

Meta-analysis of the Prevalence of Canine Heartworm Infection in Korea

Son-II Pak¹

School of Veterinary Medicine and Institute of Veterinary Science, Gangwon National University, Chunchon 200-701, Korea

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Abstract : The purpose of this meta-analysis was to collect data from the literatures reporting prevalence of canine heartworm (HW) infection in an effort to emphasize the preventive importance of the disease and to serve as baseline data for further studies related to the disease management. Fourteen studies regarding the prevalence of HW infection published during the 1996-2007 were identified using the meta-analytic approach, involving a total of 1,790 cases by microfilarial test and 3,277 cases by enzyme-linked immunosorbent assay (ELISA) antigen test for adult worms. Data analysis was performed separately for pet and military dogs. The overall apparent prevalence of HW infection in pet dogs by microfilarial test and ELISA was 11.7% (183/1,563) and 16.5% (379/2,302), respectively ($p < 0.001$). No significant difference in prevalence by microfilaria and ELISA was observed between sexes whether assessed by data separately or pooled together. Compared to before 2000, the prevalence by microfilaria after 2000 has increased from 2.5% to 13.1% ($p < 0.001$), and the rate of adult worm has also increased from 9.9% to 20.3% ($p < 0.001$). The prevalence determined by ELISA was found to increase with age: 20.7% ($n = 1,003$) of dogs older than 2 years were positive, compared with 5.0% of dogs ($n = 701$) under 2 years of age ($p < 0.001$). Also, outdoor dogs showed significantly higher prevalence than indoor dogs (37.9% versus 3.4%; $p < 0.001$). For military dogs, the overall prevalence was 38.3% (87/227; 95% CI, 32.1 – 44.7%) by microfilarial test and 38.5% (375/975; 95% CI, 35.4 – 41.6%) by ELISA. This rate was significantly higher than that of pet dogs ($p < 0.001$). Occult infection rate of 37.5% and 66.7% was found in pet and military dogs, respectively.

Key words : meta-analysis, *Dirofilaria immitis*, prevalence, dog, Korea.

Introduction

A filarial nematode, *Dirofilaria immitis* (heartworm disease) is a zoonotic, pathogenic parasite of dogs transmitted by vector mosquitoes and produces a serious clinical problem in dogs in the endemic areas (21). The parasite has occasionally been isolated from other animal species (22,31). In Korea, canine heartworm infection has been occurred since as early as 1960s and human filariasis has so far almost been eradicated except in a very restricted coastal area (11,15). During the recent past years, the prevalence of canine heartworm disease seems to be gradually decreasing, but remains high despite increasingly use of preventatives. Also the rate varies widely depending on the laboratory methods employed and characteristics of the population studied. Recently, in a study conducted between January and December 2006, 9.9% of 565 stray dogs rescued in animal shelters had adult heartworms, according to the results of necropsy findings (4). Another study reported prevalence of 40% ($n = 848$) among military dogs by antigen-ELISA test conducted between 2001 and 2002 (29). Demographic (age, sex and breed) and ecological conditions (temperature and relative humidity) are reported to be important risk factors for heartworm infection or in the

transmission of the parasite (25,29,31,33).

Prevalence estimation is an essential step for designing prevention and control measures of a specific disease of concern. Large-scaled epidemiological studies have never been performed in Korea so far and thus, prevalence of heartworm infection by demographic factors in domestic dog populations remains unclear. Therefore, the purpose of this meta-analysis was to find prevalence of canine heartworm infection based on the reliable evidences available, to investigate potential chronological changes of prevalence with year, to explore geographical foci of heartworm infection, and to determine demographic factors related to heartworm infection. The results of this analysis may serve as baseline data for further studies related to the disease management in Korea.

Materials and Methods

Literature search and identification of studies

To identify Korean- and English-language studies published from January 1996 to December 2007 on the prevalence of canine heartworm disease in Korea, the author initially performed a computer-aided search of MEDLINE and the Korean Studies Information Service System (KISS). In both databases, the English phrases 'canine heartworm', 'diagnostic test', and 'Korea' were used singly or in combination as search terms for relevant articles. In addition, the author carried out

¹Corresponding author.
E-mail : paksi@kangwon.ac.kr

a manual search of the references included in the articles retrieved in the electronic search, to identify studies that were not identified initially, from the following peer-reviewed journals: Korean Journal of Veterinary Research, Journal of Veterinary Clinics, Korea Journal of Public Health, and Korean Journal of Veterinary Service. Whether a gold test for detection of heartworm was used was not considered in the inclusion criteria of articles for further review.

Eligible criteria and data extraction

Original studies that reported on the prevalence of microfilariae or adult worms in blood samples using either the direct microscopic examination or ELISA which detect antigens produced by adult *D. immitis* were included. Articles that did not explicitly report the regions studied, sampling period, number of samples tested or laboratory methods employed were excluded. Overlapping reports from the same study population or a fraction thereof (serial reports) were excluded. All abstracts from conferences within the time period (1996-2007) were examined for analyses. After screening for eligibility, the year of publication, total number of samples tested, and region of the country where the sampling was undertaken were extracted.

Statistical analysis

Samples that showed ELISA positivity were expressed seropositive. The prevalence in this study represents apparent prevalence not taking into account for diagnostic accuracy (sensitivity and specificity) of the test kits. Prevalence was estimated separately for military dogs and other sources including stray dogs (hereafter pet dogs). Total number of animals examined and number of test positives reported by each study was extracted to calculate prevalence of microfilaria and adult worms, and the 95% confidence interval (CI) was computed using the binomial distributions. The chi-square or Fisher's exact test was used to test differences in prevalence estimates between groups. The Cochran-Armitage chi-square for trend testing was used to assess the underlying proportion of dogs with positive response increases with years.

Results

A total of 14 studies met the inclusion criteria. Table 1 presents a summary of the individual study characteristics. Of the 14 studies, 3 were epidemiologic studies targeted for military dogs. A total of 1,790 samples (including 227 military

Table 1. Studies reporting to the prevalence of *Dirofilaria immitis* in dogs using by either microscopic microfilaria or ELISA for adult worm, during 1996-2007

Year of Population	City or province	No. of dogs tested			Reference
		publication studied	Microfilaria	Adult worm (test kit) ^b	
1996	Nationwide ^a	Military	27	127 (DiroCHEK)	(13)
1996	Seoul (Gyeonggi)	General	Not tested	363 (SNAP)	(28)
1996	Gyeonggi	General	Not tested	122 (SNAP)	(28)
1998	Jeonnam	General	200	200 (DiroCHEK)	(6)
1999	Incheon (Gyeonggi)	General	Not tested	150 (ICT GOLD)	(14)
2001	Chuncheon (Gangwon)	General	175	175 (STAT-PAK)	(32)
2001	Gangwon	General	22	73 (DiroCHEK)	(27)
2001	Gyeonggi	General	92	92 (DiroCHEK)	(27)
2003	Chungnam	General	100	100 (FASTest)	(16)
2003	Nationwide	Military	100	Not tested	(17)
2003	Nationwide	Military	Not tested	848 (DiroCHEK)	(29)
2003	Daegu (Gyeongbuk)	General	220	220 (AGEN)	(19)
2004	Daejeon (Chungnam)	General	Not tested	206 (AGEN)	(10)
2005	Jeonju (Jeonbuk)	General	307	307 (Solo Step)	(18)
2007	Pusan (Gyeongnam)	General	294	294 (SNAP)	(3)
2007	Gwangju (Jeonnam)	Stray	153	Not tested	(12)
Total			1,790	3,277	

^aStudies tested for samples collected from more than 3 cities or provinces are classified as nationwide.

^bDiroCHEK (Synbiotics, USA), SNAP (IDEXX, USA), ICT-GOLD (Synbiotics, USA), STAT-PAK (Chembio, USA), FASTest (Mega Co., Austria), AGEN (Australia), SOLO STEP (Heska, Switzerland).

dogs) for microfilaria and 3,277 samples (including 975 military dogs) for adult worm were analyzed for prevalence estimation (Table 1). For pet dogs, overall prevalence rates for microfilariae and ELISA was 11.7% (183/1,563; 95% CI, 10.2 – 13.4%) and 16.5% (379/2,302; 95% CI, 15.0 – 18.0%), respectively (Table 2). No significant difference between sexes was observed in the prevalence by both microfilaria detection and ELISA. Compared to the results from before 2000, the prevalence after 2000 increased from 2.5% to 13.1% ($p < 0.001$) for microfilaria and from 9.9% to 20.3% ($p < 0.001$) for adult worms. The prevalence was significantly higher in the dogs over 2 year age group (20.7% of 1,003) compared to younger than 2 year age group (5.0% of 701) (Table 3). The difference was statistically significant ($p < 0.001$). Outdoor dogs revealed significantly high positive rate for adult worm compared to indoor dogs. Before 2000, the rate of outdoor and

indoor dogs was 40% and 1.4% ($p = 0.0052$), respectively and the corresponding rate after 2000 was 37.9% and 3.4% ($p < 0.001$), respectively. There was a decreasing trend of positive rate from 2001 to 2007 in both microfilaria and adult worms ($p < 0.001$) (Fig 1). Compared with the overall prevalence in pet dogs, the rate in military dogs was significantly higher ($p < 0.001$): 38.3% (87/227; 95% CI, 32.1 – 44.7%) by microfilarial test and 38.5% (375/975; 95% CI, 35.4 – 41.6%) by ELISA (Table 4). The prevalence determined by microfilaria and ELISA was similar in both sexes. The prevalence by ELISA in the dogs over 2 year age group was significantly higher than younger than 2 year age group (10.5% vs. 48.1%, $p < 0.001$). All together, 72 (54.5%) of the 132 antigen-positive dogs had circulating microfilariae, resulting in overall presumed occult infection rate of 45.5%: 37.5% (36/96) in pet dogs and 66.7% (24/36) in military dogs.

Table 2. Comparison of the crude and weighted mean prevalence (WMP) of *Dirofilaria immitis* microfilaria and adult worms in pet dogs according to sex

Year	Sex	Microfilaria		Adult worm	
		No. of examined	% positive	No. of examined	% positive
< 2000	Male	98	3.1	444	11.8 ^a
	Female	102	2.0	391	7.7
	Total	200	2.5 ^b	835	9.9 ^c
≥ 2000	Male	308	14.3	553	21.0
	Female	359	12.6	607	22.8
	Unknown	696	12.8	307	14.1
	Total	1,363	13.1	1,467	20.3
Total	Male	406	11.9	997	16.8
	Female	461	10.7	998	16.7
	Unknown	696	12.8	307	14.1
	Total	1,563	11.7	2,302	16.5
	95% CI		10.2 - 13.4		15.0 - 18.0

CI, confidence interval. Unknown represents a study without classifying sexes.

^a $p = 0.0331$ vs. female.

^b $p < 0.001$ vs. ≥ year 2000 for microfilaria.

^c $p < 0.001$ vs. ≥ year 2000 for adult worm.

Table 3. Comparison of the prevalence of *Dirofilaria immitis* adult worms by antigen test in pet dogs according to age and residency

Variable	Category	< year 2000		≥ year 2000		Total	
		No. of examined	% positive	No. of examined	% positive	No. of examined	% positive
Age (year)	< 2	302	1.7 ^a	399	7.6 ^b	701	5.0 ^c
	≥ 2	533	14.5	470	27.7	1003	20.7
Residency	Indoor	145	1.4 ^d	519	3.9 ^e	664	3.4 ^f
	Outdoor	5	40.0	201	37.9	206	37.9

^{a,b,c} $p < 0.001$ vs. ≥ 2 year age group.

^d $p = 0.0052$ vs. outdoors.

^{e,f} $p < 0.001$ vs. outdoors.

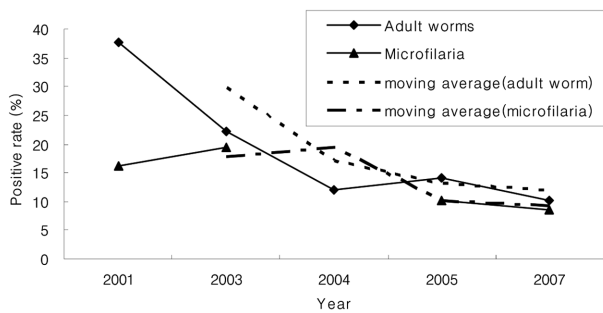


Fig 1. Positive rates of *Dirofilaria immitis* adult worms and microfilaria by year. Dotted lines represent trends using the method of moving average.

Discussion

The overall apparent prevalence of microfilariae and adult worms in all dogs were 15.1% (270/1,790; 95% CI, 13.4 – 16.8%) and 23.0% (754/3,277; 95% CI, 21.6 – 24.4%). The prevalence of 23.0% in the present study was higher than that reported in the United States for Washington (1.6% of 1,692) (30); Buenos Aires, Argentina (5.1% of 782) (25); State of Santa Catarina, Brazil (15% of 80) (1); Taipei City, Taiwan (13.4% of 664) (8); Sydney, Australia (11.4% of 404) (2); or Kayseri, Turkey (9.6% of 280) (33). On the other hand, Montoya et al (20) reported 58.9% of 2,034 dogs in Gran Canaria, Spain, markedly higher than that of this study. However, the information on the prevalence provided in this study may be biased, some of which are inherent in meta-analysis. Most importantly, the current prevalence estimates were not based on the definitive test such as necropsy. In addition, a variety of test kits (7 kits) with different diagnostic accuracy were included for prevalence estimation. Considering to the fact that the majority of test kits have greater specificity than sensitivity, the actual prevalence might be higher than the calculated prevalence due to false negative reactors. The great number of dogs used in each study were considered kept in outside without preventative medication, which may contribute to inflation of prevalence. The absence of information on the

frequency of the use of preventatives and the average worm burden in domestic dog populations which may affect sensitivity of the tests further complicated assessment of the actual prevalence. When excluding data conducted before 2000 due to great variations in the prevalence between studies, heartworm infection has apparently decreased, with a range from a high of 66.3% in Gyunggi in 2001 to 10.2% in Gyungnam in 2007. Despite all these limitations and small sample size of each study, it seems that heartworm infection is widespread throughout the country without regional foci.

The high prevalence of occult infection (45.5% of 132) in all dogs was comparable with the 49.4% in Australia (2), but was higher than the 29.6% in Turkey (33) and the 28.6% in Brazil (23). It was also found that the prevalence of heartworm in pet dogs aged over 2 years was 20.7%, while the prevalence was 5.0% for dogs in younger counterparts. This relationship has been well documented from the literatures (1,5,7-9,20,25,29). Rhee et al (24) indicated that the increased likelihood of acquiring heartworm infection in older dogs could be related to the increased length of period of exposure to the vector mosquitoes. Comparison of canine heartworm prevalence by sex has yielded contradictory results. In the present meta-analysis, no significant difference in prevalence by microfilaria and ELISA was observed between sexes for pet and military dogs or even in pooled together. Similar results were reported previously by other investigators (1,7,29). However, some other researchers have reported higher prevalence in males: ratio of 6:1 (33); 1.7:1 (25); 1.6:1 (30); or 1.3:1 (20). Ryo et al (26) also reported higher prevalence in male dogs.

In the present meta-analysis, the prevalence rate with adult worms in outdoor dogs (37.9%) was approximately 10 times higher than that of indoor dogs (3.4%), although this ratio may be exaggerated by the fact that the number of dogs subjected to antigen test which was performed before 2000 was very small. There need to be interpreted with caution when interpreting the results of sex-related risk. It is not clear that the association of male dogs with greater infection risk (prevalence) is related to genetic factor because confounding fac-

Table 4. Comparison of the prevalence of *Dirofilaria immitis* adult worms and microfilaria in military dogs according to age and sex

Variable	Category	Microfilaria		Adult worms	
		Total no. of examined	% positive	Total no. of examined	% positive
Age (year)	< 2			182	10.5 ^a
	≥ 2			666	48.1
Sex	Male	71	8.5	439	41.1
	Female	56	12.5	536	36.4
	Unknown	100	74.0		
	Total	227	38.3	975	38.5
	95% CI		32.1 – 44.7		35.4 – 41.6

CI, confidence interval. Unknown represents a study without classifying sexes.

^ap < 0.001 vs. ≥ 2 year age group.

tor(s) may attribute the relationship; more male dogs may be kept outdoors for their use to defend owner's safety and property (29) and dogs that are outdoors have greater probability of being exposed to additional risk factors (more favorable environmental conditions for the intermediate host) (25,33). Some other risk factors such as history on preventative medication, breed (mongrel, pedigree), size (large, small), environment (temperature, humidity), and time of blood collection (morning, afternoon) were not evaluated in this study because information on these covariates was not available across all studies.

In conclusion, although the prevalence of canine heartworm infection is on the decrease over the 12-year period, both serological and microfilarial examinations should be considered together when diagnosing canine heartworm disease in Korea due to high prevalence of occult infection and potentially low diagnostic performance of laboratory tests. Further comprehensive epidemiological studies are needed to determine the risk factors related to heartworm disease and to determine population dynamics of the disease.

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한국의 개 심장사상충 유병율에 대한 메타분석

박선일¹

강원대학교 수의학부 (대학) 및 동물의학연구소

요 약 : 본 연구에서는 개 심장사상충을 관리하기 위한 기초연구 자료를 확보하기 위하여 1996-2007년 동안 국내에서 발표된 논문 중 총 14편 (자충검사 1,790건, 성충에 대한 ELISA 항원검사 3,277건)의 자료에 근거하여 주요 위험 요인별 자충과 성충의 항원검사에 의한 유병율을 추정하였다. 자료분석을 위하여 일반견 (pet dog)과 군견 (military dog)을 별도로 분석하였다. 일반견에서 현성유병율은 자충검사 11.7% (183/1,563), 항원검사 16.5% (379/2,302)로 항원 검사에서 유의하게 높았으나 ($p < 0.001$) 두 검사 모두 성별에 따른 유병율의 차이는 없었다. 2000년 이전과 비교할 때 2000년 이후 자충검사와 항원검사에 의한 유병율은 2.5%에서 13.1%, 9.9%에서 20.3%로 각각 증가하였다 ($p < 0.001$). 항원검사에 의한 유병율은 2세 이하 5.0% ($n = 701$), 2세 이상 20.7% ($n = 1,003$)로 연령이 증가함에 따라 유병율이 증가하는 경향을 보였으며 ($p < 0.001$), 실외견 (37.9%)이 실내견 (3.4%)에 비하여 유병율이 유의하게 높았다. 한편 군견의 경우 유병율은 자충검사 38.3% (87/227), 항원검사 38.5% (375/975)로 일반견에 비하여 유의하게 높았으며 ($p < 0.001$), 잠복감염율은 일반견 37.5%, 군견 66.7%로 나타났다.

주요어 : 메타분석, 심장사상충, 유병율, 개, 한국.