THE EPIDEMIOLOGY OF *DIROFILARIA IMMITIS* INFECTION IN OUTPATIENT DOGS AT CHIANG MAI UNIVERSITY SMALL ANIMAL HOSPITAL, THAILAND

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Abstract. Five hundred eighty-nine dog blood samples from the small animal hospital of Chiang Mai University were examined for *Dirofilaria immitis* prevalence using a microhematocrit tube technique for microfilaria detection. In parallel, a once a month follow-up study on 36 *D. immitis* negative dogs was conducted to detect the time of acquiring infection in each animal. The diagnostic criteria for the incidence study was based on microfilaria detection or on positive findings against *D. immitis* antigen using the Witness® commercial kit. The estimated prevalence was 18.2% (15-21%; 95%CI). There was no statistical difference between male and female infection rates. The age-specific prevalence of dogs under 2 years old was 6.4%, which was lower than the 2-4 year old group and all the other age groups at a 95% confidence level. In older dogs the prevalence reached 41.5%. Most of the dogs housed outdoors had a statistically higher infection rate than the dogs housed indoors (chi-square = 9.662, 1 df, p = 0.002). Only 109 dogs received chemoprophylaxis resulting in a significantly lower infection rate than in the non-heartworm prevention dogs (chi-square =14.424, 1 df, p = 0.000). The overall incidence density and the incidence during the rainy, cool and hot seasons were 5.2, 6.9, 3.5, and 2.7 animals per 100 animal-months, respectively. The incidence rate ratio between wet/dry, rainy/cool, rainy/summer, and cool/hot seasons were 2.18, 1.98, 2.59 and 1.30, respectively. The 95% confidence interval revealed no difference among seasons. In conclusion, dogs in *D. immitis* endemic northern Thailand contract infection in about 2 years.

INTRODUCTION

Canine dirofilariasis is endemic in tropical and subtropical countries. It is caused by mosquito transmitted microfilaria of *Dirofilaria (D.) immitis* (Lok, 1988). Adult worms dwell in the right ventricle and pulmonary artery, but can occasionally be found in the epidural space (Blass *et al*, 1989), brain (Hamir, 1987), anterior chamber of the eye (Brightman *et al*, 1977) or systemic arterial system (Frank *et al*, 1997). The relatively large size (25-35 centimeters in length) of the female, together with the number of worms infested causes a chronic circulatory disorder and finally results in congestive heart failure. In addition to several vertebrate animals, cats and humans can be infected (Lok, 1988). Human pulmonary dirofilariasis is increasingly reported from several parts of the world, including Thailand (Asimacopoulos *et al*, 1992; Fleisher *et al*, 1998; Sukpanichnant *et al*, 1998). Although around 60% of human cases show no symptoms, the remaining number may have cough, chest pain, or hemoptysis (Orihel and Eberhard, 1998). The

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lesions are frequently misdiagnosed as primary or metastatic lung tumors. The diagnosis is often not established without lung biopsy, in which thoracotomy has to be performed (Bailey *et al*, 1990; Asimacopoulos *et al*, 1992). In highly endemic areas, including Thailand, human dirofilariasis has to be considered in the differential diagnosis of solitary pulmonary coin lesions (Makiya *et al*, 1987; Knauer, 1998). The burden of the worm in the surrounding environment, reflected by the prevalence in dogs, which are a main reservoir for the filaria, affects not only the health of the animal but humans as well.

The occurrence of canine dirofilariasis, based on fresh blood smears for microfilaria was reported to be 24.58-39.47% in Bangkok (Sangkavaranont, 1981) and 24.71% in Mueang District, Chiang Mai Province (Choochote *et al*, 1992). There have been few reports of the epidemiology of canine dirofilariasis in the literature. To provides data on this subject for the formation of control measures for this disease, this study was carried out to investigate the prevalence of the disease in dogs and its risk factors (sex, age, type of housing, chemoprophylaxis), as well as the incidence and its seasonal occurrence.

MATERIALS AND METHODS

Prevalence and risk factors

Dogs older than 4 months of age brought during July 2000 to July 2001 to the Small Animal Hospital of the Faculty of Veterinary Medicine, Chiang Mai University were used as subjects for the study by sampling on a fixed day of the week, once a month, with the condition that the owners agreed to participate. A total of 589 samples were used for the study. This was based on the calculated sample size, with the assumption of a 24% expected prevalence, 4% error and 95% confidence level.

Blood samples were taken from the dogs, centrifuged in a microcentrifuge tube, then a buffy coat smear were examined for microfilaria as described by Levine and Wardlaw (1988). In addition to the general recognition of no other species of dog filariasis prevailing in northern Thailand, the other two criteria of (1) skin palpation was negative for a nodule to rule out the possibly *D. repens* infection, and (2) the movement of microfilaria as described by Ducos de Lahitte *et al* (1993) and Chauve (1997) were used to confirm the diagnosis of *D. immitis* microfilaria. The animals were categorized as *D. immitis* positive when they met these two criteria.

The risk factors included age, sex, indoor *vs* outdoor housing, and chemoprophylaxis for the animal, were tested for association with disease occurrence by means of the chi-square test. The chemoprophylaxis was 6 micrograms ivermectin orally once a month.

Incidence and seasonal patterns

The incidence rate for canine dirofilariasis was investigated by longitudinal prospective study of 36 owner-cooperative dogs. The sample size was determined on the assumption of 15% expected new cases, 10% error and 90% confidence level. The animals were reared under normal conditions without heartworm prevention during the studied period of 2000-2001. The studied dogs were negative for infection at the start of the study. They were followed up for 24 months or until found positive for *D. immitis*. Whole blood and serum were collected and examined for infection once a month. The diagnostic criteria for *D. immitis* infection were either microfilaria detection using the method mentioned above, or by position test result for adult *D. immitis* antigen using the Witness® test kit, which has a 94% specificity. The incidence densities for the whole study period and for each season: *ie* hot (March-May), rainy (June-October) and cool season (November-February) were determined. The hot, rainy and cool incidences were compared with each other to calculate IRR values. Each ratio value was then esti-

mated for its 95% confidence interval using the formula:

$IRR_{L,U} = IRR \pm Z * SE(IRR)$.

where IRR = incidence rate ratio

L=lower limit, U=upper limit, SE=standard error

The month of becoming infected, which corresponded to the season, was determined by counting 7 months for the latent period back to the time the infection was diagnosed. The total animal-months at risk was used as a denominator for calculating the incidence density for each season during the studied period, and was the sum for each of the follow-up animal-months, which had its specific time depending on the yes-no status of infection and the duration each animal was exposed to that particular season.

RESULTS

Prevalence and risk factors

As summarized in Table 1, the observed prevalence was 18.2%, 107 positive out of 589 animals examined. Of the 107 infected dogs, 54 were males and 53 females, 12 were housed indoors and 95 outdoors, 6 had heartworm chemoprophylaxis and 101 did not. There was no significant difference in infection rates by sex (chi-square = 0.227, 1 df, p = 0.634). Housing indoors and having chemoprophylaxis revealed a significant lower infection rate than housing outdoors and not having chemoprophylaxis (chi-square = 9.662, 1 df, p = 0.002; chi-square =14.424, 1 df, p = 0.000, respectively).

Age-specific prevalence

In Table 2 the prevalence rose from 6.4% to more than 20% by 2 years of age. The prevalences in each age group of 25-48, 49-72, 73-96, 97-120, and >120 months old were 20.4, 33.3, 32.0, 28.6, 41.5%, respectively. The prevalence of 6.4% in dogs younger than 2 years old was lower than that of the other age groups at 95% confidence level. In the dogs older than 4 years microfilaria may reach 43-55%.

Incidence and seasonal pattern

The overall incidence density rate (IR) of 5.2 animals per 100 animal-months as well as IR for each season, *ie* IR-rainy of 6.9, IR-cool of 3.5 and IR- hot of 2.7 animals per 100 animal-months are shown in Table 3. As seen in Table 4 the incidence rate ratios (IRR) between the rainy/cool, rainy/hot and cool/hot seasons were 1.98 (0.66-5.93, 95% CI), 2.59 (0.6-11.25, 95% CI), 1.30 (0.24-7.12, 95% CI), respectively. Considering only the wet (rainy: June-October) and dry seasons (November-May or hot plus cool months), the IR-dry was 3.2 animals per 100 animal-months and the IRR-wet/dry was 2.18 (0.86-5.58, 95% CI).

Table1
Prevalence of canine dirofilariasis in relation to heartworm (HW) chemoprophylaxis, sex and housing type.

		HW chemoprophylaxis		Sex		Housing		Total
		Yes	No	Male	Female	Indoor	Outdoors	
Microfilaria detection	Positive	6	101	54	53	12	95	107
	Negative	103	379	233	249	121	361	482
Total		109	480	287	302	133	456	589

Table 2
Age-specific prevalence of canine dirofilariasis and 95% confidence interval (L = Lower and U = Upper limits).

Age (month)	No. positive	Total	Positive (%)	95% CI,L	95% CI,U
0 - 24	19	295	6.44	3.6	9.2
25 - 48	20	98	20.41	12.4	28.4
49 - 72	30	90	33.33	23.6	43.1
73 - 96	8	25	32.00	13.7	50.3
97 - 120	8	28	28.57	11.8	45.3
>120	22	53	41.51	28.2	54.8

Table 3
Overall incidence density and incidence density for each season.

	Overall	Wet period		Dry period	
		Rainy2001	Rainy2000	Cool2000	Hot 2000
No. of animals exposed to the season	36	24	36	30	26
Animal-months at risk each season	422	88	144	115	75
Newly infected cases	22	10	6	4	2
		0.114	0.042	0.035	0.027
Incidence density (IR)	0.052	6+10/144+88=0.069		4+2/115+75=0.032	
		Wet period		Dry period	

Table 4
Incidence Rate Ratio (IRR) among seasons for *D. immitis* infection in dogs during the study period 2000-2001 and 95% confidence interval (L= Lower and U = Upper limits).

	Incidence Rate Ratio	95% CI,L	95% CI,U
Wet / Dry	69/32 = 2.18	0.86	5.58
Rainy / Cool	69/35 = 1.98	0.66	5.93
Rainy/ Hot	69/27 = 2.59	0.60	11.25
Cool / Hot	35/27 = 1.30	0.24	7.12

DISCUSSION

There have been previous reports of the occurrence of dog *D. immitis* infection in Thailand. However, they used limited sample sizes and had no information regarding age of the studied animals. In this study, the prevalence of 18.2% (15.3-21.5, 95% CI) revealed a high risk for dirofilariasis for dogs in the Chiang Mai area. There is no information available regarding the ages of dogs in Thailand. In this study, dogs under 2 years of age which constituted about 50% of the entire studied dogs might be over-represented. The true prevalence in the population, in which all ages of dogs are more homogeneously represented, may be even higher than that found here. In addition, the microfilaria detection technique used could not reveal occult infection, which was reported as high as 30% of the infection (Brunner *et al*, 1998). The nocturnal nature of microfilaria is another factor influencing the low sensitivity of the technique.

The significantly higher prevalence of outdoor housing and of chemoprophylactics for

animals are not surprising. Keeping animals indoors may reduce the exposure risk to disease-transmitting mosquitoes as reported by Walter (1996) and Theis *et al* (1999). For Thailand, this result indicates that the majority of dogs are at high risk for infection, as most dogs, particularly in rural areas, are kept outside houses. The positive result for animals of 6/109 in the chemoprophylactic group and 12/133 in the indoor housing groups are additional indicators supporting the theory for these risk factors. The higher prevalence in older than younger dogs is also explainable by increased exposure risk to mosquitoes in older dogs.

The IR value of 5.2 per 100 animal-months at risk confirms the highly endemic situation of the disease in northern areas of Thailand. This reported incidence density is very close to the value found in the US (Walter, 1996). The IR value can infer that dogs kept in areas in 19 months become infected. In endemic areas, dogs older than one and a half years with no history of heartworm prevention will most likely harbor *D. immitis*. Due to the serious pathology of the disease, those infected dogs may be predicted to have a short life span.

Even the point estimate of the IRR values between seasons showed a 2 times higher chance of infection occurring during the rainy months, but this was not significantly different from the dry season. These findings show that seasonal variation may not exist, or the sample size used was too small to detect a significant difference. Preventive measures against canine dirofilariasis should be recommended year round.

Considering there are traditionally not many differences in housing for dogs and there are similar climatic conditions in the northern, northeastern, western and central regions of Thailand, the epidemiology of canine dirofilariasis reported in this study may be applicable to those areas as well. The finding of very high prevalence of the disease suggests the high chance of misdiagnosis of suspected lung tumor in human cases. Less invasive work-ups for patients with this problem may be considered if the incidence in humans is proven to be frequent.

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