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국내 개 사상충증 발생율에 관한 조사 연구

이상은, 송근호, 김덕환*

충남대학교 수의과대학

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Prevalence Study on the Canine Filariasis in Korea

Sang-eun Lee, Kun-ho Song and Duck-hwan Kim*

College of Veterinary Medicine, Chungnam National University, Daejeon 305-764, Korea

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Abstract: Prevalence of microfilariae, *Dirofilaria immitis, Dirofilaria repens, Acanthocheilonema dracunculoides* from 506 German Shepherd dogs reared in Korea was investigated by modified Knott's test to detect circulating microfilaria and by acid-phosphatase staining for differentiation of each microfilaria species. In the modified Knott's test, 74 of 506 dogs (14.6%) were microfilaria positive, and the prevelance of each species of microfilaria was 90.5% (67 of 74 samples) for *D. immitis*, 5.4% (4 of 74 samples) for duplicate infection with *D. immitis* and *D. repens* and 4.1% (3 of 74 samples) for mixed infection with *D. immitis, D. repens* and *A. dracunculoides*.

It was considered that the paying attention to the existence not only *D. immitis* but also other microfilariae were needed in canine filariasis.

Key words: microfilaria, modified Knott's test, acid phosphatase staining

Introduction

Canine heartworm disease is an obstructive pulmonary vascular disorder complicated by clinically significant disease of the several organs including the heart, lung, liver and kidneys [7].

Dirofilaria immitis, a zoonotic parasite, is transmitted by infected mosquitos. It is the most common cause of pulmonary artery hypertension, and inhibits the activity of the right ventricle in dogs. This causes edema, asthma, heart failure, renal failure or even death of the infected dogs. The heartworm, *D. immitis*, and the other filarial nematoda, *Dirofilaria repens*, *Acanthocheilonema dracunculoides* and *Dipetalonema reconditum* produce microfilaria that circulate in the blood stream of infected dogs. These nematoda are widely dispersed and are found in the tropics, subtropics and temperate zones [7] such as USA [11], Italy [4, 15], Spain [12], Japan [8] and Taiwan [16].

It may be very important to differentiate *D. immitis*, *D. repens*, Dip. reconditum and *A. dracunculoides*. Those microfilaria have a similar morphology and movement under the light microscopy and are difficult to discriminate from each other [1, 9, 11]. Some researchers described that acid-phosphatase staining method was effectively used to differentiate microfilarial species [2. 3, 9]. Especially, Peribanez [13] reported that *D. immitis*, *D. repens* and *A. dracunculoides* were discriminated by method using a commercial test kit, Leucognost-SP®.

In Korea, Park and Lee [10] firstly reported that the prevalence of microfilaria of *D. immitis* in Jinju area was

* Corresponding author: Duck-hwan Kim
Laboratory of Veterinary Internal Medicine, College of Veterinary Medicine, Chungnam National University, Daejeon 305-764, Korea
Tel: +82-42-821-6756, Fax: +82-42-822-4216, E-mail: dhkim@cnu.ac.kr

21% and Lee et al. [6] reported that positive rate of microfilaria infection by Knott's test in German Shepherd dogs was 10.2%. However, no report was found whether there were other kinds of canine microfilariae in Korea.

Therefore, the objectives of the present study are to clarify the prevalence of microfilaria and to investigate the existence of other kinds of microfilaria except *D. immitis* in dogs.

Materials and Methods

Areas examined

The areas surveyed were mountain areas (Chuncheon, Pocheon, Uoijeongbu, Injae, Wontong and Gongju) and shoreline areas or islands (Incheon, Pyoengtaek, Sokcho, Mokpo, Busan, Gangwha, Baekryeong and Sorok islands).

Experimental animals

German Shepherd dogs (n=506) raised in Korea were examined for filarial infection from March to August in 2002. Two hundred fourty female and 266 male dogs were examined. None of the dogs surveyed in this study had received prophylaxis and treatment for canine heartworm infection. The age range was from 1 to 10 years old (mean 4.0 years old).

Microfilaria detection

Five ml of blood was collected from the cephalic vein of all dogs from 08:00 to 13:00 each testing day in raising areas. Modified Knott's test was used for microfilarial detection in this study [5].

Acid-phosphatase staining

Acid-phosphatase histochemical staining kit (Leucognost-SP®, Merck, Germany) was used in this study. Each microfilaria found was differentiated according to the staining characteristics with acid-phosphatase under the light microscopy [13].

Results

Modified Knott's test

The results of modified Knott's test for microfilariae in blood samples were shown in Table 1. Seventy four of 506 (14.6%) dogs were infected with microfilariae.

Table 1. The results of modified Knott's test in 506 dogs

	Microfilaria Positive	Microfilaria Negative	Total
No. fo dogs(%)	74(14.6)	432(85.4)	506(100)

Acid-phosphatase staining

The results of the acid-phosphatase staining from 74 samples recognized as positive by modified Knott's test were shown in Table 2. The staining characteristics for each species of microfilariae by acid-phosphatase staining were as follows. The microfilaria of *D. immitis* showed two positive spots with acid-phosphatase on the site of the excretory and the anal pores (Fig. 1). The microfilaria of *D. repens* showed one positive spot on the site of the anal pore (Fig. 2), while that from *A. dracunculoides* had three positive spots on the site of the excretory pore, central body and anal pore (Fig. 3). All of the stained sites showed the spot-like red color. The single infection with *D. immitis* was 90.5% (67 of 74 dogs), double infection with *D. immitis* and *D. repens* was 5.4% (4 of 74 dogs) and mixed infection with three kinds of microfilariae, *D. immitis*, *D. repens* and *A. dracunculoides* was 4.1% (3 of 74 dogs) by acid-phosphatase staining, respectively.

Table 2. The results of acid-phosphatase staining in 74 microfilaria-positive dogs

	D. immitis	*D. immitis* and *D. repens*	*D. immitis*, *D. repens* and *A. dracunculoides*	Total
No. of positive (%)	67 (90.5)	4 (5.4)	3 (4.1)	74 (100)

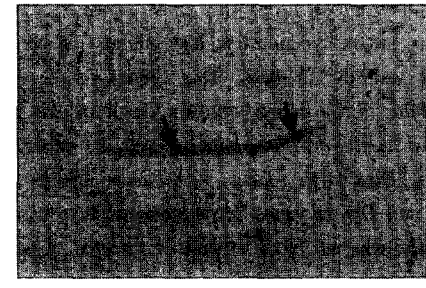

Fig. 1. Microfilaria of *Dirofilaria immitis* stained with acid phosphatase. Two arrows are excretory pore (left) and anal pore (right), ×400.

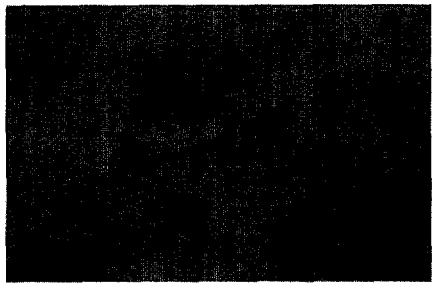

Fig. 2. Microfilaria of *Dirofilaria repens* stained with acid phosphatase. The arrow is anal pore, ×400.

Fig. 3. Microfilaria of *Acanthocheilonema dracunculoides* stained with acid phosphatase. Three arrows are excretory pore (right), central body (center) and anal pore (left), ×400.

Discussion

Heartworm infection is produced by the parasite, *D. immitis* and is transmitted to dogs by many species of mosquitoes. The diagnosis of heartworm infection is based on a positive immunodiagnostic test and/or the presence of microfilaria in the peripheral blood in dogs with or without clinical or radiographic findings consistent with the disease.

Although many heartworm-infected dogs do not have a detectable microfilaria, blood examination for microfilariae is an inexpensive and simple method of diagnosis. The positive rates of *D. immitis* infection are 14.6% (74 of 506 dogs) by modified Knott's test in the present study. This positive rate was similar to those of *D. immitis* as described by Lee et al. [6], Park and Lee [10] and Seo et al. [14] in Korea.

Discrimination of the microfilariae, such as those of *D. immitis*, instead of Dirofilaria sp. or Acanthocheilonema sp. is usually based upon the acid phosphatase stain, with different bands or spots of precipitated red-brown azo dye being shown for each species. The technique currently used to demonstrate acid phosphatase activity is the Barka method [3], which, on the basis of sediment blood smears, uses naphthol-AS-TR-phosphate as the substrate and para-rosaniline as chromogen. However, the main disadvantage of this procedure is the short period of reagents storage (from 4 weeks to 6 months), with it frequently being necessary to prepare new reagents. The kit employed in the present study, commonly used in the detection of acid phosphatase reaction in leukocytes for the diagnosis of leukemia, has the same foundation and similar reagents and has the advantage that these are easier to prepare and can be stored for at least 2 years [13]. By using acid-phosphatase staining kit in this study, only *D. immitis* infection was 13.2% (67 of 506 dogs), double infection with *D. immitis* and *D. repens* was 0.8% (4 of 506 dogs), and mixed infection with *D. immitis*, *D. repens* and *A. dracunculoides* was 0.6% (3 of 506 dogs). The prevalence of *D. repens* and *A. dracunculoides* was very low. The results of this study were similar to those of both *D. immitis* and *D. repens* (0.6%), and *D. repens* (0.8%) reported by Cringoli et al. [5]. However, *Dip. reconditum* was not detected at all in this study. Further study about *Dip. reconditum* infection by other diagnostic method should be performed. In addition, only a single infection rate with *D. immitis* was 90.5%, while the single infection with *D. repens*, *Dip. reconditum* or *A. dracunculoides* was not found in the present study.

It was considered that the paying attention to the existence not only *D. immitis* but also other microfilariae were needed in canine filariasis.

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