



Prevalence of Canine Cardiac Dirofilariasis in Nineveh Governorate of Iraq

Irak'ın Nineveh Vilayetinde Köpek Kardiyak Dirofilariosis Prevalansı

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ABSTRACT

Canine cardiac dirofilariasis is a parasitic zoonotic disease caused by *Dirofilaria immitis*. This research work is aimed to find out the prevalence of both a microfilaremic (patent infection) and occult forms (amicrofilaremic form) of cardiac dirofilariasis in stray and police dogs of Nineveh governorate (in northwestern Iraq). From October 2021 to January 2022, the blood of ninety dogs of various breeds, including 45 local breeds stray dogs and 45 police dogs, mostly of Belgian Malinois and German Shepherd breeds, with an age range of 1 to 11 years (median: 4.5 years), were examined microscopically by wet mount, acridine orange stained blood smears and modified Knott's test to detect circulating microfilaria. All the samples were further tested by a commercial antigen-enzyme-linked immune sorbent assays kit (Heartworm Ag ELISA kit, Canine, DRG International Inc., USA) in order to detect dogs with occult cardiac dirofilariasis. A total of 49 samples were microfilaria positive, giving a prevalence value of 54.4%. *D.immitis* antigens were detected in 27.8% of the examined sera, with an overall prevalence of 78.9%. Stray dogs showed a higher prevalence for both microfilaremia and occult forms of cardiac dirofilariasis compared to police dogs in Nineveh Governorate of Iraq. The agreement between the modified Knott's test (Gold standard) and wet mount, acridine orange stained blood smears, and Ag ELISA using Cohen's Kappa index was light for the wet mount (0.030) and substantial for Ag ELISA (0.462), and acridine orange stained blood smears (0.607). The findings of the present study provide evidence for the prevalence and pattern of canine cardiac dirofilariasis in dogs (both stray and police) in Iraq's Nineveh Governorate, requiring a phylogenetic analysis.

Keywords: Canine, cardiac dirofilariosis, *Dirofilaria immitis*, prevalence.

Öz

Kanin kardiyak dirofilariosis, *Dirofilaria immitis*'in neden olduğu parazitik zoonotik bir hastalıktır. Bu araştırma çalışması, Nineveh Vilayeti'nde (kuzeybatı Irak'ta) sokak ve polis köpeklerinde kardiyak dirofilariosisin hem mikrofilaremik (patent enfeksiyonu) hem de occult formlarının (amikrofilaremik form) prevalansını belirlemeyi amaçlamaktadır. Ekim 2021 - Ocak 2022 tarihleri arasında, 1-11 yaş arası (ortalama: 4,5 yaş) çoğu Belçika Malinois ve Alman Çoban ırkı olmak üzere 45 yerel sokak köpeği ve 45 polis köpeği dahil olmak üzere çeşitli ırklardan doksan köpeğin kanı, dolaşımdaki mikrofilariya'yı saptamak için wet mount, kan smeari acridine orange boyaması ve modifiye Knott testi ile mikroskopik olarak incelendi. Tüm numuneler, ayrıca occult kardiyak dirofilaryozlu köpekleri saptamak için ticari bir enzim bağlantılı immün emici tahlil kiti (Heartworm Ag ELISA kiti, Canine, DRG International Inc., ABD) ile test edildi. Toplam 49 örnek mikrofilariya pozitif ve prevalans değeri %54.4 idi. İncelenen serumun %27.8'inde *D.immitis* antijenleri tespit edildi ve genel prevalansı %78.9 idi. Sokak köpekleri, Irak'ın Ninova Vilayeti'ndeki polis köpeklerine kıyasla hem mikrofilaremik hem de occult kardiyak dirofilariyoz formları için daha yüksek prevalans gösterdi. Modifiye Knott testi (Altın standart) ile wet mount, kan smeari acridine orange boyaması ve Cohen'in Kappa indeksi kullanılarak Ag ELISA arasındaki uyum, wet mount (0.030) için hafifti ve Ag ELISA (0,462) ve kan smeari acridine orange boyaması için önemliydi (0.607). Bu çalışmanın bulguları, Irak'ın Nineveh Vilayeti'ndeki köpeklerde (hem sokak hem de polis) kardiyak dirofilariosis prevalansı ve paterni için filogenetik bir analiz gerektiren kanıtlar sunmaktadır.

Anahtar Kelimeler: Köpek, kardiyak dirofilariosis, *Dirofilaria immitis*, yaygınlık.

INTRODUCTION

Dirofilaria immitis (heartworm [HW]) is mosquito-borne filarial nematode capable of causing canine cardiac dirofilariasis commonly known as the heartworm disease owing to the location of the adult worm in the right ventricle of the heart and occasionally in the arteries of the lungs, resulting in the production of blood-circulating microfilariae^{1, 2}. HW can be diagnosed using microfilariae detection tests, serological (i.e., enzyme-linked immunosorbent assay [ELISA]) and immunochromatographic tests for the

detection of somatic and female antigens of *D. immitis* adults, radiography, and blood analysis³. Microfilariae testing may be aided by parasitological tests (wet mount, thin and thick blood smear, buffy coat method, and modified Knott's test). Microfilaria species can be distinguished using Morphological keys, histochemistry^{4, 5, 6}, and molecular tests (polymerase chain reaction [PCR])⁷. Canine heartworm antigenemia (Ag ELISA) are simple to perform, susceptible, and highly specific⁸. However, because Ag ELISA has a low rate of false positive outcomes, a positive result usually suggests a current infection^{3, 9}.

Cardiac dirofilariasis has been studied exhaustively, showing great regional and local variations in their prevalence worldwide¹⁰. The prevalence of cardiac dirofilariasis in dog population based on results of Anvari et al., (2020)¹¹ throughout the world and Asia was 10.91% and 12.07%, respectively. *D. immitis* is widely distributed in the canine population of Mediterranean and Middle Eastern countries, including Egypt¹², Turkey^{13; 14; 15}, Saudi Arabia¹⁶, Iran¹⁷, and Iraq^{18; 19}. In Iraq, the disease is diagnosed mainly in the southern provinces (Karbala, Al-Qadisiyah, and Dhi-Qar Provinces) of the country^{20; 21}. This study was designed and conducted to determine the prevalence of both microfilaremic (patent) and occult (amicrofilaremic) forms of cardiac dirofilariasis using wet mount, acridine orange stained blood smears, modified Knott's, test and antigen ELISA in stray and police dogs from Nineveh Governorate in northwestern Iraq.

MATERIAL AND METHODS

This study was approved by the College of Veterinary Medicine Committee, University of Mosul, Iraq (12 decision number in 3-10-2021).

Study Area and Sample Collection

The current study was conducted with the Nineveh Governorate in northwestern Iraq. It shares borders with Syria and several Iraqi governorates. Nineveh is the third largest governorate in terms of size. Its total land area is estimated at 37,323 km² (8.6% the total size of Iraq). The prevailing climate in Nineveh is hot and dry in summers and cool and rainy in winter.

During October 2021 to January 2022, the blood samples were collected from 90 dogs of various breeds, including 45 stray dogs of local breeds and 45 police dogs, mostly of Belgian Malinois and German Shepherd breeds, with an age range of 1 to 11 years (median: 4.5 years). All dogs recently treated with antiparasitic drugs were excluded from the study.

Blood was obtained from cephalic vein and collected in EDTA and serum tubes at morning (between 9 a.m. to 12 noon). After allowing blood to clot, sera were separated by centrifugation and stored at -20 °C until analysis.

EDTA blood samples were analyzed for the presence of peripheral blood microfilariae using the wet mount, acridine orange stained blood smears by fluorescent microscope at 100 and 400 magnifications²², and modified Knott's test as described by²³, subsequently followed by microfilaria identification using key presented by Atkins (2005)²⁴.

For detection of the circulating antigen from the ovary of mature *D. immitis* female worms in amicrofilaremic dogs (diagnosis of an occult form of cardiac dirofilariasis), all sera were examined by a commercial antigen-enzyme-linked immune sorbent assay kit (heartworm Ag ELISA kit, canine, DRG International Inc., USA) according to manufacturer's instructions.

Statistical Analysis

Data obtained from the study were input and analyzed with the online statistical program (2-way contingency table analysis, In <http://statpages.org/ctab2x2.html>. Retrieved on 20/07/10). Cohen's kappa value (κ) was calculated to determine the compatibility between all tests (wet mount, acridine orange stained blood smears, and Ag ELISA) and modified Knott's test as a gold standard. The κ values were interpreted according to as the following: poor, $\kappa < 0$; light, $0 < \kappa < 0.20$; fair, $0.21 < \kappa < 0.40$; moderate, 0.41

$< \kappa < 0.60$; substantial, $0.61 < \kappa < 0.80$; and perfect, $0.81 < \kappa < 1.00$. The difference in the percentages of infection between the various animals were assessed by using chi square test. A *P* value $< .05$ was considered statistically significant.

RESULTS

A total of 49 samples were microfilaria positive, giving a prevalence value of 54.4% (n=90, CI 40.4-61.8). *D. immitis* antigens were detected in 27.8% (N=90, CI 18.9-38.2) of the examined sera, with an overall prevalence of 78.9% (Table 1). The results showed that the prevalence of disease in dogs varies according to forms of diseases (Table 2). Stray dogs showed higher prevalence for both microfilaremic and occult forms of cardiac dirofilariasis compared with police dogs in Iraq's Nineveh Governorate (Table 3).

The agreement between the modified Knott's test (gold standard) and wet mount (Figure 1), acridine orange stained blood smears (Figure 2), and Ag ELISA using Cohen's κ index was light for wet mount (0.030) (Table 4) and substantial for Ag ELISA (0.462) (Table 1) and acridine orange stained blood smears (0.607) (Table 4).

Table 1. Overall canine cardiac dirofilariasis prevalence of 90 dogs in Iraq's Nineveh Governorate

Tests	Number of positive dogs/90 (%)	95% confidence interval	K value
Modified Knott's test	49(54.4)	40.4-61.8	Gold standard
Ag ELISA	25(27.8)	18.9-38.2	0.462
Total	71(78.9)	69.0-86.8	0.0

Table 2. Forms of canine cardiac dirofilariasis in dogs of Iraq's Nineveh Governorate

Forms of disease	Microfilaremic	Occult	Mixed
Numbers of infected animals/90(%)	46 (51.1)*	15 (16.7)	10 (11.1)
Chi square	23.632		33.414
Significance level	P = .0001		P < .0001

*Significant at *P* < .05

Table 3. The distribution of forms of canine cardiac dirofilariasis in both stray and police dogs of Iraq's Nineveh Governorate

Forms of disease	Type of dogs (N=90)		Chi square	Significance level
	Stray N/45 (%)	Police N/45 (%)		
Microfilaremic	31(68.9)*	15(33.3)	11.285	P = .0008
Occult	9 (20.0)	6 (13.3)	0.720	P = .3962
Mixed	8 (17.8)*	2 (4.4)	4.049	P = .0442
Total N/90(%)	48 (53.3)*	23 (25.6)	14.374	P = .0001



Figure 1. A wet blood smear may reveal *D. immitis* microfilariae in a dog with heartworm disease. Magnification $\times 100$ of the microscope with a phone (Infinix) camera from top

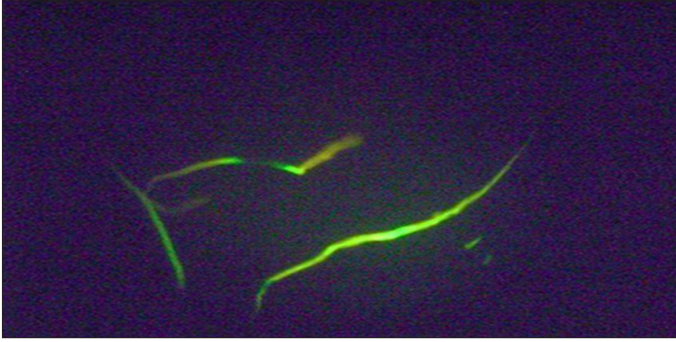


Figure 2. Microfilariae of *D. immitis* stained with acridine orange stain. Magnification $\times 100$ of the fluorescent microscope with a phone (Infinix) camera from top.

Table 4. Comparison of κ value and the compatibility between modified Knott's test (gold standard) and wet mount and acridine orange stained blood smears in its ability to detect canine cardiac dirofilariosis

Test	Number of positive/90	%	95% confidence interval	κ value
Wet mount	3	3.3	-0.042-0.030	0.030
Acridine orange stained blood smears	25	27.8	0.473-0.637	0.607

DISCUSSION

D. immitis is a widespread zoonotic filarial nematodal infection in dogs. The present study is the first one in dogs of Iraq's Nineveh Governorate. Previously, *D. immitis* has been reported in cats of Mosul city, Iraq (the same area of the present study) ²⁵. Our examination shows that canine cardiac dirofilariosis was reported with higher percentage of infection (78.9%) in Iraq's Nineveh Governorate. Our results are in agreement with previous study from Karbala province by ²⁶, who used the necropsy method and reported 73 % as a rate of infection. Iraq is situated in the southwestern part of Asia and has a favorable climatic condition with high temperature and moisture that encourages the distribution and development of the mosquito vector of *D. immitis*. In addition, Iraq is home to large numbers of stray dogs and wild carnivores that are hosts of various parasites, including *D. immitis*, that are not given anthelmintic treatment²⁷. The prevalence of canine cardiac dirofilariosis appears to be increasing globally, mainly because of climate change and the mosquito population density, mosquito fertility, environmental temperature, number of microfilaremic dogs, and diagnostic test used ^{24; 28}. In contrast, canine cardiac dirofilariosis prevalence is declining in some regions, including Japan²⁹ and northern Italy, possibly because of increased awareness and enhanced control of the disease^{10; 30}. Higher positivity for both microfilaremic and occult forms of canine cardiac dirofilariosis was observed among stray dogs in our study. It is one of the main factors that explain the wide spread of the disease in dogs of Iraq's Nineveh Governorate. This may be attributed to stray dogs living in unhygienic outdoor conditions supporting mosquitoes, lack of preventive treatment, and the activities that presumably increase its vector exposure rates ³¹. The police dogs, when imported to Iraq, spent several weeks in different patrol tasks in the entrance of the cities. Moreover, *D. immitis* has colonized these areas because of the availability of many heartworm reservoirs (the stray dogs), suitable weather, and high density of mosquitoes as the intermediate vectors.

The agreement (Cohen's κ index) between the modified Knott's test (gold standard) was 0.607 and 0.462 for the acridine orange stained blood smears and Ag ELISA, respectively.

In this study, both tests diagnosed a high percentage of infected dogs (patent and occult disease).

The modified Knott's test is the most useful parasitological method among concentration tests, being based on the detection and identification of microfilariae in blood samples ^{32; 33; 34}. The results of the modified Knott's test may be impaired by occult infections. The antigen test (Ag ELISA) was accurate enough for diagnosis of a high percentage of infected animals (occult infection); we still could not identify all cases of heartworm infection because antigen tests will only be positive if adult female worms are present, because the antigen detected is from the worm's uterus. If the heartworms were not fully mature (prepatency), if there were only male worms present (unisex infection), if there was immune-mediated clearance of microfilariae, or if there was drug-induced sterility of adult filariae, the antigen test result in infected animals would be falsely negative. This means that the test result is negative when the animal is really infected³⁵. Ogbaje and Abel-Danjuma (2016)³⁶ confirmed modified Knott's test to be the most sensitive in the diagnosis of canine dirofilariosis in dogs using parasitological technique.

Ranjbar-Bahador et al. (2007)³⁷ showed that the sensitivity and specificity of antigen detection test kit were higher 92.85% and 96.87%, respectively, when compared with modified Knott's test at 85.71% and 91.60%, respectively.

CONCLUSION

The findings of the present study provide evidence for the prevalence and pattern of canine cardiac dirofilariosis in dogs (both stray and police) in Iraq's Nineveh Governorate, requiring a phylogenetic analysis.

Ethics Committee Approval: This study was approved by the College of Veterinary Medicine Committee, University of Mosul , Iraq. (Approval No: 12, Date: 3-10-2021).

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