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SEROLOGICAL STUDY OF *DIROFILARIA IMMITIS* IN HUMAN FROM SOME VILLAGES IN AL-HINDYA PART OF KARBALA GOVERNORATE

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ABSTRACT
This study was carried out to determine the infection of *Dirofilaria* in Al-Hindya region in Karbala governorate. A total of 97 serum samples were taken from clinically healthy Iraqi people, 82 males and 15 females aged (7-57) years old and examind. 6 cases (20%) out of 30 were seropositive for IgG detected by ELISA for *Dirofilaria immitis,* infection. The nematode Ag has been identified by Elisa in the serum of these infected peoples. It's believed that the environmental factors such as temperature and humidity in Iraq are suitable for the spread of dirofilariosis.

KEYWORDS: dirofilaria IgG infection *etc.*

INTRODUCTION

Filarial diseases are a zoonostic disease it consider a major health problem in many tropical and subtropical areas. *Dirofilaria immitis* and *D. repens* are increasingly recognized as inadvertent human pathogen [1]. The first human case was reported in 1887[2]. *D. immitis* with a cosmopolitan distribution, a number of reported cases had significantly increased after 2003. In the last few years a rise of cases in both animal reservoirs and human populations of the central and Eastern European countries it has been detected [3]. Its common that parasitic disease in both domestic and wild animal around the world with canines as the principal reservoir host and mosquito as the vector containing infective L3 larvae [4]. Several studies have been aimed at improving the diagnostic tools for human dirofilariasis. Morphological identification of the worm was confirmed by polymerase chain reaction (PCR) studies [5]. Tests were designed to detect heartworm adult antigens based on ELISA or colloidal gold staining techniques that are considered a highly specific as cross reactivity with other dog's parasites does not occur. Different antigenic complexities and molecules derived from *Dirofilaria* spp. have been employed in ELISA was carried out in humans inhabiting Spain, Puerto Rico, European union, Arkansans, Brazil, France, Malaysia Tokyo,[6-11] and others. In areas in which heartworm infection in dogs is endemic, clinically healthy people are frequently found positive for antibodies against *D. immitis* antigens. Serological studies have been also shown different antibodies profiles in human: IgG, IgM and predominantly IgE antibodies against *D. immitis* antigens were detectable in healthy individual . However, the IgG, IgM and IgE responses in healthy individual varies throughout the year [12,13] were detected seasonal variations in the levels of anti- *D. immitis* antibodies in an exposed human population by using ELISA. IgM predominated in summer, during maximal vector activity, while IgG rose to a maximum in winter.

An epidemiological survey was carried out in AL- Hindya - Karbala government during 2008 – 2009 by using Knott technique, thin and thick smears, heska and ELISA for detection of nematode Ag [14&15]. The purpose of this study is to raise awareness about the importance of heartworm diagnosis and to describe one of the most efficient diagnostic tools.

MATERIALS AND METHOD

A study was carried on from April 2008 to end March 2009. A total of 97 individuals (82 males and 15 females) aged 7-57 years old were randomly selected from some villages that represent Al-Hindya - Karbala governorate of Iraq were examined. Sera were tested to detect heartworm antigen in Baghdad Central Public Health Laboratory. Five ml of blood samples was drawn from each individual in cleaned anticoagulant freed test tube. 30 serum samples selected randomly, from endemic areas of canine Heartworm [15], were analyzed by ELISA test to detect heartworm antigen using the technique described by Simón[16] . Statistical analysis was performed in chi-Square test.

RESULTS AND DISCUSSION

Canine dirofilariasis in Iraq was not reported and believed absent. High prevalence of infection (72.72%) was reported for the period between April 2008 and May 2010, at Al- Hindya region in Karbala governorate. The parasitological aspects of these cases are reviewed along with clinical, pathological, immunological and epidemiological features[14, 15&17].

Results of this study showed that healthy individuals from above areas in which canine heartworm were endemic, whereas serologically was positive for *D. immitis*. These results are identical to [18] were cases of infection with *D. immitis* in humans have been reported but are generally asymptomatic. In addition [19] reported twenty two out of 39 patients (56%) cases of Human Pulmonary Dirofilariasis were asymptomatic. Risk factors for human dirofilariasis have not been clearly defined. Dog ownership does not appear to be the most important epidemiologic factor. According to [20] the four major

factors that may influence the frequency and distribution of HD in a specific geographic area are the size of the dog population, the prevalence of canine dirofilariasis in that population, the density of the vector mosquito population, and the degree of human exposure to the bite of infected mosquitoes[1] . We believe that the size of the dog population, and prevalence of canine dirofilariasis and mosquito vector potential, play a role in transmission of *D. immitis* to human. Researchers feel that many factors may increase the incidence of heartworm disease in humans which are infected in the same way as that in animal reservoir, by the bite of a mosquito carrying infective larvae (L3), but are considered inappropriate hosts for zoonotic dirofilaria & nematodes do not normally reach sexual maturity and does not release viable Mf [21]. Vladimir *et al*. (2011) were mentioned that 10.4% of the healthy analyzed blood donors showed high levels of specific anti-*Dirofilari* spp. antibodies. The risk seems higher among persons who professionally in touch with dogs, in which seroprevalence are two times higher than that observed in the random healthy population analyzed. The majority of our patients infected with human dirofilaroiasis HD 5(83.3%) in male comparison with female (16.7%), the ratio of male: female were 5:1 of age group between (19-57) at mean of 30 years as shown in Table (1). Based on the Statistical analysis, our results showed no significant difference at the level ($P< 0.05$) which were detected between infected male and female

TABLE 1. Distribution of infected humans according to age and gender in the areas of the study

Gender	Age/year					Total (%)
	19	20	28	36	57	
Female	-	1	-	-	-	(16.7)
Males	2	-	1	1	1	(83.3)
Total	2	1	1	1	1	(100)

The predominance of male subjects is described in the literature by [1] in Brazil 17(70.1%) were male and 7(29.1%) were female [19]. Observed the same sex distributions were found 39 Americans patients are presented between 8 -80 years old, including 23 man and 16 women. Although the study population had a female: male ratio of 2.5:1, six out of the eight positive sera were from males [21]. It explained as differences in exposure to the parasite biological or hormonal characteristic [22]. Another theory involves immunologic differences and acquired immunity [21]. On the other hand [9] observed contrasted sex distribution of HD in their review of the world literature, but they stressed that differences were not significant. The prevalence of clinical cases in females 93 women out of 131(71%) contrasted with the gender distribution in the seropositive healthy blood donors, whereas 28 out of 33 (84.8%) were male found in the Rostov Region Southern Russia. This suggests the existence of an unknown factor in men [3]. The serologic examinations revealed that out of 30 healthy individuals 6 (20%), were positive by ELISA test for dirofilariasis. The optical density (OD) values, obtained with the sera from human infected with *D. immitis* reacted against the monoclonal IgG antibody for the detection of circulating antigen. As given in Table (2) any OD value greater than 0.097 was accepted as a positive result.

TABLE 2. OD values of the ELISA dirofilariasis among infected cases.

No.	OD value	ELISA Result	frequency
1	0.118 +	Positive	1
2	0.104 +	Positive	2
3	0.097	Negative	5
4	0.092	Negative	2
5	0.091	Negative	1
6	0.09	Negative	2
7	0.098 +	Positive	1
8	0.081	Negative	2
9	0.078	Negative	1
10	0.24	Negative	1
11	0.104 +	Positive	1
12	0.096	Negative	3
13	0.092	Negative	3
14	0.086	Negative	1
15	0.105 +	Positive	1
16	0.078	Negative	1
17	0.095	Negative	1
18	0.094	Negative	1

OD= optical density

Serological methods for use in humans are needed for clinical evolutions of patients living in highly enzootic *D. immitis* regions. As well epidemiological surveys are needed to determine the real extent of this zoonotic infection [23]. The authors concluded that repeated contact with *D. immitis* in this endemic population was common and began at an early age[16]. Patients showed significantly ($P< 0.05$) higher IgG titers than healthy individuals from areas in which *D. immitis* was endemic as non-endemic areas as well [24]. Furthermore serum sample from eight patients were positive for anti- dirofilarial IgG. These patients had a mean age of 54 years. The variable optical densities in the ELISA proved the role of age, indicating that repeated exposure to the parasite is the rule [9]. It is concluded that, for serodiagnosis the endemic or non endemic nature of the geographical area and the season of the year should be considered to define the basal level of positivity, and that as well as IgG, IgM should always be determine . Our previous and later data demonstrate the presence infection of *D. immitis* in canine and human population of the Karbala governorate. Because favorable conditions are available for development of mosquito vector such as a lot of water sources like rivers, lakes, they are consistently helpful the disease. Also hot climate lasts for several months during a year. For these points prophylaxis measures should be applied in dogs and control of mosquito populations to decrease the risk of infection in dogs and humans which must be discovered and treated early enough in order to prevent its spreading.

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