A Thesis
For the Degree of Master of Veterinary Science

Survey of Canine Heartworm (*Dirofilaria immitis*) Infection on Jeju Island, Korea

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(Supervised by Professor Kyoungkap Lee)

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Abstract

This study was performed to investigate the prevalence of canine heartworm (*Dirofilaria immitis*) infection on Jeju Island, Korea. Fifty-seven dogs, randomly sourced over the period from January to July 2000, were examined using a modified Knott's test and an antigen test (ICT GOLD®, Binax, USA). In addition, data for 103 patients, recorded between January 1999 and May 2000 at three veterinary clinics in Jeju City were analyzed. The veterinarians in these clinics used a direct wet smear to assess infection.

Of the 57 dogs directly assessed in this study the proportion infected with heartworm was 31.6% by a modified Knott's test, and 38.6% by an antigen test. Of 22 heartworm-infected dogs, 4 (18.2%) had occult dirofilariasis. In comparison, 31.1% (32/103) of the 103 patients independently assessed a direct wet smear were similarly infected.

D. immitis infection, according to age, was as follows: less than 2 years, 4.2%; from 2 less than 4 years, 42.1%; from 4 to 10 years, 92.9% ($p<0.01$). The prevalence of infection was influenced by the age of dog

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and where it spent the night (inside the house or out of doors). Hematological studies in heartworm-infected dogs revealed a low hematocrit, reduced red blood cell and a marked increase in eosinophil percentage ($p < 0.05$).

This study indicates that the prevalence of *D. immitis* in Jeju (38.6% by the antigen test) was higher than has previously been reported in Korea (3.1-28.3%).

Keywords: *Dirofilaria immitis*, modified Knott's test, antigen test, dog, Jeju, Korea

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Ⅰ. Introduction

Heartworm disease caused by *Dirofilaria immitis* (Leidy, 1856) is endemic in many tropical and subtropical regions of the world. It occurs in many species, such as the dog, cat, fox, wolf, black bear, sea lion and man (Soulsby, 1982). *D. immitis* is not only a veterinary problem, since it is a zoonotic infection in many countries.

D. immitis (Fam. Oncoceridae) is a filarial nematode most often found in the right heart and pulmonary arteries of dogs. Many species of mosquitoes are capable of transmitting Dirofilaria. The microfilariae that are released into the vascular system by adult females enter mosquitoes with a blood meal. In the invertebrate host, they undergo two moults before being infective for another vertebrate host. After infection of the vertebrate host, the larvae undergo two additional moults and then enter the venous system, where they are trapped in the pulmonary arteries as immature adults. Seven to eight months after infection of the vertebrate host, microfilariae can be found in the peripheral blood. Circulating microfilariae may survive for two years, while adult heartworm may survive up to six years (Rhee, 1987). Although microfilariae may be found in the blood at any time after their initial appearance, they may exhibit a periodicity that varies with geographic location (Soulsby, 1982; Rhee, 1987; Rhee *et al*, 1998).

Typically, diagnosis of dirofilariasis was confirmed by microscopic identification of microfilariae in the peripheral blood. False-negative results were common, because at any given time a substantial number of heartworm-infected dogs are amicrofilaraemic. False-positive results were also possible, because the morphology of the microfilariae of several apathogenic viviparous filarids (e.g., *Dipetalonema reconditum* and *Dirofilaria repens*) is quite similar to that of *D. immitis* (Soulsby,

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1982).

The use of serological assays to detect antigen of adult heartworms has greatly improved dirofilariasis diagnosis. With the introduction of serological methods to detect circulating antigen of adult heartworms, occult heartworm infections can now be recognized. These methods have high sensitivity and specificity (Brunner *et al*, 1988).

Butts (1979) reported that the prevalence of dirofilariasis was 10.1% in Mecklenburg County, North Carolina, based on medical records at veterinary clinics in the area. The veterinarians had used a variety of testing methods, including direct wet smears, Knott's test, and commercially available filtration tests (Difil®). Retnasabapathy and San (1976) reported an infection rate of 25.8% by necropsy in Malaysia. By the direct wet smears, 47.2% of the infected animals were identified, whereas the blood serum examination detected 57.8% of them. Wang (1997) reported an infection rate of 55% by necropsy in north Taiwan. By the modified Knott's test, 21.1% of assessed animals were identified as infected, whereas an ELISA (Snap™) kit detected 37.2%.

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In Korea, surveys of the prevalence of heartworm infection in dogs by detecting circulating microfilariae have indicated infection rates of between 3.1% and 23.0%. (Rhee, 1966; Rhee and Rim, 1970; Lee, 1971; Kim *et al*, 1985; Lee *et al,* 1992). Lee (1993) reported infection levels in Daegu of 4.8% and 7.4% by the microfilarial test (using acetone as a hemolytic reagent) and the antigen test (Assure/CH®), respectively. Lee *et al* (1996) reported infection rates in German shepherds of 10.2% and 28.3% by the modified Knott's test and the antigen test (DiroCheck®), respectively.

Because of its importance for public health, and to help prevent dirofilariasis, we initiated this study to investigate the prevalence of *D. immitis* infection in Jeju, Korea.

Ⅱ. Materials and Methods

1. Examination:

1) Experimental animals

Fifty-seven randomly sourced dogs were examined between January and July 2000 in Jeju City, Seogwipo City and other municipalities. The dogs were from 7 months to 10 years old, and most were of mixed breeding.

2) Blood sampling

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Blood samples were obtained from each dog. Blood samples were collected from the cephalic vein, using 23-gauge 1.0-inch needles affixed to 3-㎖ syringes at about 1 - 6 ㎰. Immediately after collection, samples were distributed into blood collection tubes containing ethylenediaminetetraacetic acid (EDTA) and stored in an ice chest for transport to a laboratory, where they were examined within 24 hours of collection.

3) Diagnostic methods

(1) Modified Knott's test

The EDTA blood samples were assessed by the modified Knott's test to detect microfilariae. 1 ㎖ of EDTA blood sample was mixed with 9 ㎖ of 2% formalin in a centrifuge tube and centrifuged for five minutes at 1500 rpm. After discarding the supernatant fluid, a drop of

sediment was placed on a slide; one drop of new methylene blue stain was added, and the suspension was examined under 100 × magnification with reduced light. Microfilariae were assessed morphologically as *D. immitis,* based on their tail and body appearance.

(2) **Antigen test**

The EDTA blood samples were assessed by a commercial kit (ICT GOLD®, Binax, USA) according to the manufacturer's instructions, to detect a specific circulating antigen from *D. immitis.*

4) Hematological evaluation

Six dogs selected with antigenemia of adult heartworm were evaluated by hematocrit (Ht), red blood cell (RBC), hemoglobin and white blood cell (WBC) differential count by an automatic analyzer (Coulter STKS, Beckman Coulter, USA)

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2. Data derived from medical records of patients:

1) Experimental animals

Three veterinarians in Jeju City, cooperated in a survey of canine heartworm (*Dirofilaria immitis*) infection in the area. From January 1999 to May 2000, the veterinarians recorded the following data of 103 patients tested for dirofilariasis in their clinics: location; age; whether the dog spent the night indoors or outdoors. Most of the dogs presented to the clinic for medical reasons: for preventive medical care (e.g., vaccinations), for specific health problems or for a specific

diagnostic procedure.

2) Diagnostic method

(1) Direct wet smear

One drop of blood, obtained from the cephalic vein, was placed on a glass slide. A cover slip (18 ㎜ × 18 ㎜) was placed over the drop, and the area covered was systematically examined under the low power of a compound microscope.

3. Statistical analyses:

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The data were compared using the Chi-square test and Student's t-test. Infection proportions for a given age and location were analyzed using the Chi-square test. The association of infection with spending the night outdoors or indoors was analyzed using the Chi-square test. Hematological values were analyzed using Student's t-test.

Ⅲ. Results

D. immitis infection rates, as determined by each diagnostic test, are presented in Table 1. Four (18.2%) of 22 infected dogs had occult dirofilariasis.

Table 1. *D. immitis* infection in Jeju, Korea.

Methods	No. of dogs	No. of positive dogs (%)
Modified Knott's test	57	18 (31.6)
Antigen test*	57	22 (38.6)
Direct wet smear	103†	32 (31.1)

*assessed by the antigen test (ICT GOLD®, Binax, USA).

† data obtained independently, from animals at three veterinary clinics.

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D. immitis infection by age, as determined by the antigen test is presented in Figure 1. Infection with *D. immitis* correlated with increased age (χ^2 = 29.6, p<0.01). The median age for all the dogs was 2 years, but the median age of the infected dogs was 5 years.

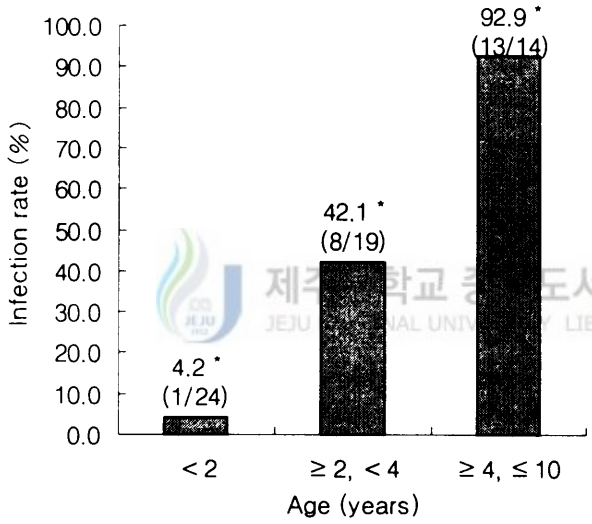

Figure 1. *D. immitis* infection by agein Jeju, Korea, as determined by the antigen test (ICT GOLD®, Binax, USA).
*significant difference (χ^2 = 29.6, p<0.01)

D. immitis infection by age, as determined by the direct wet smear, is presented in Figure 2. Microfilaria infection correlated with increased age (χ^2 =20.3, p<0.01). The median age for all the dogs was 3 years, but the median age of the infected dogs was 4.8 years.

In these results, we can find the prevalence of dirofilariasis infection increases with age.

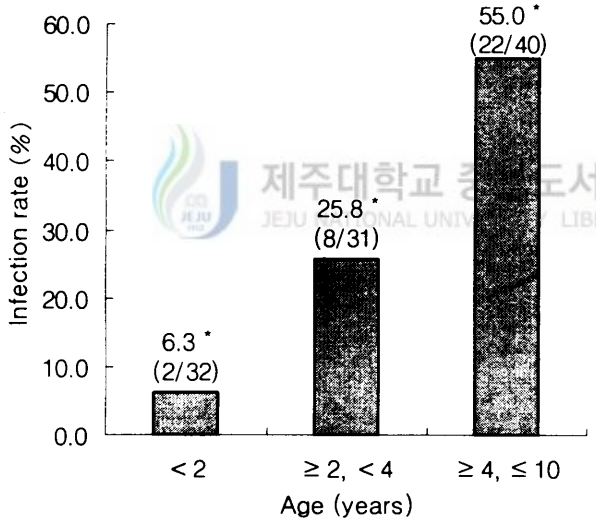

Figure 2. *D. immitis* infection by age in Jeju, Korea, as determined by the direct wet smear.

*significant difference (χ^2 = 20.3, p<0.01)

The association between location and *D. immitis* infection, as assessed by the antigen test, is presented in Table 2. The prevalence of *D. immitis* was lower in urban areas than in other areas (χ^2 = 15.6, $p < 0.01$).

Table 2. Association between location and *D. immitis* infection in Jeju, Korea.

Location	No. of dogs	No. of positive dogs* (%)
Jeju City	29	12 (41.4)
Seogwipo City	22	5 (22.7)
Other areas	6	5 (83.3)
Total	57	22 (38.6)

*assessed by the antigen test (ICT GOLD®, Binax, USA).
χ^2 = 15.6, $p<0.01$

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The association between location and *D. immitis* infection, as assessed by the direct wet smear, is presented in Table 3. The prevalence of *D. immitis* was lower in urban areas than in other areas (χ^2 = 6.1, $p<0.05$).

Table 3. Association between location and *D. immitis* infection in Jeju, Korea.

Location	No. of dogs	No. of positive dogs* (%)
Jeju City	85	22 (25.9)
Other areas	18	10 (55.6)
Total	103	32 (31.1)

*assessed by the direct wet smear.

χ^2 = 6.1, $p<0.05$

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The association between *D. immitis* infection and being outdoors or indoors at night, as assessed by the antigen test, is presented in Table 4. The results suggest that dogs spending outdoors at night may be more likely to be infected than dogs kept indoors at night, but no statistically significant difference was found.

Table 4. Association between being outdoors or indoors at night and *D. immitis* infection in Jeju, Korea.

Overnight	No. of dogs	No. of positive dogs* (%)
Outdoors	51	21 (41.2)
Indoors	6	1 (16.7)
Total	57	22 (38.6)

*assessed by the antigen test (ICT GOLD®, Binax, USA).

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The association between being outdoors or indoors at night and microfilaria infection, as assessed by the direct wet smear, is presented in Table 5. The results indicate that dogs that stay outdoors at night are more likely to be infected than dogs kept indoors at night (χ^2 = 26.7, $p<0.01$).

Table 5. Association between being outdoors or indoors at night and microfilaria infection in Jeju, Korea.

Overnight	No. of dogs	No. of positive dogs*(%)
Outdoors	61	32 (52.4)
Indoors	42	0 (0.0)
Total	103	32 (31.1)

*assessed by the direct wet smear.

χ^2 = 26.7, $p<0.01$

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Haemograms of dogs with antigenemia of adult heartworm are presented in Table 6. Hematological studies in heartworm-infected dogs revealed low hematocrit, reduced RBC and a marked increase in eosinophil percentage.

Table 6. Haemograms of dogs with antigenemia of adult heartworm (n=6 dogs).

Haemograms	dogs with antigenemia†	normal values‡	
		range	mean
Hematocrit*	19.2±15.54‖	29.8-57.5	43.7
Hemoglobin(g/dl)	14.0± 3.05	12.4-19.1	15.8
RBC($\times 10^6/\mu\ell$)**	2.7± 2.22	5.2-8.1	6.7
Total WBC($\times 10^3/\mu\ell$)	10.3± 2.72	5,4-15.3	10.4
Neutrophils(seg., %)	61.5±11.08	51-84	67.5
Lymphocytes(%)	16.5± 7.71	8-38	23.0
Monocytes(%)	6.3± 3.01	1-9	5.0
Eosinophils(%)*	14.5± 8.26	0-9	4.5
Basophils(%)	0.8± 0.98	0-1	0.5

† based on the result of an antigen test (ICT GOLD®, Binax, USA).

‡ presently being used at the University of Minnesota, Veterinary Teaching Hospital (Plumb, 1991).

‖ mean ± standard deviation.

*, **significant difference (*$p<0.05$, **$p<0.01$)

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Ⅳ. Discussion

Diagnosis of canine heartworm infection has depended on clinical signs, radiographic findings, and microscopic identification of blood samples for the presence of microfilariae in the peripheral blood. The presence of microfilariae in a blood sample is generally considered definitive proof of heartworm. However, in a high proportion of infected dogs with occult heartworm infections, circulating microfilariae cannot be demonstrated. To provide a definitive means of diagnosing these occult infections, several serological tests for heartworm antigen, or the antibody to it, have been developed.

Surveys of the prevalence of heartworm infection in dogs in Korea have indicated infection rates of 3.1% to 28.3%. The rate of infection (10.3%) determined in this study rises over this range. Differences infection rates may be related to climate (subtropical), and they cannot be said to be affected by any dirofilarial controlling agent in dogs.

Otto (1978) reported diagnosing dirofilariasis when there is no indication of microfilaremia (occult dirofilariasis) is a major problem, as this is a discrepancy that has been shown to occur in 10% to 67% of naturally infected dogs. Although reasonably accurate, necropsy examinations are only feasible in a highly selective and nonrepresentative sample of dogs and are not clinically applicable. Thilsted *et al* (1987) reported that the the antigen-detecting test gave the best agreement between serotest results and necropsy findings (97%). Poglayen *et al* (1996) reported that 17% of infected dogs had occult dirofilariasis. Polizopoulou *et al* (2000) reported that 10 (11.8%) of 85 infected dogs had occult dirofilariasis. In the present study, the serological test indicated 18.2% of the animals assessed had occult dirofilariasis, supporting the concept that occult dirofilariasis constitutes

a significant percentage of naturally occurring infections.

Lee *et al* (1996) reported that the prevalence of dirofilariasis infection increases with age, but Selby *et al* and Glickman *et al* (1980a, 1984b) reported a decreasing risk of infection beyond 8 years of age. In the present study, the percentage of infected dogs, as determined by the presence of adult parasites and microfilariae, increased with age to a maximum of 92.9% (55.0% microfilaremic) among dogs ≥4 years of age. Longer exposure to mosquito bites creates new opportunities for older dogs to acquire the disease.

Lee (1971) reported on prevalence (36.4%) in small island of Chubdo (Jindo-gun), where dirofilarial agents were not used in dog population. Park *et al* and Rhee (1962a, 1966b) reported infection rates of 21.0% and 23.0% in Jinju City and Junju City, respectively. Lee (1993) reported that rates of *D. immitis* infection were lower in urban areas than in rural areas. Although not many dogs from rural areas were examined, the percentage infected was higher than for dogs in urban areas, where the occurrence of vectors may be relatively low because of disinfection programs and environmental factors.

Poglayen *et al* (1996) reported that the prevalence of dirofilaria infection increased with increasing outdoor activity. Analysis of data from 103 patients independently assessed animals in the present study revealed similar results. Most of these dogs were of mixed breeding, which may be associated with increased time spent outdoors. The duration of exposure to the mosquito vector could explain differences in the prevalence of infection in dogs.

Hoskins *et al* (1982) reported differences in hematological values of dogs with canine heartworm disease and those of normal reference dogs. Eosinophilia is a common laboratory finding in dogs with canine heartworm infection, but is not pathognomonic for this condition. Glickman *et al* (1983) reported an increase in both eosinophils and

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basophils with dirofilaria infection while Hoskins *et al* (1982) reported increase levels of eosinophils, basophils, mature neutrophils and monocytes. Lee *et al* and Meyer *et al* (1996a, 1994b) both reported low packed cell volume and an increase in eosinophils as associated with dirofilarial infection. The results of the present study were similar, but the presence of other parasites was not determined.

Canine dirofilariasis has become an endemic disease in Jeju. Between 1989 and 1994, the following mosquitoes were collected in Jeju: *Anopheles sinensis, Culex tritaeniorhynchus, Culex pipiens* and *Aedes togoi* (Ko, 1996). These species may be considered the natural vectors of canine heartworm. Since these potential vectors are widespread throughout the Jeju area, and are commonly found near human habitations, human infection is also possible. The parasitaemia in both the canine population and the vector population must be eliminated to reduce the risk to humans. Ivermectin is the most commonly used filaricide, and it has been found effective and safe. Moreover, filaricide treatment prevents infection of new vectors, and helps to reduce the spread of the disease (Barriga and Andujar, 1988). Recently, a moxidectin formulation has exhibited a wider margin of safety than ivermectin or milbemycin in avermectin-sensitive collies (Paul *et al*, 2000). In urban areas, environmental improvements such as sewage control may help to eliminate the vector. In rural areas, natural predators reduce the numbers of mosquito larvae.

In conclusion, this study indicates that the prevalence of *D. immitis* in Jeju (38.6%) is higher than has previously been reported in Korea (3.1-28.3%). Control of this parasite in dogs should be seriously considered.

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제주지역 개 심장사상충 (*Dirofilaria immitis*) 감염률 조사

송 보 섭

(지도교수: 이 경 갑)

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초록

제주지역 개의 심장사상충 감염률을 조사하기 위하여, 2000년 1월부터 7월까지, 57두의 개를 대상으로 사상충의 자충검사(modified Knott's test) 및 성충항원검사(ICT GOLD®, Binax, USA)를 실시하였다. 또한 제주시 소재 3개의 동물병원에서, 1999년 1월부터 2000년 5월까지, 진료한 103두의 개를 대상으로 사상충의 자충검사(direct wet smear)를 실시하였다.

사상충의 자충검사법에 의한 감염률은 31.6%(18/57)이였고, 성충항원검사법에 의해서는 38.6%(22/57)을 보였다. 항원양성 반응을 보였으나 필라리아 자충이 검출되지 않은 은폐감염률은 18.2%이었다. 그리고 3개 동물병원에서 직접현미경법에 의한 감염률은 31.1%(31/103)이었다. 개의 연령별 감염률은 2년령 미만, 2년에서 4연령 미만 및 4년에서 10년령에서 각각 4.2%, 42.1% 및 92.9%로 조사되었고($p<0.01$), 숙주의 연령이 증가함에 따라 감염률이 증가되는 것으로 나타났으며 또한 밤에 실외에서 지낼 때 감염률도 증가되는 것으로 나타났다. 감염된 개의 혈액검사는 적혈구용적률과 적혈구 수는 감소하고, 그리고 백혈구중 호산구 비율은 증가한 것으로 나타났다($p<0.05$).

이 연구 결과 제주지역 개의 심장사상충 감염률 (38.6%: 성충항원검사)이 국내의 다른 지역에서 보고된 것(3.1-28.3%) 보다 높게 나타났다.

중심어: 심장사상충, 항원검사법, 필라리아자충검사법, 개, 제주, 대한민국