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Role of anti-inflammatory interleukin 10 in asymptomatic heartworm infection (Dirofilariasis) in dogs

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Abstract

Background: *Dirofilaria immitis* causes heartworm disease (HWD), a vector-borne zoonotic disease that primarily affects dogs and cats. Occasionally, human beings were reported to be infected as well. The current study aims to discover the asymptomatic dirofilariasis infection in dogs. In addition, to determine the prevalence of heartworm disease and the role of anti-inflammatory interleukin 10 (IL10) in developing the disease. Household dogs were selected from 10 veterinary clinics throughout Basrah, south of Iraq.

Methods: The study included 117 dogs older than 12 months, none of them had received heartworm vaccinations, and all of them lived in their owners' houses for at least 9 months. Animal ethics instructions were followed after the owner's consent was obtained. Physical and biochemical examinations were conducted including the examination of circulating antigens of microfilaria. The levels of anti-inflammatory IL10 and pro-inflammatory IL17, IL4, and IFN-γ were measured using ELISA tests. Descriptive statistics were used to evaluate the prevalence and the clinical and immunological results of the study.

Results: Canine heartworm disease prevalence was 29.05% (34 out of 117). The physical examination showed normal vital signs for both infected and non-infected dogs. A significant elevation in the total WBC count was noticed in the infected group. On the other hand, a significant decrease in RBCs count and hemoglobin was found in the infected group. There were neither changes in the platelet count nor the liver enzymes concentration between infected and non-infected groups. A significant increase in anti-inflammatory interleukin 10 level and a significant decrease in pro-inflammatory IL17, IL4, and IFN-γ were noticed in the infected dogs.

Conclusion: It is concluded that dirofilariasis infection is considered to be a serious life-threatening disease for dogs in Iraq. Therefore, a periodic test for heartworm infection every six months is recommended to eradicate heartworm infestations. The infected animals must be treated according to the American Heartworm Association recommendations.

Introduction

Dirofilariasis is one of the most common infectious heart diseases in dogs [1]. Due to the location of Iraq in southwest Asia with a warm climate, it has an ideal environment for the spread of heartworm disease (HWD). Clinical signs are generally related to cardiovascular and pulmonary systems. The severity of the signs depends on the worm' burden, the host's immune response, and the ability to restrict exercise. Hematological abnormalities such as anemia and leukocytosis with eosinophilia are common in dogs with clinical signs of HWD [2-4]. Effective management of canine heartworm diseases necessitates a full comprehension of the host-parasite interaction. As one might anticipate, there is a direct relationship between the number of worms and the severity of the disease. There is a lack in the relationship between heartworm count and pulmonary vascular resistance in spontaneously infected dogs. However, the host immunity interaction with the parasites plays a substantial role in the severity of the disease. Given that D. immitis worms develop a variety of immune evasion strategies over their lifetime despite being continuously exposed to the host immune system [5]. A short-term immune evasion strategy involves the release of surface antigens by infective larvae, whereas a long-term immune evasion strategy involves the adsorption of different molecules and cells from the host. In addition, controlling the production of cytokines from many regulatory cell types, including the overexpression of tumor necrosis factor (TNF-γ) [6] and interleukin 10 (IL10) [5, 7] and the downregulation of IL-4 [8] IL-5, and IL-13[9]. Although studies on the role of anti-inflammatory responses for filarial nematode have been reported, the role of immune responses remains poorly understood. The present study aimed to investigate the asymptomatic infection of canine Dirofilariasis in Basrah and evaluate the anti-inflammatory immune response and its relation to the clinical Dirofilariasis symptoms.

Methods

Animals of the study

Household dogs were selected from 10 veterinary clinics distributed throughout Basrah City south of Iraq. The dogs selected for this study had all been in their owners' homes for more than 9 months and neither received a heartworm treatment nor vaccine during that period. The study obtained an ethical committee clearance in compliance with BCVM regulations of the College of Veterinary Medicine University of Basrah.

Clinical Examination

Animals underwent a routine clinical examination emphasizing HWD diagnostic clinical indicators, such as dyspnea, cough, gastrointestinal symptoms, and other signs.

Blood collection

Syringes (5mL) with twenty-three-gauge sterile needles were used to collect blood samples via cephalic venipuncture. Samples were divided into two parts; the first part was placed in an anticoagulant vial for hematological analyses. While the other part was placed in a vial without an anticoagulant for biochemical analyses.

Detection of the dirofilariasis infection

VETSCAN® canine heartworm rapid test was purchased from Zoetis (USA). The kit was prepared and used according to the instructions of the manufacturer. Blood samples were added to the sample wells of the testing casket followed by the testing buffer solution. Results were recorded within 8 to 10 minutes. A commercial antigen-enzyme-linked immunosorbent assay kit (heartworm Ag ELISA kit, canine, DRG International Inc., USA) was used to confirm the positive results.

Determination of some hematological and biochemical parameters

A complete blood count (CBC) was performed on EDTA anticoagulated blood samples using a VetScan® automated hematology analyzer. For biochemical data, the blood was allowed to clot at room temperature (25±1°C) for 30 minutes and then centrifuged at 3000 rpm for 15 minutes. Sera were carefully harvested into labeled vials, and then immediately analyzed. Commercial test kits (Agappe, India) were used to measure the levels of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase; following the manufacturer's instructions.

Evaluation of serum IL-10, IL-17, IL-4 and Interferon-gamma by ELISA

Serum levels of interleukin 17 (IL-17) and interleukin 10 (IL-10) was determined. Canine IL-10 ELISA kit for dogs (ab193685) and canine IFN-γ ELISA Kit (ab193684) were purchased from Abcam® (Cambridge, United Kingdom). Canine Interleukin 17 ELISA kit (KitMBS901921) was purchased from Innovative Research (Vancouver, British Columbia, Canada). Canine IL-4 DuoSet ELISA kit (DY754) was purchased from R&D Systems Europe Ltd (northeast Minneapolis, USA). All kits were prepared and used according to the instructions of the manufacturers.

Statistical analysis

GraphPad Prism® 8 software (San Diego, CA) was used for the statistical analyses. The experiments were

conducted in triplicates and data was normalized with MS-Excel 2019. Unless otherwise indicated, the Unpaired t-test was used for statistical comparisons, and the Mann-Whitney test was applied to analyze differences between non-parametric groups. The significant differences were determined at P<0.05.

Results

The current study included 117 pet dogs that were tested for heartworm disease. All dogs included in this study were free of clinical signs, such as cough, dyspnea, gastrointestinal signs, and other clinical signs. Overall, the prevalence of asymptomatic canine heartworm infection was 29.09% (34/117) whereas the prevalence of non-infected dogs was 70.94% (83/117) as can be seen in figure1 A and B.

Vital parameters of the infected dogs

All vital parameters of infected animals showed no significant changes compared with the parameters of non-infected including (body temperature, respiratory rate, heart rate, and SPO2) as can be seen in Figures 2 A, B, C, and D respectively.

Changes in the hematological and biochemical levels

A complete blood count (CBC) showed that the total WBCs, lymphocytes, neutrophils, and eosinophils, except monocytes of infected animals, were significantly higher than those of non-infected animals (Fig. 3 A, B, C, D and Fig. 4 A, B). In contrast, the red blood cells and hemoglobin concentration were significantly lower in the infected animal groups as can be noticed in Figures 4 C and D.

In addition, dogs with symptomatic infection had no change in ALT, AST, and ALP levels compared to non-infected dogs as in Figure 5 A, B, and C respectively.

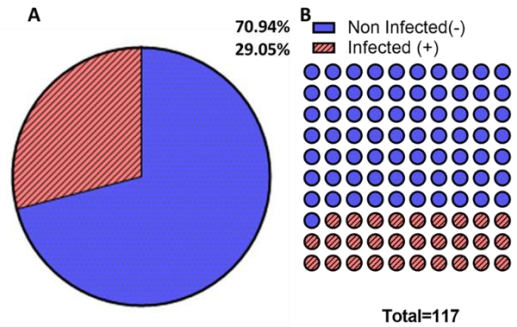

Figure 1: The prevalence rate of morbidity of canine dirofilariasis in Basrah City - Iraq. Charts A & B represent the number and the percentage of infected (34, ≈ 30%) and non-infected dogs (83, ≈ 70%) (total n = 117).

Changes in serum inflammatory biomarkers

As a result of measuring serum interleukin 17 (IL-17), a common pro-inflammatory biomarker, the results showed that levels were decreased in dogs with asymptomatic dirofilariasis (P < 0.05). However, when compared with non-infected groups, asymptomatic dirofilariasis groups had significantly higher levels of interleukin 10 (IL-10) and interleukin 4 (IL-4) as seen in figures 6 A and C) respectively.

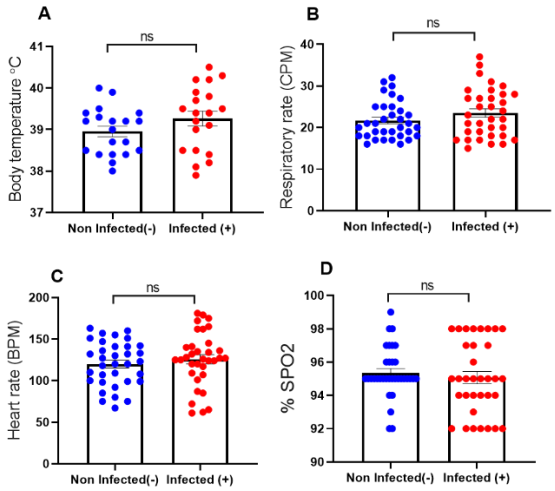

Figure 2: Changes in the vital parameters between non-infected and infected dogs with dirofilariasis. There were no changes in the vital signs indicating an asymptomatic infection. A) Body temperature in Celsius (n=20). B) Respiratory rate in cycles per minute (n=34). C) Heart rate in beats per minute (n=34). D) Percentage of blood oxygen saturation (n=34). Values were reported as means ± SEM. The unpaired T-test was used to establish significance when two-tailed P < 0.05. Mann-Whitney test was used to compare the % SPO2 (D).

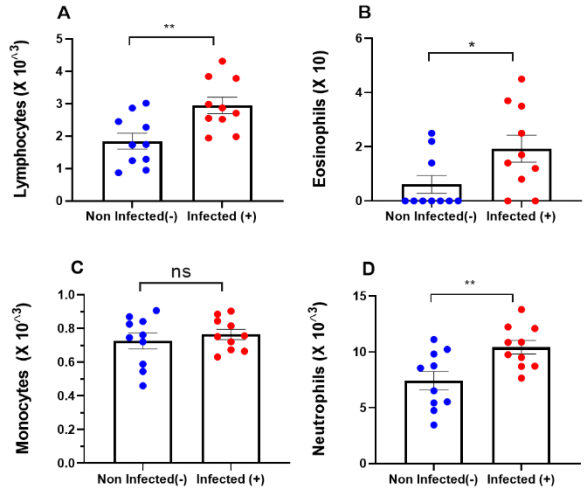

Figure 3: Changes in the differential leukocyte count between non-infected and infected dogs with dirofilariasis. A) Lymphocytes. B) Eosinophils. C) Monocytes. D) Basophils. All parameters (A-D) are measured as an absolute cell number /µl (n=10). Values are reported as means ± SEM. The unpaired T-test was used to establish significance when two-tailed P < 0.05.

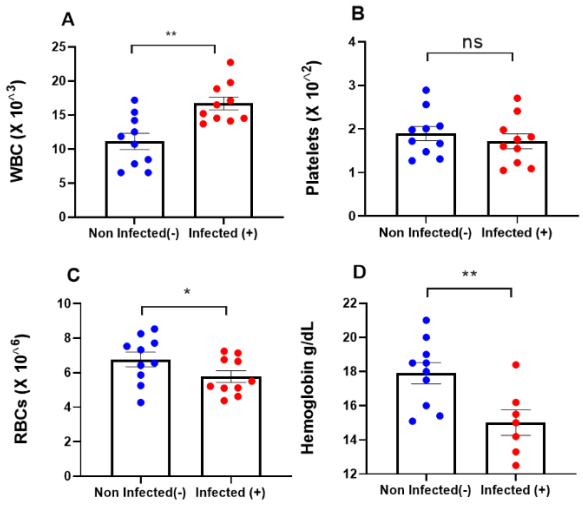

Figure 4: Changes in some hematological parameters between non-infected and infected dogs with dirofilariasis. A) Total white blood cell count measured as an absolute cell number /µl (n=10). B) Platelets count measured as an absolute number /µl (n=10). C) Red blood cells measured as an absolute cell number /µl (n=10). D) Hemoglobin (g/dl) (n=10). Values are reported as means ± SEM. The unpaired T-test was used to establish significance when two-tailed P < 0.05.

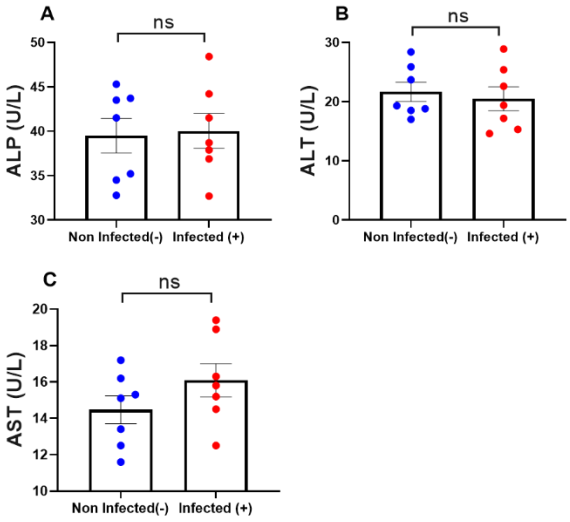

Figure 5: Changes in some biochemical parameters between non-infected and infected dogs with dirofilariasis. A) Alkaline phosphatase. B) Alanine transaminase. C) Aspartate aminotransferase. All parameters (A-C) are measured as units per liter (n=7). Values are reported as means ± SEM. The unpaired T-test was used to establish significance when two-tailed P < 0.05.

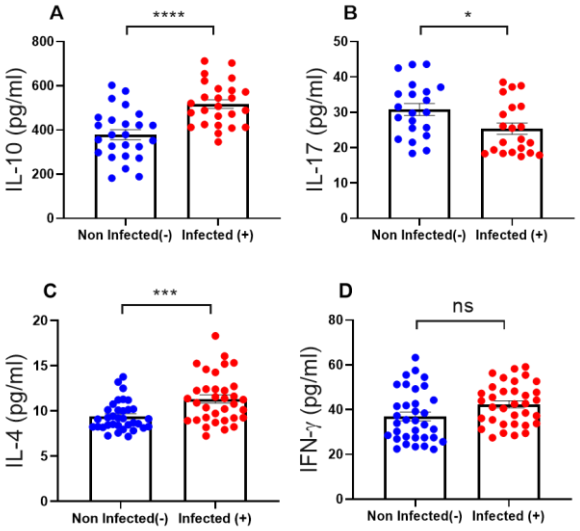

Figure 6: Changes of some serum inflammatory biomarkers between non-infected and infected dogs with dirofilariasis. A) Interleukin 10 concentration (n=25). B) Interleukin 17 concentration (n=21). C) Interleukin 4 concentration (n=34). D) Interferon-gamma concentration (n=34). All parameters (A-D) are measured as picograms per milliliter. Values are reported as means ± SEM. The unpaired T-test was used to establish significance when two-tailed P < 0.05. Mann-Whitney test was used to compare the IL-4 (C).

Discussion

Infections caused by Dirofilaria immitis are widespread among dogs worldwide. As the best of our knowledge, the current study is the first to be conducted on dogs in Basrah City Iraq. Infections with *Dirofilaria immitis* have previously been reported in dogs of other cities of Iraq such as Mosul and Karbala. A percentage of asymptomatic infection 29.09% (34/117) among household dogs in Basrah city was recorded in this study. These results are not in consistent with previous studies from other provinces in Iraq [10, 11], which reported a rate of infection of 73 % based on necropsy.

Since this study proved that there are no significant changes in the vital parameters and the clinical signs between non-infected and infected dogs; therefore, this disease is called asymptomatic.

Previous studies reported that dogs with heartworm disease are more likely to have mild non-regenerative anemia, neutrophilia, eosinophilia, basophilia, and thrombocytopenia [4]. Anemia combining dirofilariasis in dogs has been reported in several retrospective studies such as [12-14]. In this study, the overall prevalence of anemia was significantly high in infected dogs which completely agrees with recent studies that demonstrated the prevalence of anemia to be 14.5% [3] and another study 15.3% [15] in dogs. The major causes of anemia in dogs could be due to hemorrhage, hemolysis, and decreased production, as a result of chronic inflammation. Despite a previous study [16]

suggesting that mild non-regenerative anemia is more common in dogs with severe dirofilariasis, it is believed that hemolytic anemia is more likely to have a regenerative response.

It has been shown in recent research that dirofilariasis is primarily associated with a complex immune response. This might be because *Dirofilaria immitis* can infect different hosts, achieving different levels of immunity and causing different pathologies. Furthermore, the presence of symbiotic bacteria in the larvae as well as the adult worms [12, 13]. The inflammatory response commonly seen in cases of dirofilariasis and the sorts of reactions caused by the two antigenic sets are associated with parasite viability [13].

In general, IFN-γ and IL17 are important cytokines related to proinflammatory activity, whereas IL-10 is involved in the immunoregulatory functions. Th1-mediated responses have been associated with parasitic infection control [17, 18]. On the other hand, IL-10 prevents the host tissue damage that results from the exacerbated inflammatory response. In addition, IL-10 inhibits macrophagesand dendritic cells responses to parasitic infection which facilitates the parasites asymptomatic infection.

At the cytokine and antibody levels, *Dirofilaria immitis* displayed a simultaneous Th1/Th2 immune response. Th2 response is primarily triggered by *Dirofilaria immitis* antigens, while Th1 response is usually triggered by symbiotic bacteria [14]. Infections with *Dirofilaria immitis* in dogs are associated with Th2 responses characterized by the production of IL-4 and IL-10 Messenger RNAs and Immunoglobulin G1 (IgG1). In contrast, microfilaremia canine infections trigger a Th1 response characterized by a lack of IL-10 expression and an increase in IgG2 production and inducible nitric oxide (NO) synthase (iNOS) expression [19]. It has been shown that the absence of cellular response in helminth infections is associated with the production of anti-inflammatory cytokines IL-10 and transforming growth factor beta [20], and IL-10 expression is associated with hypo-responsiveness in lymphatic dirofilariasis [15, 21, 22]. It seems that in dogs with heartworm disease, circulating worms are not inducing the host immunity (Th2), allowing adult worms to survive for a long period [18].

Competing Interest

The authors declare no conflict of interest.

Author Contributions

Haider Rasheed Alrafas, Jala Amir Salman Alahmed, and Israa Muhsen Essa contributed to the overall research design and manuscript preparation, Original Manuscript Draft, Writing – Editing, and Revisions. Haider Rasheed Alrafas performed the majority of experiments and the analysis of some data presented in the manuscript. Jala Amir Salman Alahmed contributed by preparing and providing physiological and biochemical blood examinations for all experimental groups. Hassan M. Al-Tameemi, Mohammed R. Abduljaleel, and Murtakab Younis Al-Hejjaj assisted with collecting data for animal experiments including performing and measuring the clinical signs collecting serums for ELISA explant, and preparation of samples for analysis. Sabah Zyara Kadhim and Farhan Zameer assisted in the statistical analysis and interpretation of some data. All authors read and approved the final manuscript.

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