

Short Paper

Survey of canine *Dirofilaria immitis* infection in Caspian provinces of Iran

Malmasi, A.^{1*}; Hosseini, S. H.²; Aramoon, M.¹;
Bahonar, A.³ and Seifi, H. A.⁴

¹Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; ²Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; ³Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; ⁴Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

*Correspondence: A. Malmasi, Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. E-mail: amalmasi@ut.ac.ir

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Summary

Between July and August 2009, a total of two hundred crossbreed dogs in three contiguous littoral provinces of Guilan, Mazandaran and Golestan were sampled. Circulating antigens and/or microfilariae of *Dirofilaria immitis* were found in 25.5% of dogs in the whole studied area. The highest infection rate was found in Guilan (51.42%), followed by Golestan and Mazandaran provinces (15.38% and 7.69%, respectively). Prevalence values were not influenced by age and sex in the whole area; the values detected in low altitude regions were 4.6 times the values found in high altitude areas. This is the first detailed survey on canine dirofilariasis in these coastal provinces of Iran. Guilan province, with its certain hyper endemic counties, was shown to be the most endemic for canine heartworm infection in the country. Chemoprophylaxis should be considered for the dog population in these areas.

Key words: *Dirofilaria immitis*, Heartworm, Canine, Caspian provinces, Iran

Introduction

Heartworm is a worldwide disease caused by the parasite *Dirofilaria immitis*. Mammals, mainly carnivores are definitive hosts and mosquitoes from the family Culicidae act as intermediate hosts or vectors (Genchi *et al.*, 2009).

Transmission of dirofilariasis is dependent upon the presence of sufficient numbers of infected microfilaraemic dogs, susceptible mosquitoes, and suitable climatological conditions (Medlock *et al.*, 2007; Genchi *et al.*, 2009).

Although some reports focusing on *Dirofilaria immitis* and its epidemiology in some localities of Iran have been published (Bokaie *et al.*, 1998; Meshgi *et al.*, 2002; Ranjbar-Bahadori *et al.*, 2007; Razi Jalali *et al.*, 2010), its provincial or countrywide

prevalence has not been studied. The importance of canine heartworm disease and the lack of information regarding its distribution in Iran's three Caspian provinces with their favourable climatological conditions inspired us to conduct this survey for the first time.

Materials and Methods

Area of the study

This study was conducted between July and August 2009 in three contiguous provinces of Guilan, Mazandaran and Golestan, located in the southern coasts of the Caspian sea (Fig. 1). These provinces have temperate climates and encompass several geographical regions including sea shores, plains, mountains, forests and even deserts. Climatological conditions of these

provinces are provided in Table 1. For each province, five counties and in each county, 1-4 villages (totally 31 villages) were selected from various geographical and climatological zones (Fig. 1).

Selection of dogs

A total of two hundred large crossbreed dogs {Guilan (n = 70), Mazandaran (n = 65) and Golestan (n = 65)} which were usually kept in the open air were selected. They were all asymptomatic and older than 1 year. No dogs in these provinces receive prophylactic medications for heartworm disease. One hundred fifty eight of these

dogs were male and 42 female, with ages ranging between 1-13 years; based on their age, the dogs were divided into three classes of: 1-3 (n = 118), 4-6 (n = 54) and >6 (n = 28) years old (Table 2).

Sample preparation

Approximately 3-5 ml of blood was withdrawn from the cephalic vein of each dog, collected in sodium citrate vacuum tubes and stored under refrigeration (+4°C) in local laboratories. Immediately after testing with commercial rapid *D. immitis* Ag kits, blood samples were prepared for modified Knott's test and were kept until



Fig. 1: Map of Iran. Three studied Caspian provinces with selected counties (spots) are shown

Table 1: Climatological data of three Caspian provinces of Iran (Meteorological Organization of Iran)

| Province | Mean annual temperature (C°) | Mean summer temperature (C°) | Mean annual precipitation (mm) | Mean annual humidity (%) | Coordinates | Area (km ²) |
|------------|------------------------------|------------------------------|--------------------------------|--------------------------|-----------------------------------|-------------------------|
| Guilan | 16.6 | 25 | 1438 | 82 | 36°34'38",27 N; 48°34'50",36 E | 14711 |
| Mazandaran | 17.2 | 26.3 | 952 | 82 | 35°47'36",35 N; 50°34'56",14 E | 23756 |
| Golestan | 18.5 | 27.1 | 446 | 71 | 36°24'38",5 N; 53°51'56",14 E | 20437 |

Table 2: Distribution of the dog population in the studied provinces based on their age, sex and canine heartworm infection

| | Guilan | | | Mazandaran | | | Golestan | | | Total | | |
|------------------|--------|----|------|------------|---|------|----------|----|------|-------|----|------|
| | No. | P | % | No. | P | % | No. | P | % | No. | P | % |
| Sex ^a | | | | | | | | | | | | |
| Male | 55 | 27 | 49 | 57 | 4 | 7 | 46 | 8 | 17.3 | 158 | 39 | 24.6 |
| Female | 15 | 9 | 60 | 8 | 1 | 12.5 | 19 | 2 | 10.5 | 42 | 12 | 28.5 |
| Age ^b | | | | | | | | | | | | |
| 1-3 | 39 | 20 | 51.2 | 44 | 5 | 11.3 | 35 | 2 | 5.7 | 118 | 27 | 22.8 |
| 4-6 | 20 | 12 | 60 | 14 | - | - | 20 | 6 | 30 | 54 | 18 | 33.3 |
| >6 | 11 | 4 | 36.3 | 7 | - | - | 10 | 2 | 20 | 28 | 6 | 21.4 |
| Total | 70 | 36 | 51.4 | 65 | 5 | 7.6 | 65 | 10 | 15.3 | 200 | 51 | 25.5 |

^a: Differences among sexes were not statistically significant, ^b: Differences among three age classes were not statistically significant, and P: positive antigen and/or modified Knott's test

analysed later in the parasitology laboratory of the Faculty of Veterinary Medicine, University of Tehran.

Antigen test

To detect the presence of adult female *D. immitis* circulating antigen the Anigen rapid canine *Dirofilaria immitis* Ag Test kit (Anigen Animal Genetics, Inc., 476-1 Pajang-dong, Jangan-gu, Suwon-si, kyonggi-do, Korea) was used. The test is based on rapid chromatographic immunoassay technology, which detects *D. immitis* antigens in dog's blood or serum.

All samples were tested as described by the protocol of the manufacturer.

Modified Knott's test

To detect the circulating microfilariae, all blood samples were analysed by the modified Knott's technique (Bowman, 2009). Subsequent identification to the species level was based on morphological and morphometric characteristics (Bowman, 2009); however, microfilariae of *Acanthocheilonema* (*Dipetalonema*) *reconditum* were not included in the statistical analysis and of *D. repens* were not

found.

Statistical methods

Statistical analyses were performed using SPSS (17.0) statistical software (SPSS Inc., Chicago, Illinois, USA). The prevalence of infection was compared by Chi-square tests. A $P < 0.05$ was considered significant.

Results

Table 3 summarizes our findings. Of a total of 200 dogs tested, 51 exhibited positive results for *D. immitis* infection with antigen detection kit and/or modified Knott's test (microfilariae) resulting in a prevalence of 25.5% (CI, 95%). Of the 51 infected dogs 42 (82.3%) had patent and 9 (17.7%) had occult infections. The highest infection rate was found in Guilan (36/70; 51.42%), followed by Golestan (10/65; 15.38%) and Mazandaran (5/65; 7.69%) provinces ($P < 0.0001$) (Table 3). Among fifteen counties selected for the study, the highest values were, surprisingly, detected in Fouman (100%), Rasht (86.6%) and Rudsar

Table 3: Distribution of canine heartworm infection among 15 counties in three Caspian provinces of Iran

| Province | No. of examined dogs | No. of infected dogs | Antigen (+) microfilariae (+) | Antigen (-) microfilariae (+) | Antigen (+) microfilariae (-) |
|-----------------|----------------------|-----------------------|-------------------------------|-------------------------------|-------------------------------|
| Guilan | | | | | |
| Rasht | 15 | 13 | 11 | — | 2 |
| Rudsar | 13 | 10 | 9 | — | 1 |
| Siahkal | 18 | 1 | 1 | — | — |
| Talesh | 13 | 1 | 1 | — | — |
| Fouman | 11 | 11 | 8 | — | 3 |
| % | 70 | 36 (51.4%) | 30 (83/3%) | 0% | 6 (16/6%) |
| Mazandaran | | | | | |
| Sari | 6 | — | — | — | — |
| Kiasar | 14 | — | — | — | — |
| Amol | 11 | 1 | 1 | — | — |
| Larijan | 8 | — | — | — | — |
| Chaloos | 26 | 4 | 3 | 1 | — |
| % | 65 | 5 (7.6%) | 4 (80%) | 1 (20%) | 0% |
| Golestan | | | | | |
| Bandar torkaman | 29 | 3 | 3 | — | — |
| Kordkuy | 8 | 3 | 2 | 1 | — |
| Ramian | 10 | 3 | — | — | 3 |
| Gonbad qabus | 8 | 1 | 1 | — | — |
| Minudasht | 10 | — | — | — | — |
| % | 65 | 10 (15.3%) | 6 (60%) | 1 (10%) | 3 (30%) |
| Total | 200 | (25.5%) ⁵¹ | (78.4%) ⁴⁰ | (3.9%) ² | (17.7%) ⁹ |

(77%) counties (all in Guilan province) ($P < 0.0001$) (Table 3).

Regarding age and sex, no significant differences were found among dogs (Table 2). Considering the living places of dogs, while 9 out of 99 dogs (9%) living in mountain and high altitude regions in the study area were found infected, the values for those living in plain, shoreline and low altitude regions were 42 out of 101 (41.5%), which were significantly different ($P < 0.0001$).

Discussion

This survey demonstrated that the overall prevalence of canine heartworm infection in the entire study area is high (25.5%), although it varied between provinces from 7.6 to 51.4% ($P < 0.0001$). The prevalence in the whole study area and the values found within Mazandaran and Golestan provinces (7.69 and 15.38%, respectively) can be compared with data obtained from previously reported areas of Iran (Bokaie *et al.*, 1998; Meshgi *et al.*, 2002; Ranjbar-Bahadori *et al.*, 2007; Razi Jalali *et al.*, 2010) or with many other Middle Eastern (Yildirim *et al.*, 2007), Asian (Boonyapakorn *et al.*, 2008) and European (Tasić *et al.*, 2008) countries; however, the prevalence detected in Guilan province (51.4%) has not previously been signaled neither in Iran nor in the region.

Based on our preliminary study, it would appear these provinces, especially Guilan with its certain hyper endemic localities and villages having up to 100% infection rates (Table 3) should be regarded as new endemic areas of canine heartworm in the country.

The significantly higher prevalence found in Guilan province (3-6.5 times more than the other two provinces) ($P < 0.0001$) shows other environmental and geographical differences may exist among these provinces which need more investigation.

The prevalence in the 4–6-year age group (33.3%) was more than that detected in the other two classes; similar results have been found in Turkey (Öncel and Vural, 2005). Finding non-significant difference in prevalence by sex among dogs is in agreement with other investigators (Simsek

et al., 2008; Tasić *et al.*, 2008). In the whole studied area, prevalence values for dogs sampled in low altitude regions such as plain and shoreline areas (82.3%) were 4.6 times more than values detected in high altitude regions (17.7%) ($P < 0.0001$), which is in agreement with the findings of other workers who found significantly higher prevalence in coastal rather than urban and mountain areas (Rossi *et al.*, 1996; Genchi *et al.*, 1998).

Guilan, Mazandaran and Golestan provinces have mean annual and summer temperatures in optimum range for mosquito development; mean temperatures (Table 1) in all these provinces never drop below the threshold of 14°C from May to November and below 25°C during summers, which easily provide the 130 HDUs (Heartworm Development Unit) required for larval development in mosquitoes (Bowman and Atkins, 2009). Mean annual rainfall of Guilan and Mazandaran provinces is high; however, the mean annual precipitation in Golestan is much less than the two other provinces (Table 1). The mean annual humidity of both Guilan and Mazandaran provinces is high and even in summer will not drop below 77 and 79%, respectively, however the mean for Golestan is less than the two others (Table 1). Accordingly, it seems these provinces, especially Guilan, based potentially on summer steady temperatures above the threshold and the abundance of several Culicidae mosquito species previously reported from the area (Azari-Hamidian, 2007) have favourable climates for canine *D. immitis* infection in Iran. Resident dogs and dogs visiting these regions, especially in the summer months are at higher risk of mosquito-borne *D. immitis* infections and should be treated and prophylactically protected which is not yet established in these touristic provinces.

Many culicid species which are well known as vectors of *D. immitis* have been found in Guilan and Mazandarn provinces (Azari-Hamidian, 2007), but no work has been done on their vectorial potential with regard to *D. immitis* infection in these regions.

Regarding various epidemiologic aspects of canine heartworm disease throughout these provinces, extensive investigations are

needed.

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