

## The Occurrence of Microfilaria and the Response of Microfilaria and Gut Nematodes to Ivermectin Therapy in Myanmar Timber Elephants

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### Introduction

Filariasis is a parasitic nematode infestation characterized by the presence of microfilaria, an embryonic stage between the eggs and the larvae (Haleem *et al.* 2002). They occur in all orders of vertebrates and are obviously of considerable antiquity, but have no close relations among the other parasitic nematodes. Little is known about their evolutionary history. They live within the body tissues of host animals. The microfilariae are removed from the body by blood-sucking insects. In some species there is a peculiar and as yet unexplained periodicity of their appearance in the peripheral blood-vessels of the host. In some cases, they appear during the hour of darkness, in others during daylight (Cameron 1965). The appearance of microfilaria is thought to be influenced by environmental conditions such as sunlight, temperature and humidity (Oishi 1975).

Filariasis denotes the presence of microfilaria in the blood and tissue of the host. Filarial parasites of African elephants are; *Dipetalonema loxodontis*, *Dipetalonema asiatica*, and *Stephanofilaria* sp. the latter two being also found in Asian elephants. Microfilaria vary from 0.137-0.255 mm, and 0.007-0.011 mm in diameter. Microfilaria are concentrated in the blood oozing from nodules that form in the skin of infected elephants. Presumably blood-sucking insects transmit the parasite in elephants also. The life cycle in the insect is unknown (Fowler and Mikota 2006). Filariasis causes a chronic progressive dermatitis in elephants and is prevalent among Myanmar timber elephants. In Myanmar, filarial infestation has been found in about 50-55% of Myanmar Timber Enterprise owned elephants before 1986. Mortality is negligible, but there is significant economic loss from decreased working capacity.

The objectives of this study were to investigate the occurrence, density, and periodicity of microfilaria, and the effectiveness of ivermectin in treating microfilaria infection and in gut nematodes of elephants.

### Materials and Methods

Sixty elephants owned by Myanmar Timber Enterprise with ages ranging from 9 to 54 years were used in the study.

We employed two methods for examination of blood samples for microfilaria:

1. Direct examination of microfilaria from wet blood films: Blood samples were dropped directly onto glass slides, a wet-smear made, and the slide searched for microfilaria under the microscope at 100x magnification.
2. Examination by staining: A sample of venous blood was collected and was processed promptly, not allowing it to clot. One milliliter of whole blood was placed in 10 ml, 2% formalin and was mixed gently by inverting the closed tube twice and centrifuging five to eight minutes at 1500 rpm. The supernatant fluid was discarded by careful inversion of tube and the sediment stained with an equal amount of 1:1000 methylene blue. The mixture was pipetted onto two glass microscope slides; a cover slip placed, and examined at 100X. Blue stained, elongated microfilariae were readily visible, and length and width was measured using a micrometer (Embert 1967).

### Experimental design

Sixty elephants from Pinyinmana North and Pinyinmana South Timber Extraction agency were tested for microfilaria. From those found to be infected with microfilaria, ten were selected

randomly and divided into two groups of  $\square$  ve:  $I_1$  and  $I_2$ ; Five elephants were randomly selected from the elephants not detected as being infected with micro $\square$  laria, which formed a control group - C. Group  $I_1$  was treated with ivermectin at day 0 and group  $I_2$  was treated at day 0 and day 15. The group C did not receive treatment.

#### *Ivermectin treatment*

Ivermectin 10mg/ml (1% w/v) was administered subcutaneously to elephants naturally infected with micro $\square$  laria in a dosage of 1 ml/100 kg of body weight. The body weight of treated elephants was assessed by using the formula described by Hile *et al.* (1997):

$$\text{Body weight [kg]} = 18.00 \times \text{HG [cm]} - 3336$$

(HG = heart girth)

#### *Testing effectiveness of treatment*

Blood samples were collected from the 15 experimental animals on days 0, 15, 30, 60, 90 and 120 to determine micro $\square$  laria titers and to assess blood parameters; Packed Cell Volume (PCV), Haemoglobin (Hb), Total Protein (TP), erythrocytes (RBC) and leukocytes count (WBC), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC). Four or 5 ml of blood were collected from the ear vein of each elephant by using a 5 ml disposable syringe and 18 gauge needle and put into labeled bottles containing 1 mg EDTA powder per 1 ml of blood (Chakrabarti 2002).

#### *Counting of micro $\square$ laria*

With the help of a haemoglobinometer pipette 20 mm<sup>3</sup> of blood was placed on a clean glass slide, dried as a thick  $\square$  lm, dehaemoglobinised and stained in the usual manner. The total number of micro $\square$  laria in the thick smear multiplied by 50 was taken as the number per milliliter of blood (Chatterjee 1976).

#### *Quantitative examination of faeces Modified McMaster technique*

Twenty-eight ml of  $\square$  otation  $\square$  uid (saturated common salt- NaCl, solution) was added to 2 g

of feces. The feces and  $\square$  uid were mixed until all lumps were broken down and the mixture sieved with a coarse sieve (tea strainer). The sieve containing coarser material was then removed leaving the  $\square$  otation  $\square$  uid and smaller fecal material. The mixture was then thoroughly stirred and each chamber of a McMaster counting-slide  $\square$  lled using a pipette. The counting-slide was covered with a coverslip and left aside.

After 5-8 minutes, parasite eggs rise in the solution and come to lie against the under surface of the glass coverslip covering the top of the counting chamber. The slide was then examined under a microscope using 100x magni $\square$  cation. The total number of eggs were counted and multiplied by 50 to give a count of eggs per gram of faeces (Pomroy 2000). Faecal egg counts of all 15 elephants were done at days 0, 15, 30, 60, 90 and 120.

#### *Statistical analysis*

The data were analyzed by one-way analysis of variance (ANOVA) and differences between means were tested for signi $\square$  cance by Tukey test using SPSS version 11.00 (Coakes & Steed 2003).

## **Results**

#### *Morphology of micro $\square$ laria*

The length of the micro $\square$  laria observed was  $182.00 \pm 37.96 \mu\text{m}$ , tail length was  $17.92 \pm 4.13 \mu\text{m}$  and width  $5.12 \pm 1.28 \mu\text{m}$ . The head of the micro $\square$  laria were prolonged and the tail was curved and pointed. Nuclei were seen in the body of micro $\square$  laria (Figs. 1-3).

#### *Examination of micro $\square$ lariae*

Micro $\square$  laria were detected in 46.6% (n=28) of the 60 elephants screened in daytime, 51.66% (n=31) of those screened in early-night and 53.33% (n=32) of those screened at midnight. There was no signi $\square$  cant difference in the occurrence during early night and midnight but a signi $\square$  cant difference between day and night time.

## Response to treatment

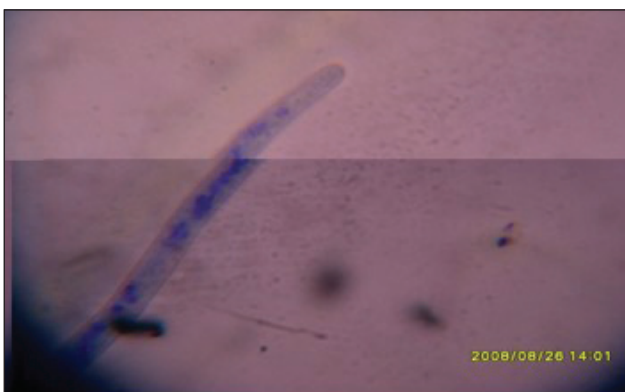
The density of microfilariae in groups I<sub>1</sub> and I<sub>2</sub> were 152 and 184 per ml of blood before the treatment period at day 0. Group C remained free of microfilariae infestations on days 0-90. After treatment, microfilariae disappeared in both groups I<sub>1</sub> and I<sub>2</sub> by day 15 and were not detected at days 30 and 60. On day 90, microfilariae were observed in I<sub>1</sub> group and on day 120 in the I<sub>1</sub>, I<sub>2</sub> and C groups. On day 120, the densities of



**Figure 1.** Microfilaria in elephant blood.



**Figure 2.** Tail of microfilaria: methylene blue stain.



**Figure 3.** Head of microfilaria: methylene blue stain.

microfilariae were 52 mf/ml of blood in I<sub>1</sub>, and 12 mf/ml of blood in I<sub>2</sub> and C.

## Faecal worm egg counts

The mean±SE of epg for the 3 groups on day 0 was 1256±123.7. On day 15, 30, 60, 90 and 120 were 684±283.67, 370±122.23, 95±35.41, 70±31.22 and 330±123.76 respectively. The mean value of faecal worm egg counts (epg) between group I<sub>1</sub> and group C were not significant, but the mean values of epg in group C were significantly ( $p<0.05$ ) higher than that of group I<sub>2</sub> at day 0. At day 15, group C was significantly ( $p<0.05$ ) higher than that of mean (epg) of group I<sub>1</sub>. From day 30 to day 120, mean values of (epg) in C group were significantly ( $p<0.05$ ) higher than that of groups I<sub>1</sub> and I<sub>2</sub> (Figs. 4 & 5).

## Discussion

The occurrence of microfilaria using the direct wet blood film and staining methods, showed that more than half the elephants were naturally infected with microfilaria. This result is consistent with what has been observed previously for MTE elephants.

The appearance of microfilaria in the peripheral blood of elephants was observed to be somewhat higher nocturnally in this study, consistent with a daily periodicity. Rhee, Yang and Kim (1998) from a study of species other than elephants in Korea, stated that periodicity of microfilaria depended on geographic location. They also stated that the maximal counts of microfilaria were found at 21:00 hrs and minimal counts at 11:00 hrs. Euzéby & Laine (1951) postulated that the lowest counts of microfilaria occurred at 08:00 hrs and the highest counts at 20:00 hrs in France. Webber & Hawking (1995) stated that the

**Table 1.** Observation of microfilarial infection in 60 elephants.

Observation period	Microfilariae status			
	Mf (+)		Mf (-)	
	m*	f*	m*	f*
daytime (12 - 14)	14	14	8	24
early night (20 - 22)	13	18	9	20
midnight (00 - 02)	14	18	8	20

\* m = male, f = female

**Table 2.** Haematological profile of the experimental groups.

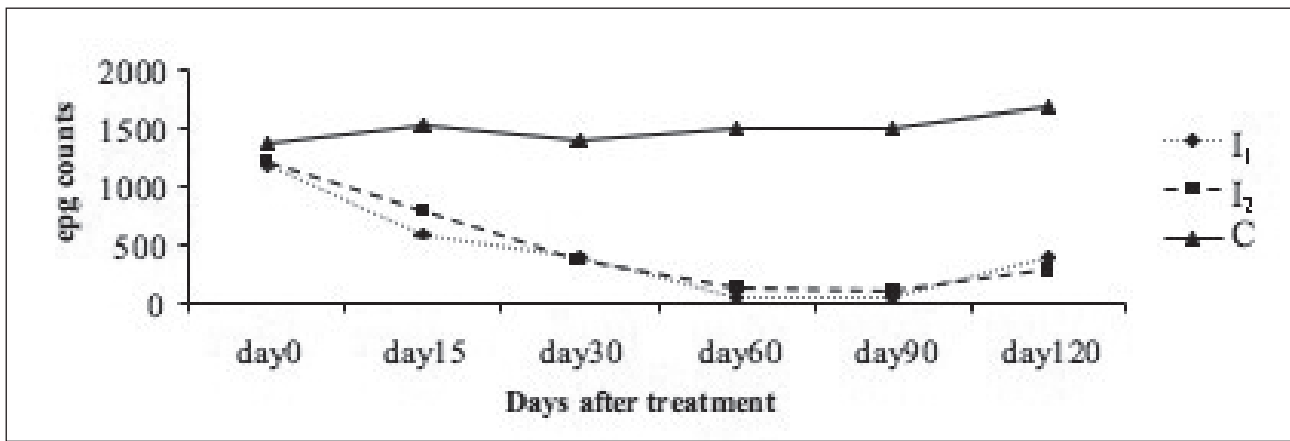
Parameter	Observed values										*Normal values			
	Groups I <sub>1</sub> and I <sub>2</sub> (n=10)					Group C (n=5)								
	Day #	0	15	30	60	90	120	0	15	30		60	90	120
Total RBC counts (x10 <sup>12</sup> /l)		2.3	2.3	2.6	2.9	3.0	3.3	2.3	2.7	3.1	2.6	3.4	3.2	2.5-5.0
Total leukocyte counts (x10 <sup>9</sup> /l)		14.4	12.4	5.5	5.7	8.3	10.5	12.7	8.1	8.5	7.1	15.8	17.5	10-18
PCV (%)		34.0	34.5	27.5	28.5	33.9	27.2	33.0	28.0	26.4	29.8	30.4	28.0	30-40
Hb (g %)		10.6	10.0	11.4	12.1	13.9	14.5	13.2	9.2	9.3	11.2	12.8	13.9	12-14
Total plasma protein (g/dl)		7.3	7.3	6.5	6.2	7.0	5.9	7.5	7.1	6.6	6.2	7.5	6.6	6-12
MCV (fl)		174.6	159.6	108.5	101.0	101.0	114.8	148.1	105.8	91.4	124.1	124.6	89.9	80-160
MCHC (g/dl)		34.2	33.9	41.7	43.2	43.2	43.5	36.7	32.3	44.7	38.1	38.1	42.4	25-40
Lymphocytes (%)		55.4	46.9	49.2	51.8	51.0	42.5	58.4	53.2	48.6	49.0	54.5	39.4	40-60
Neutrophils (%)		34.8	30.2	33.1	29.0	35.8	36.6	29.8	34.2	29.4	34.4	35.0	36.6	22-50
Eosinophils (%)		5.6	11.3	8.6	5.4	4.2	6.1	6.0	6.6	4.4	6.8	4.6	7.2	6-15
Monocytes (%)		3.2	11.8	12.1	12.3	9.0	15.7	5.0	7.0	17.6	9.8	7.8	16.8	1-3

\*Source: K.K. Sarma (2004)

minimum parasitemia occurred at 06:00 hrs and the maximum at 18:00 hrs in China. Therefore the results from previous studies are consistent with our finding that the occurrence of microfilaria was higher in early night and midnight than in daytime.

The dosage of ivermectin used by us was half the usually recommended dosage. The results of this study showed that all microfilariae were cleared off in the peripheral blood of experimental animals after treatment at day 15. Thereafter, microfilariae infestation was not observed in the treated groups until day 90. On day 120, both treatment groups were re-infected with microfilaria. Thong Nyunt *et al.* (1987) described that all treated elephants were free from microfilaria at the 12<sup>th</sup> day after treatment and that reinfection occurred eight months after treatment. So, the results of Thong Nyunt *et al.* (1987) did not agree with the findings of the present study. Gaysorn *et al.* (2005) reported that after a single oral dosage of ivermectin (200 µg/kg) and diethylcarbamazine, rapid suppression of microfilaria was achieved within 30 days. The authors also stated that ivermectin is effective in clearing microfilaria within 30-60 minutes, but cannot eliminate adult worms, and therefore, microfilaria can recur within 1-2 months. The haematological values did not show any clear differences between the treated and untreated groups during the period of examination.

Mean values of epg significantly ( $p < 0.05$ ) decreased after injection of ivermectin in the treatment groups (Fig. 4). Burkholder *et al.* (2004) reported that ivermectin, a broad spectrum endo- and ectoparasitic antihelminthic, is recommended as a preventive for meningeal worm in cattle at a dosage of 200 µg/kg subcutaneously administered once in 3 weeks. Soulsby (1968) stated that in equines, 500 epg was observed in mild infection, 800-1000 epg in moderate infection and 1500-2000 epg in severe infection. The gastrointestinal tract of elephants is similar to that of horses. Therefore, using the values given by Soulsby (1968) in the present study, the mean values of epg were severe at the start of the study and then decreased to moderate and mild infection with ivermectin treatment.



**Figure 4.** Faecal worm egg counts of experimental elephants.

## Conclusions

According to the results obtained from this study, it can be concluded that;

1. The diagnosis of microfilariae can be performed anytime of the day.
2. A single dose of ivermectin (1%) administered subcutaneously, not only reduced the density of microfilariae in elephant's peripheral blood but also significantly ( $p < 0.05$ ) decreased gastrointestinal nematodes.
3. A single dose of ivermectin (1%) in half recommended dose is effective for the clearance of microfilaria for up to 3 months. Therefore the drug should be administered four times per year to elephants.

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**Figure 5.** Strongyloides egg in elephant faeces.

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Burmese veterinarian doing elephant inspection in a camp  
Photo by Peter Leimgruber