

CHAPTER III

RESULTS

Prevalence study

To find the prevalence of dirofilariosis among dogs in Small Animal Hospital, 589 blood samples were examined, of the subjects, 287(48.73 %) were from male dogs and 302(51.27%) were from females. Microfilaria was detected in 107 of the 589 buffy coat smears, resulting in a total prevalence of 18.2%. Of the 107 infected dogs, 54 were males and 53 were females. The difference in prevalence between male and female dogs was not statistically significant (Chi-square test, $p=0.634$). One hundred and thirty-three of all dogs were housed indoors and 456 were housed outdoors, the difference of the infection rates (9.9% in indoor and 20.83% in outdoor dogs) was significant (Chi-square test, $p=0.002$). The 109 dogs having participated in a heartworm prevention program also had a significantly lower prevalence (5.50%) than the 480 dogs, which never had received anti-heartworm drugs (21.04%) (Chi-square test, $p=0.000$). (Table4)

The youngest group had the lowest infection rate. The age-specific prevalence of 6.4% of dogs younger than 2 years of age was significantly lower than of older dogs (Chi-square test, $p = 0.00$). The monthly prevalence of dirofilariosis for the study period of 14 months is illustrated in Figure4. As can be seen, highest prevalence was detected from February (32.4%) to April 2001 (42.9%). The prevalence ratio (PR) between summer and rainy, summer and winter were $31.03/13.76=2.3$ and $31.03/13.93= 2.2$ respectively.

Table2: Numbers of dirofilariosis positive and negative dogs in categories of heartworm prevention, sex and housing

Capillary tube test	HW prevention program		Sex		Housing		Total
	Yes	No	Male	Female	Indoor	Outdoor	
Positive	6	101	54	53	12	95	107
Negative	103	379	233	249	121	361	482
Total	109	480	287	302	133	456	589

Table3: Result in crosstabulation between heartworm prevention program and rearing condition

HW Prevention program	Rearing		Total
	Indoor	Outdoor	
Yes	36	73	109
No	97	383	480
Total	133	456	589

Table4: Results of Pearson Chi-square between results in capillary haematocrit test and the factors

Test	Chi-square test		
	Value	df	Asymp.Sig(2side)
Results in capillary haematocrit test and heartworm prevention program (yes/no)	14.702	1	0.000
Results in capillary haematocrit test and sex (male/female)	0.227	1	0.634
Results in capillary haematocrit test and housing (indoor/outdoor)	9.662	1	0.002

Table5: Prevalence of dirofilariasis according to age groups of dogs

Age (Month)	Results		Total (%)
	No. Positive (%)	No. Negative (%)	
0-24	19(6.44)	276(93.56)	295(100)
24-48	20(20.41)	78(79.59)	98(100)
48-72	30(33.33)	60(66.67)	90(100)
72-96	8(32.00)	17(68.00)	25(100)
96-120	8(28.57)	20(71.43)	28(100)
>120	22(22.00)	31(78.00)	53(100)
Total	107(18.17)	482(81.83)	589(100)

Table6: Prevalence of dirofilariosis according to seasons (May, 2000-
May2001)

Season	Results		Total (%)
	Positive (%)	Negative (%)	
Summer	18 (31.03)	40 (68.97)	58 (100)
Rainy	26 (13.76)	163 (86.24)	189 (100)
Winter	28 (13.93)	173 (86.07)	201 (100)
Total	72 (19.15)	376 (80.85)	448 (100)

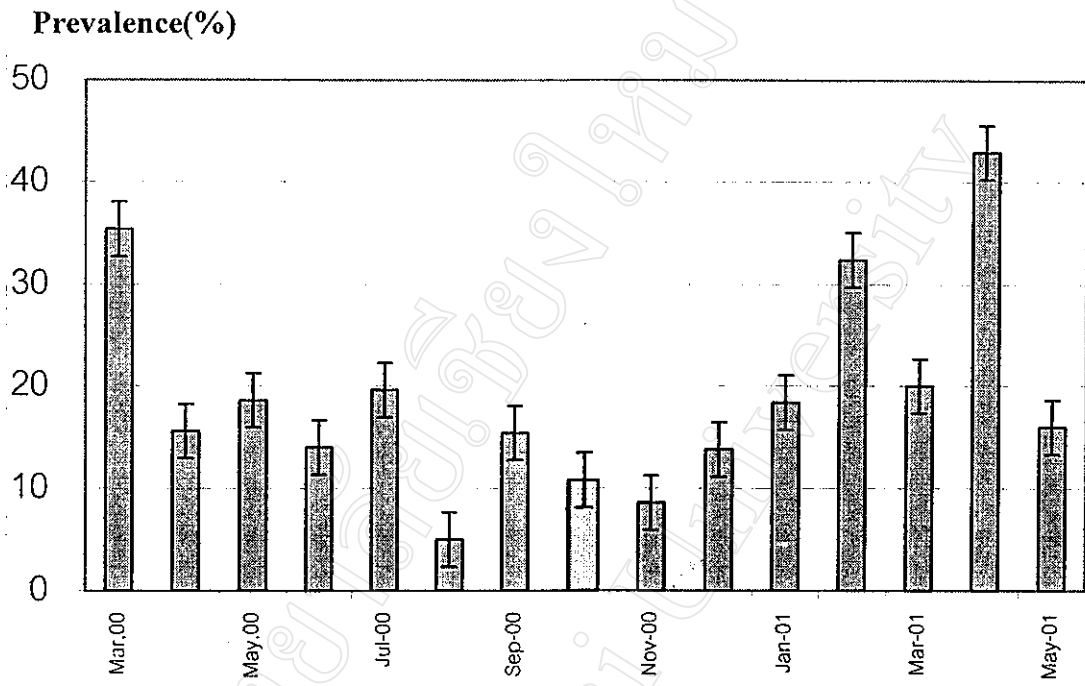


Figure4: Monthly prevalence of dirofilariosis in dogs, March 2000 – May 2001.

Incidence study

The study was started in August, 2000. There were 36 subjects in this study, 20 subjects were male (55.56%), and were females 16(44.44%). The youngest animal was 5 months and the oldest was 24 month (mean 10.64 month) at the beginning of the study. All of the subject were housing outdoor and negative for heartworm antigen at the beginning of the study. The results of the test showed that 22 of 36 were infected, 6 were positive in May 2001 (27.27 %), 2 were positive in June 2001 (9.09%) one each were positive in August 2001 (4.55%), September 2001 (4.55%), October 2001 (4.55%), November 2001 (4.55%), January 2002 (4.55%), February 2002 (4.55%), March 2002 (4.55%) and 7 were positive in April 2002 (31.8%)(Figure5). All infections were caused by *D. immitis* as diagnosed by specific movement of the microfilaria on the slide. Among the infected dogs, the youngest was 12 month old, and the oldest was 29 month old (mean 19.45 month). Twelve infected dogs were male (54.5 %) and 10 were female (45.5%). Table 8 shows the test results of each animal in the incidence study. All of the infected dogs did not show any sign of heartworm infection at the time. Among the infected dogs, the result of test show that 15 cases are positive with serological technique and negative with haematocrit tube technique (68.18%), 3 cases positive with haematocrit tube technique and serological technique at the same time (13.64%) and 4 cases positive with haematocrit tube technique but negative with serological technique (18.18%).

Table 8 shows risk time of each animal categorized to each season during the year 2000 –2001. The grand total of animal-months at risk calculated from the result of monitoring the infection status during the study period are 507 animal-months.

Deviding the 507 risk time with 22 newly infected cases found in the study results in incidence density rate of canine dirofilariasis in the Chiangmai province around the value of 0.043 animals per animal-month; or 4.3 animals per 100 animal-months.

The incidence density of each season are summarized in Table 9 .The highest rate is in rainy season and then decreases sharply in winter and then lowest in summer seasons. The values are 0.069, 0.035 and 0.027 animals per animal-month. The relative risk affected by seasons in which the animals exposed to is demonstrated by the calculation of Incidence Rate Ratio. The ratio of the rate in rainy by winter, rainy by summer and winter by summer are 1.99, 2.57 and 1.30 times respectively. The calculation and its results are summarized in Table 10.

Blood picture of D. immitis infected dogs compared with non-infected dogs

The results of haematology showed that the mean of pack cell volume among male dogs was 43.95%(34-52, SD=5.21), female dogs were 43.31%(30-57, SD=6.96) and mean of the group was 43.67%(30-57, SD=5.97). The comparative haematological value between infected groups and non-infected group is shown in table11. The mean pack cell volume of the infected group was 43.55%(30-57) and non-infected group was 43.86(38-52). The number of white blood cell count of the infected group was 16542.77 cells/mm³ (10010-27690, SD=4255.57) and non-infected group was 16393.93 cells/mm³ (10270-31200, SD=5806.29). The number of neutrophil of the infected group was 7816.23 cells/mm³ (4141-13291, SD=2291.31) and non-infected group was 8656 cells/mm³ (4724-19344, SD=4069.38). The number of lymphocyte of the infected group was 4396.05 cells/mm³ (1841-8861, SD=1764.81) and non-infected group was 4786.07 cells/mm³ (2585-7459, SD=1559.93). The number of monocyte of

the infected group was 526.41 cells/mm³ (0-1775,SD=461.72) and non-infected group was 575.5 cells/mm³ (103-1560, SD=412.21). The number of eosinophil of the infected group was 3784.59 cells/mm³ (200-10453, SD=2401.75) and non-infected group was 2376.57 cells/mm³ (527-4721,SD=1219.65). Only the eosinophil value that was statistically difference between infected group and non- infected group. (Table12)

Fecal examinations among the infected dogs revealed 10 of 22 dogs (45.45%) had intestinal parasitic infection. Three groups of parasitic egg: hookworm, whipworm and tapeworm, were found by floatation technique and the entire fecal samples were negative in sedimentation technique. The clinical blood picture between the heartworm infected without intestinal parasite and heartworm infected with intestinal parasite is shown in table 1. There was not statistically difference between 2 groups.

Case No.	sex	Test results	
		Antigen test	Hematocrit tube test
1	male	negative	negative
2	male	positive	negative
3	male	positive	negative
4	female	positive	negative
5	male	positive	negative
6	male	negative	negative
7	female	positive	negative
8	male	negative	negative
9	male	negative	negative
10	male	negative	negative
11	female	negative	negative
12	female	negative	negative
13	female	negative	negative
14	male	negative	positive
15	male	negative	negative
16	male	positive	negative
17	male	positive	negative
18	female	positive	positive
19	female	positive	negative
20	male	positive	negative
21	male	positive	negative
22	female	negative	negative
23	male	positive	negative
24	female	positive	negative
25	female	negative	positive
26	female	negative	negative
27	female	negative	negative
28	male	negative	positive
29	female	positive	negative
30	male	positive	negative

31	female	positive	negative
32	male	positive	negative
33	female	positive	negative
34	male	negative	negative
35	male	negative	negative
36	female	negative	positive

Table7: Test results of *D.immitis* infection and follow up period of each animal.

Table8: Risk time and risk season in which each investigated animal in incidence study falls in and incidence density.

Animal No.	Infected animal (yes/no)	Total month at the follow terminate (month)	Month at the follow up terminate		Age being infected	Time(months) animal exposed to each season					
			noninfected	infected		Before Rainy00	Rainy00	Winter00-01	Summer01	Rainy01	
1	n	25-7=18	Sep-01	-	-	2	5	4	3	4	
2	y	14-7=7		Dec-00	7	0	5	2*	0	0	
3	y	12-7=5		Oct-00	5	0	5*	0	0	0	
4	y	25-7=18		Sep-01	18	2	5	4	3	4*	
5	y	23-7=16		Sep-01	16	0	5	4	3	4*	
6	n	22-7=15	Sep-01	-	-	0	4	4	3	4*	
7	y	22-7=15		Sep-01	15	0	4	4	3	4*	
8	n	22-7=15	Sep-01	-	-	0	4	4	3	4	
9	n	20-7=13	Sep-01	-	-	0	2	4	3	4	
10	n	20-7=13	Sep-01	-	-	0	2	4	3	4	
11	n	20-7=13	Sep-01	-	-	0	2	4	3	4	
12	n	23-7=16	Sep-01	-	-	0	5	4	3	4	
13	n	29-7=22	Sep-01	-	-	6	5	4	3	4	

14	y	24-7=17		Jun-01	17	4	5	4	3	1*
15	n	21-7=14	Sep-01	-	-	0	3	4	3	4
16	y	19-7=12		Jan-01	12	4	5	3*	0	0
17	y	19-7=12		Jul-01	12	0	3	4	3	2*
18	y	28-7=21		Dec-00	21	14	5	2*	0	0
19	y	22-7=15		Sep-01	15	0	4	4	3	4*
20	y	14-7=7		Sep-00	7	3	4*	0	0	0
21	y	19-7=12		Sep-00	12	8	4*	0	0	0
22	n	21-7=14	Sep-01	-	-	0	3	4	3	4
23	y	20-7=13		Feb01	13	4	5	4*	0	0
24	y	15-7=8		Sep-00	8	4	4*	0	0	0
25	y	21-7=14		Sep-01	14	0	3	4	3	4*
26	n	25-7=18	Sep-01	-	-	2	5	4	3	4
27	n	28-7=21	Sep-01	-	-	5	5	4	3	4
28	y	30-7=23		Sep-01	23	7	5	4	3	4*
29	y	19-7=12		Oct-00	12	7	5*	0	0	0
30	y	16-7=9		Oct-00	9	4	5*	0	0	0
31	y	17-7=10		Apr-01	10	0	4	4	2*	0
32	y	19-7=12		Jul-01	12	0	3	4	3	2*
33	y	15-7=8		Mar-01	8	0	3	4	1*	0
34	n	32-7=25	Sep-01	-	-	9	5	4	3	4

35	n	20-7=13	Sep-01	-	-	0	2	4	3	4	
36	y	18-7=11	Aug-01	11	0	1	4	4	3	3*	
Animal - months at risk in each season											
85											
No. of likely infected animals in each season											
6											
Incidence density											
0.042											
0.035											
0.027											
0.114											
22											
0.043											

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Table 9: Overall incidence density in each season

	season			Overall
	Rainy	Winter	Summer	
Animal - months at risk in each season	232	115	75	507
No. of likely infected animals in each season	16	4	2	22
Incidence density	0.069	0.035	0.027	0.043

Table 10: Incidence Rate Ratio between seasons of *D.immitis* infection in dogs
in the study period of 2000 – 2001

	Incidence Rate Ratio
Rainy / Winter	$69/35 = 1.99$
Rainy / Summer	$69/27 = 2.57$
Winter / Summer	$35/27 = 1.30$
Rainy / Out of rainy	$69/32 = 2.18$

Table 11: Comparative haematological values between infected and non-infected group

	Infected group (n=22)				Non-infected group (n=14)			
	Min	Max	Mean	SD	Min	Max	Mean	SD
PCV	30	57	43.55	6.99	38	52	43.86	4.09
Wbc	10010	27690	16542.77	4255.57	10270	31200	16393.93	5806.29
Seg	4141	13291	7816.23	2291.31	4724	19344	8656.00	4069.38
Lymph	1841	8861	4396.05	1764.81	2585	7459	4786.07	1559.93
Mono	0	1775	526.41	461.72	103	1560	575.5	412.21
Eos	20	10453	3784.59	2401.75	527	4721	2376.57	1219.65
Baso	0	0	0.00	0.00	0	0	0.00	0.00

Table12: t- test of the haematological values between infected and non-infected group.

	Test Value=0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
PCV	0.000	32.507	1.000	.00	-3.81	3.81
Wbc	-.149	24.858	.883	-258.48	-3839.06	3322.11
Seg	-1.072	19.964	.297	-1217.59	-3587.56	1152.38
Lymph	-.659	33.103	.515	-364.93	-1492.10	762.24
Mono	-.215	32.922	.831	-31.37	-328.15	265.41
Eos	2.145	30.874	.040	1334.79	65.62	2603.96

Other intestinal parasite	Other intestinal parasite											
		PCV	WBC	SEG	LYMP	MONO	EOS	BASO				
Positive	1	50	11700	7371	2808	0	1521	0				
	2	51	15340	10278	1841	307	2915	0				
	3	48	15015	6006	5405	601	3003	0				
	4	42	12708	7498	3177	0	2033	0				
	5	46	14495	7392	4349	725	2029	0				
	6	30	19500	5850	6435	1755	5460	0				
	7	35	16055	9633	3050	803	2569	0				
	8	47	27690	13291	8861	277	5261	0				
	9	41	18200	9646	4368	546	3640	0				
	10	39	24310	8265	5105	486	10453	0				
	Total	10	10	10	10	10	10	10				
Negative	1	48	10010	6506	3203	100	200	0				
	2	44	16510	4623	7595	660	3632	0				
	3	34	12220	4277	3788	244	3910	0				
	4	56	15925	4141	5415	319	6052	0				
	5	43	15730	8337	4404	157	2831	0				
	6	35	12285	7494	2457	614	1720	0				
	7	49	21515	10112	4303	645	6024	0				
	8	44	13780	7441	3583	551	2205	0				
	9	57	14203	8238	2557	426	2983	0				
	10	37	17750	6923	6568	1775	2485	0				
	11	41	18980	7023	3037	190	8731	0				
	12	41	20020	11612	4404	400	3604	0				
	Total	12	12	12	12	12	12	12				
	N	22	22	22	22	22	22	22				

Table3: Comparisons of haematological values between animals with and without additional infection of gastrointestinal

helminths among infected group