Short Communication

Diurnal variation in microfilaremia in cats experimentally infected with larvae of *Dirofilaria immitis*

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Abstract

In five healthy mongrel female cats used, four cats (Cats 1- 4) were experimentally inoculated with 100-123 larvae (L_3) of *Dirofilaria immitis*. Cat 5 was uninfected control. Only Cat 1 became microfilaremic on Day 201 after inoculation and the diurnal changes in microfilaria population were monitored every two hours for 24 hr on Day 237 when a sufficient number of microfilaria were detected in the circulation. The maximum number of microfilaria in the blood (1,350/ml) occurred at 9:00 PM and then gradually decreased to the minimum of

300/ml at 7:00 AM , indicating that the number of microfilaria shows a nocturnal sub-periodic pattern of diurnal rhythm even in peripheral blood of cats as an abnormal host. In postmortem examination, 10 live adult worms (3 males and 7 females) in Cat 1 and one live adult worm (1 male) in Cat 2 were detected.
Key word: cat*, Dirofilaria immitis*, microfilaria, periodicity

1. Introduction

The cat is regarded as an aberrant or abnormal host of canine heartworm, *Dirofilaria immitis,* with low infection susceptibility. However, the number of infected cats has been increasing (Rawlings, 1986; Roncalli et al., 1998). In this report, we describe successful experimental infection of cats with *D. immitis* and discuss microfilarial periodicity.

2. Materials and Methods

Five healthy mongrel cats, with clinically assumed age of 1 to 5 years were used (Table 1) and verified to be free of intestinal parasites by repeated fecal examination. Specifically, the cats were free of adult *D. immitis* infection as confirmed by Knott test for microfilaremia, physiological examination, radiological examination, ultrasonography, and antibody-based enzyme-linked immunosorbent assay (ELISA) conducted routinely in our laboratory. Four cats (Cats 1- 4, Table 1) were experimentally inoculated with 105, 100, 123 and 104 larvae (L_3), respectively, using the procedure described previously (Hayasaki, 1982). Cat 5 was an uninfected control. Cats were housed in a standard laboratory animal room with a large window under natural daylight conditions. The number of microfilaria in the peripheral blood was counted by Knott test and blood smear method (20 μl of blood) from Day 169 after inoculation throughout the experimental period.

3. Results and Discussion

For Cat 1, microfilaria were first detected in the circulation on Day 201 (1995 March 13) after inoculation, which approximately corresponds to the prepatent period in a previous report of infected dogs as a normal host (180 to 200 days) (Hayasaki, 1982). The number of microfilaria increased gradually to 708 microfilaria/ml on Day 243 after inoculation, although their number fluctuated during this period (Fig. 1A). In order to monitor the diurnal changes in the microfilaria population, we conducted microfilaria counts in Cat 1 every two hours

for 24 hr on Day 237 (1995 April 18) after inoculation, when a sufficient number of microfilaria were detected in the circulation (714 microfilaria/ml). The maximum number of microfilaria in the blood (1,350/ml) occurred at 9:00 PM and then gradually decreased to the minimum of 300/ml at 7:00 AM (Fig. 1B). The ratio between the maximum and minimum number was 4.5 (Table 2) which was similar to that observed in dogs (Ohishi, 1986). In dogs, the highest number of microfilaria was noted at 10:00 PM and the lowest at 10:00 AM. On the other hand, the peak number of circulating microfilarial in cats experimentally infected by transplanting juvenile *D. immitis* through a large cervical vein occurred at 10:00 PM but the lowest was recorded at 2:00 PM (Nogami et al., 2000). It is likely that in the study by Nogami et al. (2000) biological and immunological stimulation affecting the host during the penetration of larval worms of various host organs over a period of three to four months did not occur due to the artificial method of inoculation. Therefore, diurnal changes in microfilaria should be evaluated under the natural biological condition by *D. immitis* L_3 infection. There are few studies in which the diurnal pattern of microfilaria of *D. immitis* was monitored in the circulation of cats inoculated with *D. immitis* L_3 (to mimic natural infection). Although a low peak of diurnal pattern of microfilaria count was observed in our study at 1:00 PM, this is non-specific or simply represents biological fluctuation based on our research experience, because such low peaks were often encountered when counting the number of circulating microfilaria within short intervals, e.g., 30 min, in dogs infected with *D. immitis*.

We also examined the level of specific IgG antibody, as shown in Fig. 2. ELISA, using a crude antigen (protein concentration, 10 μg/ml) extracted from *D. immitis* by phosphate buffered saline (PBS, pH 7.2, 0.1 M), serum samples diluted 1/400 and peroxidase-conjugated goat IgG fraction to cat IgG whole molecule (Cappel Lab., Inc. Malvern, PA) diluted 1/800, showed persistently high levels of the antibody in Cats 1 and 2, until the end of the experiment (although only one worm was detected in Cat 2, see below). In contrast, in Cats 3 and 4, in which no adult worms were detected, the antibody levels were high only during the early phase of infection, indicating that migrating larvae may be dead after this period. Cat 5 as the negative control showed no increase in antibody throughout the experimental period.

For recovery of adult worms, the cats were sacrificed by pentobarbital sodium on Day 243 (Cats 1 and 3) or on Day 245 (Cats 2, 4 and 5). Postmortem examination of Cat 1 revealed the presence of 10 adult worms (3 males and 7 females) in the right ventricle and pulmonary arteries and 2 fragmented worms at peripheral parts of the pulmonary arteries [recovery rate, 11.4 % (12 worms)] (Table 1). Similar examination of Cat 2 showed 1 male worm in the right ventricle and pulmonary arteries and one fragmented worm in the peripheral pulmonary arteries [recovery rate, 2% (2 worms)]. The average lengths of adult males and females in Cat 1 were 15.4±1.6 cm and 22.3±2.7 cm, respectively. In comparison, the lengths of adult worms recovered from infected male and female dogs reported previously by Hayasaki (1982) were 17.2±1.6 cm and 27.6±3.2 cm, respectively.

These results indicate near-normal growth of worms detected in Cat 1. As shown in Table 1, no worms were present in Cats 3 and 4, and of course Cat 5.

In conclusion, we have demonstrated in the present study that, when *D. immitis* could attain near-normal growth to sexual maturation in cats, the number of microfilaria also shows a nocturnal sub-periodic pattern of diurnal rhythm as a specific biological phenomenon of microfilaria, even in peripheral blood of cats as an abnormal host. Such diurnal change may be depend on mainly factor(s) endogenous of microfilariae including phototaxis-like behavior, rather than on the host immune response. Further study is needed to test this hypothesis.

References

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Table 1. Experimental infection of *Dirofilaria immitis* in cats and dogs.

	Cat 1	Cat 2	Cat 3	Cat 4	Cat 5
Breed	Mo	Mo	Mo	Mo	Mo
Sex	F	F	F	F	F
Body weight (kg)	2.9	2.8	3.1	2.5	2.7
Assumed age (yrs)	5	5	2	1	3
No. of inoculated L_3	105	100	123	104	-
Duration of infection at necropsy (day)	243	245	243	245	-
Total worms recovered (M/F)	12 (3/7)	2 (1/0)	0	0	-
Recovery rate (%)	11.4	2	0	0	-
Worm length (cm)	M: 17.5,15.4,13.5 F: 26.5, 24.5, 24.0, 23.0, 21.5, 20.5, 18.0 2 dead in PA	M: 10.5 1 dead in PA	-	-	-

PA: Pulmonary arteries, Mo: Mongrel, M: male, F: Female

Table 2. Microfilarial counts during 24 h in Cat 1.

Time	Number of mf/ml in blood	Ratio (%)[a]
AM 7:00	300	42.1
9:00	350	49.1
11:00	500	70.1
PM 1:00	900	126.3
3:00	600	84.2
5:00	600	84.2
7:00	1200	168.4
9:00	1350	189.4
11:00	750	105.2
AM 1:00	850	119.2
3:00	600	84.2
5:00	550	77.1

[a]: Data are percentages of mean microfilarial counts (712.5)

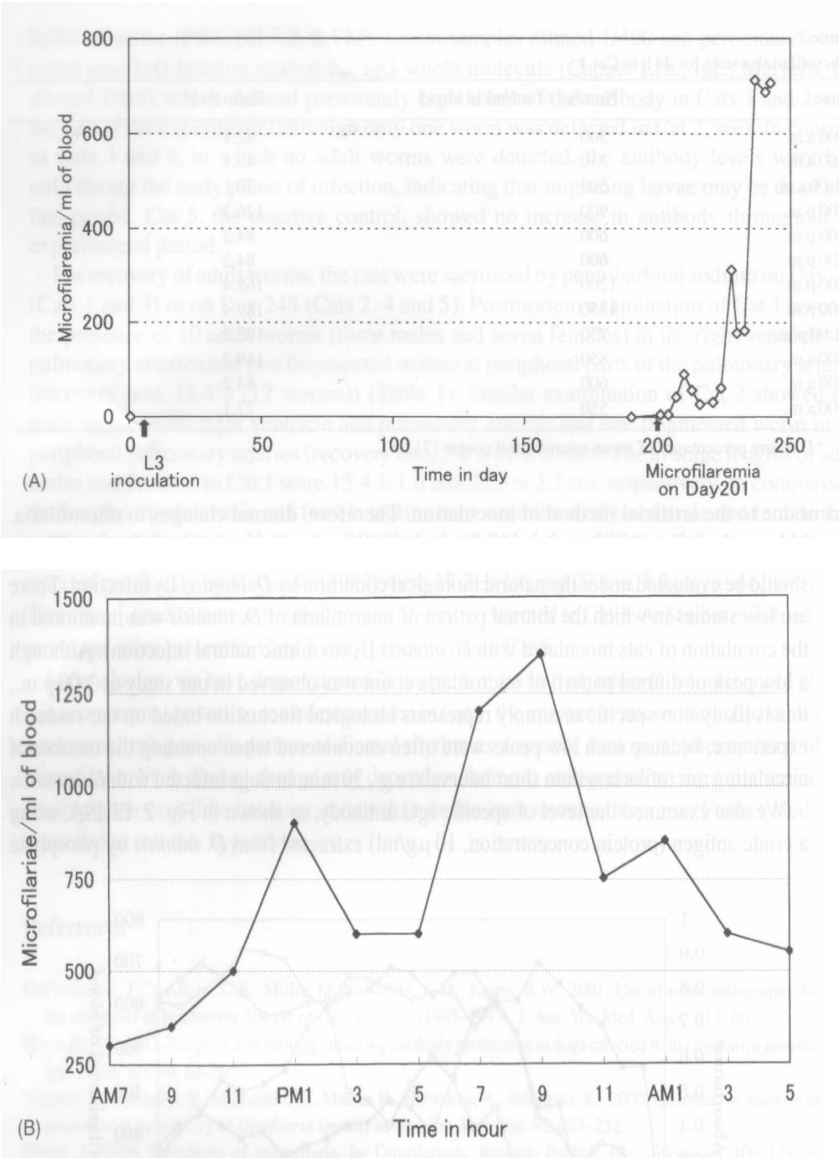

Figure 1. A: Serial changes in the number of microfilaria of *D. immitis* in the peripheral circulation of Cat 1. B: Diurnal changes in microfilarial counts of *D. immitis* in Cat 1.

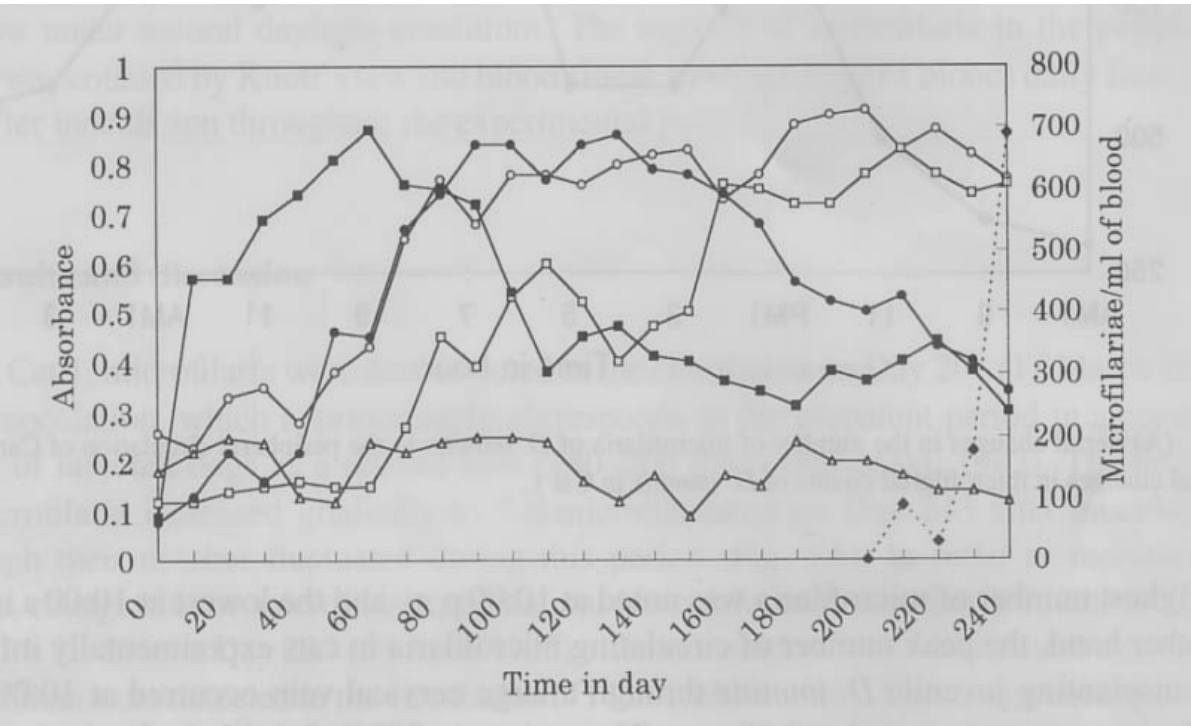

Figure 2. Anti-*D. immitis* antibody production in four infected cats (Cats 1-4) and control cat (Cat 5) as detected by ELISA, and microfilaria counts (Cat 1). Cat 1 (○), Cat 2 (□), Cat 3 (●),Cat 4 (■) and Cat 5 (△). Microfilaria counts (◆).