

## Dirrofilariasis in Shepherd Dogs of High Altitudes Areas in West Azerbaijan-Iran

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### Abstract

Although the biology and ecology of the arthropod vectors are different, some factors, such as global warming, the increasing abundance of mosquitoes, the movement of domestic hosts, and the abundance of wild reservoirs, can act as favourable factors for the distribution of infections. The aim of this study was to determine the prevalence of *Dirrofilaria immitis* infection in shepherd dogs living in the high altitude of mountainous area (i.e.1200 meters above the sea level). The study group was comprised of 160 shepherd dogs living in 4 mountainous regions (Targavar, Margavar, Kolshin and Hovarchin) of west Azerbaijan where continuous movement of sheep and goat flocks resulted to have a little information about shepherd dogs in these regions. Additionally, arduous pathways have made impossible any access by car to some territories of these areas. The dogs were mostly mixed raced with different ages (from 1 to 10 years) and sexes (male = 136, female = 24). Blood samples were collected from cephalic vein. Direct thin and thick blood smears and modified knott's technique were used for detecting *D.immitis* microfilariae and other blood parasites. The results indicated that 40 (25 %) of dogs were infected with *D. immitis* microfilariae. In examination of the dogs, no severe life threatening feature of the disease was diagnosed. There were no significant differences ( $P > 0.05$ ) of *Dirrofilaria* infection among gender, age groups and geographical areas. High prevalence of asymptomatic Dirrofilariasis in shepherd dogs in this area highlights the need of controlling and preventive programs.

**Key words:** *Dirrofilaria immitis*, Shepherd dogs, High altitudes

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## Introduction

*Dirofilaria immitis* (*D. immitis*), the etiological agent for heartworm (HW) disease, is a zoonotic infection transmitted by various mosquito species and generally resides in the pulmonary arteries and the right ventricle of the heart of dogs. Many organs such as the lungs, heart, liver, and kidney may be affected by the infection. There is a report of presence of adult *D. immitis* in the bronchus of a dog.<sup>1</sup> In humans there are many reports of dirofilariasis infecting frequently pulmonary and subcutaneous tissues<sup>2,3</sup> In addition, there are a few reports of human dirofilariasis in unusual organs, such as large vessels, mesentery, peritoneal cavity, spermatic cord and liver.<sup>4-7</sup>

Several epidemiological surveys have determined the rate of infection to *D. immitis* in Iran. The infection rate has been reported 1.2 % of dogs in Tehran area<sup>8</sup> and 15.5 % in Golestan province.<sup>9</sup> This parasite has worldwide distribution; however enzootic areas are those of with a hot and humid climate which favor the mosquitos existence and heartworm transmission. Its prevalence is varied from country to country based on climatic conditions and other factors. Mosquito species and its role as vector for *Dirofilaria* infection could influence the risk and the distribution of the infection. Climatic and environmental variables, such as temperature, humidity, rainfall, vegetation index, land cover, elevation and slope could also influence the seasonal occurrence of HW transmission and its distribution.<sup>10</sup>

There is a lack of knowledge about the status of infection of shepherd dogs living in mountainous and cold conditions of west Azerbaijan. Highland mountainous area, outdoor living and continuous movement of sheep and goat flocks of this region have resulted in a little information about shepherd dogs. Furthermore, arduous pathways have made impossible any access by car to some territories of this

area. Large population of flocks reared in these mountainous areas plays a major role in the economy of the population in this region. Shepherd dogs accompanying these flocks have an important role in guarding and guidance of these flocks. These dogs are not often on routinely preventive parasites programs including *dirrofilaria*. The aim of this study was to assess the status of infection to *D. immitis* in shepherd dogs living in high altitude mountainous areas.

## Materials and Methods

**Area of study:** Urmia in West Azerbaijan is semi-humid mountainous area, with a mean yearly rainfall of about 350 mm, the maximum mean monthly temperature of 28.3°C in August and the minimum mean monthly temperature in January -3°C. Four mountainous regions (Targavar, Margavar, Kolshin and Hovarchin) of high altitude (above 1200 m) in Urmia and Oshnavieh were chosen for this study. **Sampling:** Shepherd dogs accompanying moving flocks in mountainous regions of Urmia were the target population. In order to access to the flocks and sampling from shepherd dogs, long walks, climbing and sleeping over were necessary. A total number of 160 dogs (aged between 1 and 10) were sampled in a period of 6 months from March to August in 2010. History taking and complete clinical examinations were carried out. Information collected from each dog included age, sex, history of previous diseases, and any antiparasitic treatments. None of these dogs had a history of any heartworm prophylactic treatment. Blood samples were collected via cephalic venipuncture into EDTA coated tubes and submitted in ice to the parasitology laboratory of the Faculty of Veterinary Medicine of Urmia University. Thin and thick blood smears were prepared and examined under light microscopy for detecting of blood protozoa and *Dirofilaria* microfilariae.

Furthermore, the modified Knott's technique was performed to confirm the status of infection to *Dirofilaria immitis*. Statistical analysis: The dogs were divided according to their sex, age (< 3, 3- < 6, 6-9 and > 9 years old) and location of the flocks (Targavar, Margavar, Hoorgochan, Kolshin). The differences of relative frequencies of infection to *dirofilaria immitis* between groups were analyzed using Chi-square test. Differences between the results of direct blood smears and Knott's technique of infected dogs were also analyzed by Chi square test. Significance was defined at a value of  $P < 0.05$ .

## Results

Examination of the direct thin and thick blood smears revealed no infection to *Ehrlichia*, *Babesia* and *Tripanosoma* spp.. In thin blood smears, only 6 (4.1 %) of the samples were positive To *D. immitis*. Thick blood smears with modified Knott's technique showed that 40 out of 160 of the samples (25 %) were positive to *D. immitis* microfilariae in thick blood or Knotte. Differentiation from *Acanthocheilonema* (formerly *Dipetalonema*) reconditum was carried out by measurement of diameter the microfilarers (medium size was 309) by

micrometer. Evaluating of the relative frequency of infection to heartworm by sex showed no significant difference ( $P > 0.05$ ) between males and females (Table 1). In addition, no significant difference ( $P > 0.05$ ) was observed between age groups (Table 2). There was also no significant difference ( $P > 0.05$ ) in relative frequency of infection between different areas where all had an altitude of higher than 1200 meters.

## Discussion

The results, as expected, showed that modified Knott's technique is a more sensitive and reliable screening test to identify microfilaria of *D. Immitis* compared to direct (thin and thick) blood smear examination. A climate that provides adequate temperature and humidity to support a viable mosquito population, and also sustain sufficient heat to allow maturation of ingested microfilariae to infective, third-stage larvae (L3) within this intermediate host is a pivotal prerequisite for heartworm transmission to occur. Laboratory studies indicate that development and maturation requires the equivalent of a steady 24-h daily temperature in excess of 18°C for approximately 1 month.

**Table1.** Infection to microfilaria of *D. immitis* in different sexes of shepherd dogs in west Azerbaijan number of samples

	Male	Female	Total
Healthy	105 (66%)	15 (9%)	120 (75%)
Infected	31 (19%)	9 (6%)	40 (25%)
Total	136 (85%)	24 (15%)	160 (100%)

**Table 2.** Infection to microfilaria of *D. immitis* in different ages of shepherd dogs in west Azerbaijan Age of the dogs (years)

	< 3	3 - < 6	6 - 9	> 9	Total
Healthy	67 (42%)	25 (16%)	18 (11%)	10 (6%)	120 (75%)
Infected	22 (14%)	9 (5%)	5 (3%)	4 (2.5%)	40 (25%)
Total	89 (56%)	34 (21%)	23 (14%)	14 (8.5%)	160 (100%)

Intermittent diurnal declines in temperature below the developmental threshold of 14°C for only a few hours retard maturation, even when the average daily temperature supports continued development. At 27°C, 10–14 days are required for development of microfilariae to the infective stage.<sup>11</sup> Both *Dirofilaria* species are transmitted by different genera and species of mosquitoes (Culicidae), which act as either intermediate hosts or vectors, whereas *Acantocheilonema* species are transmitted by ticks and flies. Although the development of *D. immitis* larvae in the mosquito does not occur below the threshold temperature of 14.8°C, the spread of HW infection in southern Switzerland is a good example of the “new” epidemiologic trend of the arthropod-borne infections. In 1995, 4 out of 371 stray, unwanted dogs tested positive for HW infection in southern Switzerland.<sup>12</sup>

The results of this study on 4 areas with altitude of above 1200 meters showed that the rate of infection of shepherd dogs to *D. immitis* in cold climatic condition of mountainous areas was remarkable indicating that similar to the low altitude and flat regions, the extreme climate of mountainous areas was also favourable for the development of larvae of *D. immitis* in their mosquito vectors. Prevalence rate of *D. Immitis* infection in dogs living in mountainous area of west Azerbaijan (25 %) was lower than that of reported from a mountainous area of the south Korea (43.2 %).<sup>13</sup> This can be explained by higher altitude of the area of our study (above 1200 m) compared to study carried out in Korea (400 m). Such a difference could be attributed to less favourable environmental conditions for the intermediate host (mosquitoes) in the higher altitudes. Age is an important risk factor for infection with *D. immitis* microfilaria. In general, older animals have more time and more exposure to the mosquito bites and have more opportunity to become infected. We

did not find any significant relationship between age and the rate of infection which was not in agreement with other reports, which showed higher *D. immitis* infection in older dogs.<sup>13-15</sup>

The relative frequency of infection in male and female dogs showed no significant differences. This finding was in agreement with previous reports.<sup>13-16</sup> However in other studies heartworm infections tended to be more prevalent in male dogs than in females.<sup>17,18</sup> As mostly male dogs are picked up by shepherds to secure flocks, large number of shepherd dogs in our study was male rather than females. The lower samples of females in our study could be an explanation that the difference did not reach to a significant level. In conclusion, the data of this survey shows that the rate of infection to canine heart worm disease is widely distributed in the shepherd dogs population of Urmia but the parasitized dogs did not have any remarkable clinical signs of infection. As the treatment and the following probable surgical procedure of heart worm disease is expensive and requires educated staff,<sup>19</sup> using preventive medications are of paramount importance in controlling the disease. In order to identify the potential transmission period and the risk areas for HW in Iran, more information about the mosquito species involved in HW transmission, their biology, and competence as vectors of *Dirofilaria* species would be useful.

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