

A serological survey of *Dirofilaria immitis* infection in pet dogs of Busan, Korea, and effects of chemoprophylaxis

Kang Hyun BYEON¹⁾, Bong Jin KIM¹⁾, Sun-Mi KIM¹⁾, Hak Sun YU²⁾, Hae Jin JEONG²⁾
and Mee-Sun OCK^{1)*}

¹⁾Department of Parasitology, Kosin University College of Medicine, Busan 602-703,

²⁾Department of Parasitology, Pusan National University College of Medicine, Busan 602-739, Korea

Abstract: The status of *Dirofilaria immitis* infection was assessed in pet dogs of Busan, Korea, and chemoprophylactic effects of microfilaricidal medication were evaluated. A total of 294 pet dogs older than 6 mo were examined, 217 of which had been maintained indoors, and 77 had been kept outdoors. The Snap^R kit and direct microscopic examinations of the peripheral blood were used. The mean overall parasite positive rates were 10.2% and 6.5%, respectively. Outdoor dogs evidenced adult worm infection rate of 31.2% and microfilaria infection rate of 18.2%. The indoor dogs, however, evidenced adult worm infection rate of 2.8% and microfilaria infection rate of 2.3%. The prevalence in males was more than 2 times that of females. The changing pattern of infection rates by age evidenced a gradual increase, from 2- to 6-year-old dogs, after which, a decrease in infection rates was noted. With regard to chemoprophylaxis, the infection rates of complete and incomplete chemoprophylaxis groups were found to be 2-3 times lower than that of the non-chemoprophylaxis group. The results of the present study indicate that the risk of exposure to *D. immitis* in pet dogs is quite high, particularly in male outdoor dogs, and chemoprophylactic measures were quite effective.

Key words: *Dirofilaria immitis*, microfilaria, dog, prevalence, chemoprophylaxis

INTRODUCTION

Dirofilaria immitis, a mosquito-borne filarial nematode, typically inhabits the right ventricle and pulmonary arteries of dogs. *D. immitis* has also been associated with human dirofilariasis, as a result of accidental infection. Cases of human pulmonary dirofilariasis have been increasingly reported worldwide (Darrow and Lack, 1981; Echeverri et al., 1999; Foroulis et al., 2005). The first Korean case of human

pulmonary and hepatic dirofilariasis was recently reported (Lee et al. 2000; Kim et al., 2002).

The prevalence of dirofilariasis in Korean dogs was 10.2% on a microfilarial test and 28.3% on an antigen test, in a series of studies conducted from 1994 to 1995 (Lee et al., 1996). Recently, the prevalence of heartworm infection in several regions of Korea was found to correspond to an infection rate of 40.0%, according to the results of antigen tests conducted between 2001 and 2002 (Song et al., 2003). These reports showed that Korea is an endemic area of dirofilariasis. However, information regarding the status of dirofilariasis infection in Korea is relatively scarce, despite

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*Corresponding author (e-mail: msock@ns.kosinmed.or.kr)

the rapid increase of animals diagnosed and treated in veterinary clinics. Moreover, coastal regions, such as Busan, have been identified as endemic dirofilariasis areas, as these regions normally experience heavy annual precipitation and exhibit conditions suitable for mosquito breeding and migration.

The objective of the current study was to determine the rates of microfilarial and adult worm infection of pet dogs in Busan, using a commercial antigen detection kit (Heartworm Snap^R kit, IDEXX Laboratories, USA), as well as a direct microscopic examination of blood smears. In addition, we evaluated the chemoprophylactic effects of microfilaricidal medication administered a year before.

MATERIALS AND METHODS

Dogs

A total of 294 pet dogs, older than 6 mo, were examined between May and July of 2002. Among them, 217 dogs were maintained indoors and 77 were kept outdoors. The dogs were brought to 1 of 3 veterinary clinics selected by our research group for routine health care examinations.

Definition of chemoprophylaxis

The complete chemoprophylaxis program was arranged as follows: dogs were administered with anti-microfilarial medication in more than 6 dosages of ivermectin/pyrantel, or milbemycin or selamectine (one dosage/month) in the previous year (Clemence et al., 2000; McCall et al., 1996; Tagawa et al., 1993; Grieve et al., 1991). The incomplete chemoprophylaxis group received less than 6 dosages. The non-chemoprophylaxis group received no microfilaricidal medication.

Identification of adult worms and microfilariae

In order to identify adult worm infections, the Snap^R kit (Heartworm Snap^R kit, IDEXX Laboratories, USA) was applied. The positive results were represented semi-quantitatively in 2 ways, namely with low and high antigen levels. Both were considered positive reactions. For identification of microfilariae, 1

drop of blood (0.05 ml whole blood) was dropped onto a glass slide and examined under a microscope.

Autopsy

Autopsies were conducted on 3 heavily infected dogs, after obtaining a dog-owner's approval. Infections were confirmed, and the size and number of worms were determined.

Statistical analysis

Differences in infection rates between dogs according to sex and age were analyzed for statistical significance using the chi-square tests and Fisher's exact tests.

RESULTS

General characteristics of dogs

Of the 294 privately-owned dogs, 139 were males and 155 females. The complete chemoprophylaxis group included 76 dogs, and the incomplete group was 57 dogs; the other 161 were included in the non-chemoprophylaxis group. The age distribution of dogs ranged from 6 mo to 12 years.

Prevalence by age and sex of dogs

The mean overall positive rates of adult worms and microfilariae, as determined using the Snap^R kit and via direct microscopic examination, were 10.2% and 6.5%, respectively (Table 1). The prevalence of adult worms was 16.4% in males and 6.5% in females. The positive rate of microfilariae was lower than that of adult worms; 9.4% in males and 3.9% in females. The positive rates of adult worms and microfilariae by age group were as follows; 4.9% and 3.3% in the dogs of younger than 2 years, 6.6% and 1.3% in the 2-4 year age group, and 23.8% and 16.7% in the 4-6 year age group, and 16.7% and 13.0% in dogs older than 6 years, respectively.

Prevalence according to keeping conditions

Outdoor dogs revealed the adult worm positive rate of 31.2%, and microfilarial infection rate of 18.2% (Table 2). The difference in infection rates between

Table 1. Prevalence of *Dirofilaria immitis* microfilariae and adult worms among dogs according to age and living conditions

		No. of dogs examined	Positive rate (%) ^{a)}	
			Adult worm	Microfilaria
Total		294	30 (10.2)	19 (6.5)
Rearing condition	Indoor	217	6 (2.8)	5 (2.3)
	Outdoor	77	24 (31.2)	14 (18.2)
Gender	Male	139	20 (16.4)	13 (9.4)
	Female	155	10 (6.5)	6 (3.9)
Age (year)	0.5~2	122	6 (4.9)	4 (3.3)
	2~4	76	5 (6.6)	1 (1.3)
	4~6	42	10 (23.8)	7 (16.7)
	> 6	54	9 (16.7)	7 (13.0)

^{a)}positive rate of adult worms was based on the result of test with the adult worm antigen (Heartworm Snap^R kit, IDEXX Laboratories, USA), and microfilariae were detected by the direct microscopic examination of blood.

Table 2. Comparison of the prevalence of *Dirofilaria immitis* adult worms and microfilariae in indoor and outdoor dogs by age group

		Indoor			Outdoor		
		No. of dogs examined	Positive rate (%)		No. of dogs examined	Positive rate (%)	
			Adult worm	Microfilaria		Adult worm	Microfilaria
Total		217	6 (2.8)	5 (2.3)	77	24 (31.2)	14 (18.2)
Gender	Male	104	3 (2.9)	3 (2.9)	35	17 (48.6)	10 (28.6)
	Female	113	3 (2.6)	2 (1.8)	42	7 (16.7)	4 (9.5)
Age (year)	0.5~2	105	0 ^{a)}	0	17	6 (35.2)	4 (23.5)
	2~4	54	1 (1.9)	0	22	4 (18.2)	1 (4.6)
	4~6	27	3 (11.1)	3 (11.1)	15	7 (46.7)	4 (26.7)
	> 6	31	2 (6.5)	2 (6.5)	23	7 (30.4)	5 (21.7)

^{a)}Means no detection of adult worms or microfilariae.

males and females was clear; adult worm positive rate was 48.6% in males and 28.6% in females, and microfilaria infection rate was 16.7% in males and 9.5% in females. The 4-6 year age group represented the highest rates of infection with adult worms and microfilariae, 46.7% and 26.7%, respectively, and this dropped to 30.4% and 21.7%, in dogs older than 6 years.

The positive rate of adult worms was 2.8% in indoor dogs, and the positive rate of microfilariae in indoor dogs was 2.3% (Table 2). No significant differences were detected between males and females. No infection was detected in dogs of younger than 2

years, whereas only adult worm infection (1.9%) was verified in the 2-4 year age group. The positive rates of adults and microfilariae were the same in the 4-6 year age group, 11.1%. These rates equally decreased to 6.5% in dogs of older than 6 years.

Differences in the prevalence after chemoprophylaxis

Obvious differences in infection rates were observed between the chemoprophylaxis and non-chemoprophylaxis groups (Table 3). The infection rates were much higher in the non-chemoprophylaxis

Table 3. The effects of chemoprophylaxis with microfilaricides among complete, incomplete and non-chemoprophylaxis groups of dogs

	No. of dogs examined	Positive rate (%)	
		Adult worms	Microfilaria
Total	294	30 (10.2)	19 (6.5)
Complete chemoprophylaxis ^{a)}	76	3 (4.0)	0 ^{d)}
Incomplete chemoprophylaxis ^{b)}	57	3 (5.3)	0
No chemoprophylaxis ^{c)}	161	24 (14.9)	19 (11.8)
Indoor			
Complete chemoprophylaxis	63	1 (1.6)	0
Incomplete chemoprophylaxis	32	0	0
No chemoprophylaxis	122	5 (4.1)	5 (4.1)
Outdoor			
Complete chemoprophylaxis	13	2 (12.5)	0
Incomplete chemoprophylaxis	25	3 (15.4)	0
No chemoprophylaxis	39	19 (48.4)	14 (35.9)

^{a)}One of *Dirofilaria immitis* microfilaricides (ivermectin/pyrantel, milbemycin, or selamectine) was prescribed more than 6 times a year before.

^{b)}Prescription of microfilaricides was less than 6 times.

^{c)}no preventive medication.

^{d)}no detection of adult worms or microfilariae.

group (14.9%) than in complete (4.0%) and incomplete chemoprophylaxis groups (5.3%). In the case of indoor dogs, no microfilariae were detected in complete and incomplete chemoprophylaxis groups, and only 1 dog (1.6%) was infected with adult worms in the complete chemoprophylaxis group, which contained 63 dogs. Neither adult worms nor microfilariae were detected in the incomplete chemoprophylaxis group. However, the prevalence of adult worms and microfilariae were 4.1% and 4.1%, respectively, in the non-chemoprophylaxis group. The prevalence in outdoor dogs was not significantly different from that of indoor dogs. We detected only adult worms in complete (12.5%) and incomplete chemoprophylaxis groups (15.4%); however, in non-chemoprophylaxis group, the adult worm positive rate was 48.4% and the microfilarial positive rate was 35.9%.

Autopsy results

Postmortem examinations were conducted on 3 dogs among the Snap^R test-positive dogs, and all of them were confirmed to be infected with the heartworms. An average of 3.7 male and 5.7 female adult worms were retrieved per dog. The mean lengths of

female and male adult worms were 26 cm and 16 cm, respectively.

DISCUSSION

Although dirofilariasis has been detected in a variety of mammals, including humans, the dog is the most suitable host, in which *D. immitis* can complete its life cycle. Recently, pet dogs became increasingly popular in the Republic of Korea, and are also becoming more popular as companion animals. This increase of dogs as pets will inevitably eventuate a rise in zoonosis. In this study, we surveyed of *D. immitis* infection in pet dogs in Busan, Republic of Korea, in order to determine the prevalence depending on environment, in which dogs were maintained, and to determine the effects of chemoprophylactic measures applied a year prior to the study.

We utilized the Snap^R kit for detection of adult worm antigens. This kit has been recognized to possess a high degree of sensitivity and specificity. It is relatively simple to use, and can also be applied in the field. In order to identify microfilariae, direct blood smears, a modified Knott's test, or filter tests are com-

monly employed. We used the direct smear method, as it is difficult to acquire sufficient volumes of blood in the case of small dogs, although the modified Knott's test and filter test were reported to achieve detection rates slightly higher than that could be obtained by the direct smear method.

The total rates of infection with adult worms and microfilariae were 10.2% and 6.5%, respectively. This discrepancy can be explained by occult infections and/or differences in sensitivity of detection methods employed. The occult infection rate was reported to be between 10% and 67% (Martherne et al., 1988; Lee et al., 1996). Courtney and Zeng (2001) reported that direct blood smear examinations (0.05 ml of whole blood) were capable of detecting all 134 cases, in which microfilaremia was present at a concentration greater than 50 microfilariae/ml, but only 31 of 70 cases, in which microfilaremia was present at concentrations of less than 50 microfilaria/ml. As post-mortem examinations had verified infections with male and female heartworms in this study, this discrepancy could be partly attributed to the paucity of microfilariae in the peripheral blood.

Depending on living conditions of dogs, the rate of infection with adult worms in outdoor dogs was 10 times that of indoor dogs. It is generally assumed that outdoor dogs are more frequently bitten by mosquitoes than are indoor dogs. A survey by Song et al. (2003) confirmed that the prevalence of dirofilariasis in shoreline areas was significantly higher (69.5%) than that in other areas (urban or mountain areas). Outdoor dogs in Busan also evidenced the highest prevalence (48.4% of adult worms and 35.9% of microfilaria) among dogs examined in this study. There are some causes of a high rate of *D. immitis* infection in coastal areas, including high annual temperatures, suitable mosquito reproduction environment, and frequent migration of mosquitoes from other areas (Ahid et al., 1999). The dogs evaluated in this study were uniformly kept in houses near the downtown area, in which preventive measures against epidemics are regularly applied. More effective mosquito control is clearly required, as the prevalence of dirofilariasis in pet dogs was fairly high, despite the systemic sanitary

measures taken in these areas. Microfilarial infection also evidenced a pattern similar to that of adult worms. Differences between males and females couldn't be recognized in indoor dogs, but were in excess of 2 times that of males in the outdoor group. These differences, however, were not significant when the amount of outdoor activity and age factors were considered (Grieve et al., 1983).

The prevalence, depending on the age of dogs, increased gradually until the dogs reached 6 years of age, but decreased after that in this study. Many studies have noted that the incidence of dirofilariasis increased until middle age (about four to eight years), with a subsequent decline occurring in later years (Capelli et al., 1996; Glickman et al., 1984; Graham, 1974). This pattern of prevalence was suspected to be the consequence of the selective death of infected dogs, or attributable to the treatment and removal of worm burdens and microfilariae (Grieve et al., 1983).

The rate of adult worm infection varied according to whether or not chemoprophylactic measures had been taken in a year prior to the study. The non-chemoprophylaxis group (14.9%) evidenced infection rates in excess of 2-3 times that of complete (4.0%) and incomplete chemoprophylaxis groups (5.3%). These results indicate that chemoprophylactic measures were quite effective ($P = 0.003$). However, the 4.0% infection rate observed in the complete chemoprophylaxis group could be attributed to several factors, including the followings: 1) veterinarians may not have conducted tests for adult worms prior to chemoprophylaxis, or 2) problems inherent to direct microscopic examination for microfilariae, or 3) low sensitivity of the antigen test. In the case of microfilariae, none were detected in the complete and incomplete chemoprophylaxis groups. The non-chemoprophylaxis group, however, evidenced an infection rate of 11.8%. The absence of microfilariae in the incomplete chemoprophylaxis group can be attributed to the fact that, although the recommended dose of microfilaricide is 6 single doses per mo, the effects of the medication can persist for approximately 4 mo, depending on the type of medication utilized. Furthermore, Ivermect* solution (ivermectin 1.0% w/v), a type of dirofilariasis

chemoprophylactic medicine, is currently being widely prescribed for the treatment of ectoparasites in dogs.

These results collectively imply that *D. immitis* infection is widespread in Busan, Republic of Korea, particularly in male outdoor dogs. The prevalence of *D. immitis*, depending on the age of dogs, evidenced a gradual increase up to the age of 6 years, but declined afterwards. In addition, whether or not chemoprophylactic medicine was administered in a year prior to the study can significantly affect the prevalence of *D. immitis*. Therefore, chemoprophylactic medication should be administered every year to prevent an increase in canine heartworm infections, particularly for male outdoor dogs. In addition, vector mosquito control protocols should clearly be implemented. Information regarding dirofilariasis infection rates will provide us with new insights into measures for the prevention and control of dirofilariasis.

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