



**In Iraq, The First Application of Serological SNAP ELISA
Technique In Detection of Canine Heartworms (*Dirofilaria immitis*)
In Herder Dogs of Al-Qadisiyah and Dhi-Qar Provinces**

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Abstract

Canine heartworm disease is one of the most pathogenic parasitic infections in the temperate countries, which had importance large effects on public health as could be infecting human. Serologically, this study is the first one in Iraq, which dealt with detection of the disease in dogs by using of SNAP ELISA technique. The overall seroprevalence for (172) herder dogs submitted to this study, was (40.12%), comprising (42.48%) and (35.59%) in Al-Qadisiyah and Dhi-Qar provinces, respectively. Also, the severity of infection in seropositive dogs had been estimated as (42.59%) for low infection level and (53.62%) for high *D. immitis* infection level. In association with the sex factor, the seropositive rates were (39.53%) for females and (41.86%) for males. Whilst, in relation to age factor, (32.35%) of less than 4 years dogs and (45.19%) of more than 4 years dogs, were infected, serologically. Statistically, the significant differences were showed between the seropositive results of examined provinces, intensity of infection, as well as the risk factors (age and sex) at a level of $P \leq 0.05$.

Keywords: Canine heartworms, *Dirofilaria immitis*, SNAP ELISA, Al-Qadisiyah, Dhi-Qar

**في العراق ، الاستخدام المصلي الاول لتقنية سناب اليزا في تشخيص ديدان القلب الكلابية
(دايروفيلاريا ايميتس) في كلاب الرعي لمحافظة القادسية وذي قار**

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الخلاصه:

يعتبر مرض ديدان القلب الكلابية واحد من اكثر الاصابات الطفيلية ذات الامراضية العالية في البلدان الدافئة ، والذي يمتلك تأثير كبير ومهم على الصحة العامة بسبب امكانية اصابته للأنسان . مصليا ، تعتبر الدراسة الحالية الاولى في العراق التي تناولت تشخيص المرض في الكلاب باستعمال تقنية سناب اليزا . كانت نسبة الاصابة الكلية ل (172) كلب رعي خضع لهذه الدراسة هو (40.12) % ، تضمنت (42.48) % و (35.59) % في محافظتي القادسية وذي قار ، على التوالي . كذلك ، تم تقدير شدة الاصابة في الكلاب الموجبة مصليا للاصابة وكانت (42.59) % لمستوى الاصابة المنخفضة و (53.62) % لمستوى الاصابة العالية بالدايروفيلاريا ايميتس. فيما يتعلق بعامل الجنس ، كانت نسب الاصابة الموجبة مصليا (39.53) % للاناث و (41.86) %

للذكور . اما فيما يتعلق بعامل العمر ، (32.35%) من الكلاب التي تقل اعمارها عن 4 سنوات و (45.19%) من الكلاب التي تزيد اعمارها عن 4 سنوات ، كانت مصابة مصليا .
الكلمات المفتاحية : ديدان القلب الكلابية ، دايروفيلاريا ايميتس ، سناب اليزا ، القادسية ، ذي قار

Introduction

Canine heartworm is a vector-borne disease of domesticated canines and felines as well as humans, due to the infection with a roundworm parasite, *Dirofilaria immitis*, belongs to phylum Nematoda of Onchocercidae family (1). The disease is counted as one of the most life-threatening pathogenic problems in dogs and appears that all individuals of the genus, *Canis*, engaged in expansion of disease and act as reservoirs (2, 3). Globally, *D. immitis* reported, firstly, in dogs of United States in 1847, while in Iraq; it's first recorded in dogs in 2009, then in humans in 2013 (4, 5, 6). As well as, its exist in most continents and thought to be that the origination began from Asia and transmitted to the Americas by the dogs of early scouts and emigrants (7). Epidemiologically, several studies demonstrated that the heartworm's infections stay increasing, dramatically, and more than (70) species of mosquitoes serve as intermediate hosts (8). During feedings and activeness season, the parasitized mosquitoes have the ability for transmitting the infection to final host at any period and the severity of disease depend, mainly, upon the number and location of adult worms (9). Clinically, the disease perhaps be, completely, symptomatic or appearing the signs, slowly and gradually, and cannot depending on it (10). The accuracy of diagnostic methods is very importance factor in demonstration of microfilariae worms in infected dogs and several detectable techniques are available and can provided a precise recommended data (11). Recently, Snap 4Dx ELISA test is approved and

used, regularly, to detect the circulating antigens (proteins) which released by the reproductive tracts of mature female worms (12). With very low worm burdens (≤ 2 worms), the sensitivity of test in dogs is 91.7% and with (> 2 worms) was 100 %, while the specificity of test was 100% (13).

The objectives of the present study were to:

1. First detection of canine heartworms in some rural areas of Al- Qadisiyah and Dhi-Qar provinces, by using a new advance technique (Snap ELISA test) that used for first time in Iraq.
2. Provide further information about the prevalence of infection in Iraq to for futurism studies and to take a new control measures to prevent spreading for more dogs and/or humans.

Materials and Methods

In some rural areas of Al- Qadisiyah and Dhi-Qar provinces, and during the period (August 2015 to May 2016), a total of 172 adult herder dogs were submitted to this study including both sexes (129 females and 43 males), which divided into two age groups (68 dogs with less than 4 years and 104 dogs more than 4 years). From each dog, about 3 ml of blood sample was taken from cephalic vein by using vacuum tubes. The serums were obtained after centrifugation of blood samples, packaged into a numbered 1 ml micro-tubes, and then frozen at (-20°C) (14).

According to manufacturer's instructions, the SNAP 4Dx Enzyme Linked Immunosorbent assay (ELISA, IDDEX Laboratories, USA) kits were designed to detect the presence of

circulating *D. immitis* antigens in dogs. According to the severity of infection, the seropositive results were divided into two levels (high and low) depending on the color development as a response to the proportion of heartworm antigen concentration in positive samples (15).

Statistically, all attained data were analysed by application of Chi-square at a significant difference's level $P \leq 0.05$ of with using the IBM SPSS v.23 program. The seropositive rates of disease incidence were compared with the severity of infection, variations in prevalence between the two examined provinces and the risk factors (age and sex) (16).

Results and Discussion

Canine heartworm is a life-threatening disease that affecting, mainly, the dogs and other individuals of canine and

feline as well as human (17). "The relocation of infected dogs appears to be the most important factor contributing to further dissemination of the parasite (18). The ubiquitous presence of one or more species of vector competent mosquitoes makes transmission possible wherever a reservoir of infection and favorable climatic conditions co-exist" (19, 20). However, the specificity and the sensitivity of SNAP ELISA test, which used in this study, was ranged (95-100) % and the false-negative results can be occur due to the low worm counts, immature infections, and all-male infections (21).

The results of (Table 1) revealed a totally seropositive rate of infection was 69 (40.12%), detailed as 48 (42.48 %) and 21 (35.59 %) in Al-Qadisiyah and Dhi-Qar provinces, respectively

Table (1): Seroprevalence of canine heartworm infection according to examined provinces

Province	Positives	Negatives
Al-Qadisiyah (113)	48 (42.48 %) ^a	65 (57.52)
Dhi-Qar (59)	21 (35.59 %) ^b	38 (64.41)
Total (172)	69 (40.12 %)	103 (59.88)

The difference in small letters, vertically, referred to a significant difference at level $P \leq 0.05$

In Iraq, only, one study dealt with the detection of heartworms in dogs of some regions in Karbala province by (5) through using the necropsy method, and reported (73 %) as a rate of infection. The variation in prevalence of heartworm infection among dogs presented to this study in the two provinces might be due to canine population, regional variation in reservoir species and mosquito vector, and climatic or ecological elements (22). It is widely anticipated that climate change will impact the spread of vector-borne diseases because the weather not influences on the development and maintenance of the

vectors, but also, influence on vector habitat, and the weather should be warm, sufficiently, to allow for presence of mosquitoes (23, 24). "Although, the risk of infection in dogs was varies from one region to another (in the same country) and even from one community to another, one fact remains heartworm disease is a threat to unprotected dogs in every state (25). The unprotected dogs, foxes, coyotes, and wolves act as reservoirs, or sources, for the spread of this serious disease (26). The relocation of dogs, as with humanitarian efforts following natural disasters, can introduce the heartworm disease into the parts of

country where it is not normally found (10). Furthermore, unprotected dogs traveling with their owners to areas where heartworms exist will be at risk for heartworm exposure. Heartworm disease is a complicated and deadly illness - the best approach is prevention” (27).

According to the manufacturer’s instructions, the kit of SNAP ELISA test had the ability for showing two levels of reaction (color intensity) that depended on the proportional of

heartworm antigen concentration in positive samples. The results of (Table 2) demonstrated that 32/69 (46.38%) had low antigen levels and 37/69 (53.62%) had high antigen levels. The prevalence of high antigen levels in seropositive dogs could be explained by the situation of infection (patent or occult), severity of infection, failure of immunity and/or persistence of abundance vectors or reservoirs (28, 29).

Table (2): Levels of infection’s severity in seropositive dogs

Total Seropositive Dogs	Low severity	High severity
69 (42.59 %)	32 (46.38 %) ^b	37 (53.62 %) ^a

The difference in small letters, horizontally, referred to a significant difference at level $P \leq 0.05$

In (Table 3), the seroprevalence of canine heartworm according to sex was 51/129 (39.53%) in females and 18/43 (41.86%) in males, while in association to age, the incidence of infection was 22/68 (32.35%) and 47/104 (45.19%) in less than and more than 4 years, respectively.

Table (3): Seroprevalence of infection according to sex and age

Risk Factors	No.	Seropositive Dogs	Seronegatives Dogs
Sex	Female (129)	51 (39.53 %) ^b	78 (60.47)
	Male (43)	18 (41.86 %) ^b	25 (58.14)
Age	Less than 4 years (68)	22 (32.35 %) ^b	46 (67.65)
	More than 4 years (104)	47 (45.19 %) ^a	57 (54.81)

The difference in small letters, vertically, referred to a significant difference at level $P \leq 0.05$

Generally, the present study indicated that mean seroprevalence of *D. immitis* infection in dogs increased with age. Similar results were recorded, previously, by many studies (30, 31, 32). A possible explanation for the higher seroprevalence of *D. immitis* infection in aged dogs could be the results of their prolong exposition to danger factor, vector (33). Also, (34) indicated that the age of dogs was an important risk factor and determined by time of exposure in the endemic area. Moreover, the higher infection rate in male dogs had been postulated to be due to their stronger attraction to mosquitoes (34, 35). The large extent

of seroprevalence in males may, also, due to the fact that” more males’ dogs are kept outdoors for their use to defend safety and property” (36). But it is more difficult to explain why males are more subject to infection than females. However, when estimating the seroprevalence of disease according to sex, no significantly variation was noted between both sexes of several studies done by (37, 38).

In conclusion, the present study showed that the disease of canine heartworms might be endemic in dogs of the rural areas in Al-Qadisiyah and Dhi-Qar provinces, and the infection is more severe than expect, more

prevalence in males than females, and increased with age

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