3 were not significantly different (p > 0.05). However, the average densities of the microfilariae in the cats were not related to the amount

Table 1

Microfilarial rate, pre-patent period and microfilarial density of experimental cats infected with *B. pahangi* at 100, 300 and 500 L3.

Number of L3 (experimental cats)	100 L3 (n = 6)	300 L3 (n = 6)	500 L3 (n = 6)
Positive cats	5	5	6
Microfilarial rate (%)	88.3	88.3	100
Pre-patent period (days) (means±SD)	63 - 154 (99 ± 44)	63 - 98 (76±13)	56 - 70 (63 ± 5)
Microfilarial density (mf/1ml blood) (means±SD)	17 - 1,842 (501 ± 551)	6 - 1,314 (600 ± 475)	3 - 8,083 (3,678 ± 2,722)

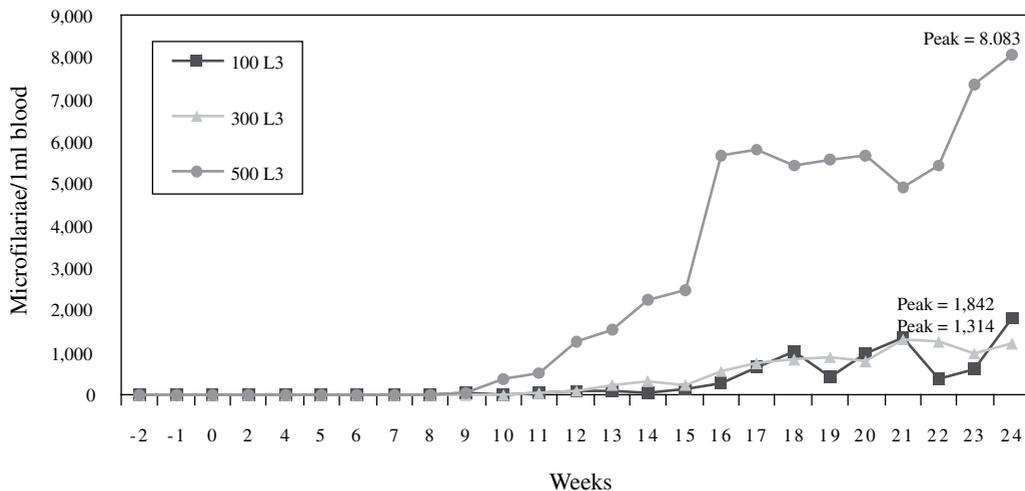


Fig 1- Microfilarial density of cats infected with groups that received 100, 300 and 500 L3 of *B. pahangi*.

of L3 received by the host.

The pre-patent course was determined. Cats that received 100 L3 became patent for microfilariae. The range of the pre-patent period in 6 cats infected with 100 L3 was 63-154 days (mean₁₀₀ = 99±44 days), while for cats receiving 300 and 500 L3 the periods were 63-98 days (mean₃₀₀ = 76±13 days) and 56-70 days (mean₅₀₀ = 63±5 days). In conclusion, the pre-patent period of *B. pahangi* was somehow shorter in cats with 300 L3 infection, and the shortest pre-patent period was seen for 500 L3 (Table 1).

DISCUSSION

Five-hundred L3 from mosquitoes gave the highest microfilarial rate in cats (Table 1), which is the result of the high level infectivity of *B. pahangi* found in the dissected larvae. This result agreed with Lawrence and Denham (1991), who reported a much higher rate of infection when the inoculated dose of L3 was raised substantially. When microfilarial density was determined, we found that the amount of microfilariae produced in these groups of animals was not related to the amount of L3 received (Fig 1). The sex ratio among the adult worms may play a crucial role, thus determining the number of microfilariae in the bloodstream. This was previously described (Wilson and Ramachandran, 1971). We premised that the 1:1 ratio between male and female worms should be corrected to produce the highest number of microfilariae.

The peak microfilarial density of cats infected with 500 L3 was reached faster than cats infected with 300 and 100 L3 (Fig 1 and Table 1). Similar results were indicated previously (Ewert and Singh, 1969; Wilson and Ramachandran, 1971; Denham *et al.*, 1972). The longer pre-patent period of *B. pahangi* in cats receiving 100 L3 is due to the slower developmental process of the adult worms compared with the cats receiving the higher dose of L3. However, this experiment revealed the biological distribution of *B. pahangi* in cats; the higher the number of L3 received, the shorter the

patent period detected.

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