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Serorevalence of *Dirofilaria immitis* in dogs and cats in Riyadh City, Saudi Arabia

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Abstract. The aim of present study was to determine the seroprevalence of *Dirofilaria immitis* in dogs and cats from Riyadh, Saudi Arabia. Blood samples were collected by cephalic and jugular venipuncture from 294 dogs and 190 cats from Riyadh. Serum samples were tested against circulating *D. immitis* antigen using DiroCHEK®, SYNBIOTICS Corporation, San Diego, CA 92127, USA and anti-*D. immitis* antibodies using Green Spring *D. immitis* IgG antibody ELISA test kit, Shenzhen Lvshiyuan Biotechnology Co., Ltd. Guangdong, China. *D. immitis* antigens were detected in 7.82% of the dogs and in 1.58% of the cats, while antibodies against *D. immitis* were detected in 13.61% of the dogs and 2.11% of the cats. In outdoor dogs, antigens of *D. immitis* were detected in 10.33% while antibodies were detected in 16.85%. A significant difference in the seroprevalence of *D. immitis* antibodies in dogs was reported in the summer compared to the winter ($p<0.05$). There was no significant difference observed in the seroprevalence between males and females dogs. Older dogs (3-6 years) showed higher prevalence of *D. immitis* antigen compared to younger dogs, in the contrary *D. immitis* antibody prevalence was higher in younger dogs (1-3 years) compared to old dogs. In cats, the prevalence of *D. immitis* antigen and antibody was high in males compared to the females, however, the difference was not statistically significant. There was no effect for the season and age in the prevalence of *D. immitis* in cats.

INTRODUCTION

Dirofilaria immitis is a filarial nematode of the genus *Dirofilaria* commonly known as the heartworm due to the location of the adults in the arteries of the lungs and occasionally in the right ventricle of the heart. It is found in over 30 mammalian species including dogs, cats, wolves, ferrets, coyotes, foxes and other wild carnivores (Otto, 1975). Dogs are the definitive host and serve as the main source of infection, with the heaviest worm burden. *D. immitis* has also been reported in felids but the low microfilaraemiae indicates that feline hosts play no significant role in the transmission of this parasite (Genchi *et al.*, 1988). Heartworms can also be transmitted to humans but worms cannot reach maturity and pre-adult worms are responsible for pulmonary dirofilariasis (Genchi *et al.*, 1988). *D. immitis* is transmitted by culicid mosquito vectors such as *Culex*, *Aedes*, *Anopheles* and *Culiseta* (Morchon *et al.*, 2012; 2012). The prevalence and transmission of *D. immitis* in dogs and cats depends on the presence of dogs infected with adult worms producing microfilariae. The

prevalence can vary largely in different regions of the world probably due to certain epidemiological factors such as the distribution of the mosquito species (vector), mosquito population density, mosquito fertility, environmental temperature, animal behavior, living conditions and the average age of the susceptible host (Atkins, 2005). The choice of diagnostic methods and situation of infection patency or occult heartworm infection may also affect the prevalence. *D. immitis* is widely distributed in the canine population of Mediterranean and Middle Eastern countries, including Egypt, and Turkey (Al-Kappani *et al.*, 2011; Yaman *et al.*, 2009). It also occurs in the north-western part of Iran (Azari-Hamidian *et al.*, 2009). In Europe, the most endemic area is the Po river valley in northern Italy, where the prevalence of *D. immitis* infection in dogs was 40–80% and 24% in cats (Kramer and Genchi, 2002; Genchi *et al.*, 2005).

D. immitis has been studied exhaustively, showing great regional and local variations in their prevalence worldwide (Genchi *et al.*, 2005). The involvement of vectors in this parasite life cycle makes its transmission and distribution correlated with global climate change, and rates have undergone rapid and significant changes in defined geographic regions in recent years. In cats with patent heartworm infections, microfilariae are also detectable seven to nine months post-infection. However, microfilaremia occurs only in 20 percent of cats with mature heartworms (McCall *et al.*, 1992). In cats, adult worms survive for only two to four years whereas in dogs, they survive five to seven years (Venco *et al.*, 2008).

High prevalence rates of *D. immitis* infection in dogs was reported from Malaysia (70%) (Genchi *et al.*, 2001), Japan (46.8% and 59%) (Fujinami *et al.*, 1983; Nogami & Sato, 1997), South Korea and Taiwan (28.3- 40-57%) respectively (Lee *et al.*, 1996; Song *et al.*, 2003; Wu and Fan, 2003). Various methods were used in the diagnosis of *D. immitis* infection in dogs and cats and these methods include: detection of circulating antigen and antibody, concentration tests for microflariae, as well as PCR, radiography and echocardiography (Rubin *et al.*, 2010).

In Saudi Arabia, there were no report regarding heartworm (*D. immitis*) infection in dogs or cats. However, only few scattered reports on *D. repens* in dogs have been published from the Middle Eastern countries and only one report from Saudi Arabia (Tarello, 2003; 2008; 2011). Natascia & Tarello (2011) identified 3 microfilariae of *D. immitis* in a 20-month old female saluki in Qatar which was imported from Syria using the modified Knott test. The disease was confirmed by using a rapid assay test system (IDEXX SNAP) and x-ray. There was no report of feline dirofilariasis in the Middle East; except for the report by Al-Kappany *et al.* (2011) who reported 3.4% of *D. immitis* infection in feral cats in Egypt.

In the present study an attempt was made to investigate the seroprevalence of *D. immitis* infection using antigen and antibody ELISA in dogs and cats from Riyadh city in Saudi Arabia.

MATERIAL AND METHODS

Study area

This study was carried out in the Riyadh city, central region of Saudi Arabia. Riyadh lies at the center of the Arabian Peninsula on latitude 34°–38° N and longitude 46°–43° E approximately 1,950 feet (600 meters) above sea level. Riyadh is a city with a population of 5.7 million inhabitants. It is noticeable that people in Riyadh are developing an impressive interest in the acquisition of pet animals especially dogs and cats due to the changes in lifestyle. Several expatriates in the country bring their pets with them which may probably lead to the emergence of new diseases never experienced before, such as filariasis.

The Riyadh climate is marked by extremes of temperatures, with low humidity throughout the year, particularly in the summer season. The temperature varies greatly between night and day. In the summer, the highest average temperature ranges between 40°C and 43°C. Humidity ranges from 10% to 13% (www.pme.gov.sa). In the winter, it is cold, with the highest temperature ranging between 20°C and

28°C, and the lowest between 8°C and 14°C. The temperature in the winter occasionally goes down to as low as -2°C, while the humidity ranges between 40% and 49%. Rainfall ranges from 4.9 to 5.2 inches. Climatic conditions in farms where hunting dogs are kept for breeding provide ideal situation for the development of culicid mosquito vectors of *Dirofilaria* spp.

Selection of animals

Blood sample were collected from 484 animals during the study (294 from dogs and 190 from cats). Of dogs investigated in the present study, 110 were kept indoor and 184 were left outdoor. Similarly, cat samples were from 160 indoor and 30 were from outdoor cats. For each individual age, sex, breed, and rearing condition were recorded. Blood samples were collected from dogs and cats by cephalic or jugular venipuncture using 20 g x 1/2 inch needles. Blood samples were collected in plain vacutainer tubes (without anticoagulant). Serum was collected after centrifugation of the clotted blood and stored at -20°C till use.

Indoor dogs are those brought by owners to the private clinics in Riyadh for routine vaccination, minor surgeries, issuance of health certificate, training, grooming, and for boarding. Outdoor dogs are those kept in farms as guard dogs and some were stray dogs. Indoor cats are also those brought by owners to the clinics for routine clinical examination or vaccination while stray cats are those found in streets, parks and the vicinity of places such slaughterhouses, restaurants and residential compounds. These cats were baited with food containing a sedative (Acepromazine 12.5 mg; Veto-quinol, France) or darted with a combination of xylazine hydrochloride 2% (Rompun®, Bayer, Leverkusen, Germany) and ketamine 10% (Ketaminol vet.; MDS Animal Health, Intervet Boxmeer, The Netherlands) using a blowpipe.

All dogs and cats which were less than 1 year were excluded from the study. Dogs and cats which receive any regular deworming agents were also excluded from the study.

Antigen ELISA (Direct ELISA)

Antigen from the ovary of mature *D. immitis* female worms was detected by using an enzyme-linked immunosorbent assay (ELISA) kit (DiroCHEK®, SYNBIOTICS Corporation, San Diego, CA 92127, USA), following the manufacturer's instructions. All samples collected from dogs and cats were subjected to the antigen ELISA using DiroCHEK. Blue color development indicates the presence of heartworm antigen in the sample, and the test has high sensitivity and specificity in canine and feline serum.

Antibody ELISA (Indirect ELISA)

Serum samples collected from dogs and cats were tested for the presence of anti *D. immitis* antibodies using the indirect ELISA method. The test was performed using the Canine HD Ab (Green Spring *D. immitis* IgG antibody ELISA test kit, Shenzhen Lvshiyuan Biotechnology Co. Ltd. Guangdong, China) for the determination of HD Ab concentration in canine and feline sera. The test was performed according to the manufacturer's instructions. The optical densities were measured at 480 nm using Microplate Reader (Molecular Devices Spectramax 190, Bio-Rad Laboratories, Hercules, California, USA). Cut-off points of enzyme-linked immunosorbent assay (ELISA) for *D. immitis* was 0.8.

Specificity and sensitivity

The specificity and the sensitivity of tests were calculated according to the following formulae:

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive + False negative}} \text{ X } 100$$

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative + False positive}} \text{ X } 100$$

Statistical analysis

Statistical comparisons were carried out using Chi square test in GraphPad statistical software (Prism 6.0). The statistical significance level was set at $p<0.05$.

RESULTS

Dirofilaria immitis antigen was detected in 23 (7.82%) while antibodies against the same worm were detected in 40 (13.61%) of the dogs investigated (Table 1). The association between *D. immitis* infection and risk factors (sex, age, rearing condition and season) was shown in Table 2. There was no significant difference in the seroprevalence of *D. immitis* antigen between males (7.91%) and females (7.74%) dogs investigated. The same was found in the Ab ELISA test where 11.61% of males and 15.83% of females dogs investigated were positive. There was no significant difference in the seroprevalence between young and old dogs ($p>0.05$). Outdoor dogs showed significantly high seroprevalence compared with indoor dogs on both Ag and Ab ELISA ($p=0.05$). There was a significant difference in the prevalence in the summer compared with the winter in both methods with the seroprevalence higher in the summer in both methods ($p<0.05$).

The seroprevalence of *D. immitis* in cats was found to be 1.58% and 2.11% using Ag and Ab ELISA respectively (Table 1). There was no difference in the seroprevalence in the three risk factors categories using both methods (Table 3).

The sensitivity of the antigen ELISA test in dogs cats was found to be 100% whereas the specificity was found to be 88.6 for the dogs and 97.9% for the cats.

DISCUSSION

The results of the present study represent the first evidence of the occurrence of *Dirofilaria immitis* in dogs and cats population in Riyadh City, Saudi Arabia. Previously *Dirofilaria (Nochhtiella) repens* has been reported from cutaneous lesions in

Table 1. Results of the seroprevalence of *D. immitis* in dog and cats in Riyadh using Antigen and Antibody ELISA

Animals Examined	Results of Ag ELISA		Results of Ab ELISA	
	Positive (%)	p value	Positive (%)	p value
Dogs (294)	23 (7.82%)	0.003	40 (13.61%)	0.0001
Cats (190)	3 (1.58%)		4 (2.11%)	

Table 2. Results of the Antigen and Antibody seroprevalence of *D. immitis* in dogs, correlation with sex, age, rearing condition and season in Riyadh

Variable	No Examined	Results of Ag ELISA		Results of Ab ELISA	
		Positive (%)	p value	Positive (%)	p value
Sex					
Male	155	12 (7.74%)	1.0	18 (11.61%)	0.31
Female	139	11 (7.91%)		22 (15.83%)	
Age (Years)					
1-3	181	11 (6.08%)	0.18	24 (13.26%)	0.86
3-6	113	12 (10.62%)		16 (14.16%)	
Rearing condition					
Indoor	110	4 (3.64%)	0.04	9 (8.18%)	0.03
Outdoor	184	19 (10.33%)		31 (16.85%)	
Season					
Summer	152	19 (12.5%)	0.002	29 (19.08%)	0.006
Winter	142	4 (2.82%)		11 (7.75%)	

Table 3. Results of the Antigen and Antibody seroprevalence of *D. immitis* in cats, correlation with sex, age, rearing condition and season in Riyadh

Variable	No Examined	Results of Ag ELISA		Results of Ab ELISA	
		Positive (%)	p value	Positive (%)	p value
Sex					
Male	109	3 (2.75%)	0.26	3 (2.75%)	0.63
Female	81	0		1 (1.23%)	
Age (Years)					
1-3	120	3 (2.5%)	0.29	2 (1.67%)	0.62
3-6	70	0		2 (2.86%)	
Rearing condition					
Indoor	160	3 (1.86%)	1.0	4 (2.5%)	1.0
Outdoor (stray)	30	0		0	
Season					
Summer	106	3 (2.83%)	0.25	3 (2.83%)	0.63
Winter	84	0		1 (1.19%)	

dogs and humans in Saudi Arabia, however, there was no evidence of *D. immitis* involvement in those studies (Tarello, 2001; 2008). The failure to detect *D. immitis* during those studies was explained by the absence of a suitable vector or the adverse conditions prevailing in Saudi Arabia which may have affected the persistence of the vector during the time those studies were conducted. Various species of culicid mosquitoes (*Culex* spp., *Aedes* spp., *Anopheles* spp.) act as vectors for the transmission of *D. immitis*. Some species of *Aedes*, *Culex* and *Anopheles* have been reported in Saudi Arabia (Alahmad *et al*., 2009; 2011; Al-Khreji, 2005).

Similar results to what have been obtained in the present study have been reported from different countries (Yildirim *et al*., 2007; Maia *et al*., 2015). *D. immitis* was prevalent in 9.6% of dogs in Turkey while it was in 9.4% in Portugal (Yildirim *et al*., 2007; Maia *et al*., 2015). Lower results were reported by various authors from different countries (Ng *et al*., 2012; Xia *et al*., 2012; McCown *et al*., 2014; Vieira *et al*., 2015). Considerably high seroprevalence as high as 27.3% have been reported from Portugal (Vieira *et al*., 2014).

Evidence of *D. immitis* antibodies were found in 13.61% of the dogs investigated in the present study, and this is less than 34.7% reported by Glickman *et al*. (1984) in USA. The presence of antibodies only indicates that an infection occurred and does not provide a guarantee that it still exists, while a positive antigen test result is indicative of an active adult infection (McCall *et al*., 2008). Positive antigen test has shown to be highly specific, but sensitivity may decline in dogs with worm burdens of two female heartworms or fewer, although it is more closely related to the actual weight of worm present (Cardoso *et al*., 2010). The results of the present study revealed that there is no significant difference in the seroprevalence of *D. immitis* between males and females dogs using Ag and Ab ELISA. This finding is similar to other previous studies (Panday *et al*., 1981; Ryo *et al*., 1992; Fan *et al*., 2001; Yaman *et al*., 2009; Borthakur *et al*., 2015).

There are contradictory reports with regards to relevance of heartworm infections and age (Martin & Collins, 1985; Song *et al*., 2003; Fan *et al*., 2001). The risk of dogs getting infected will probably stay throughout life and the likelihood of acquiring infection with *D. immitis* increases with age and length of exposure to the infected mosquitoes (Rhee *et al*., 1998). Thus, older dogs will have longer time of exposure and more have more opportunities to become infected with heartworm. However, Selby *et al*. (1980) indicated that the age of dogs are an important risk factor and also determined at the time

of exposure in endemic areas. In the present study, positive antigen ELISA was reported from all age groups studied which is in congruence with what has been recorded by previous investigators (Martin & Collins, 1985). Our findings are contrary to what has been suggested by other investigators who strongly stressed that there is a positive correlation between age and heartworm infections in dogs with high prevalence in older dogs (Song *et al.*, 2003; Fan *et al.*, 2001). We have detected antigens of *D. immitis* in dogs which are 1-3 years as well as in older dogs (3-6 years). Logically, *D. immitis* should infect any age group provided that there is a source of infection as well as a suitable vector. The prepatent period of the heartworm is 5-6 months and it is probable that the source of the infection in our study is from within Saudi Arabia (Pantchev *et al.*, 2009).

Our results showed a significant difference in the seroprevalence between outdoor and indoor dogs. This finding is in accordance with the results obtained by other investigators (Montoya *et al.*, 1998; Yildirim *et al.*, 2007; Yaman *et al.*, 2009; Liu *et al.*, 2013). The outdoors dogs live in conditions which are conducive to infection as they are exposed to mosquitoes which may be harboring infective third stage larvae (L3). The finding of positive results in indoor dogs would probably be explained by the fact that the indoor dogs might be accidentally bitten by some mosquitoes which harbored infective L3.

The seroprevalence of *D. immitis* in dogs was found to be higher (both in Ag and Ab ELISA) in the summer compared with the winter and this can be explained by the high abundance of mosquito vectors in summer than in other parts of the year. The intensity of mosquito is increased during January and we expect the animals get infected during this time and then start producing microfilariae during the summer (Al-Khreji, 2005). It is important that the model of *D. immitis* seasonality can be used for timing of *D. immitis* chemoprophylaxis and scheduling of diagnostic testing. Seasons are critical factors in the prevalence of *D. immitis* infection due to the availability of the vector involved in the transmission. Moisture and moderate temperature are also considered important factors in determining the survival and availability of mosquitoes. The environmental temperature is an important factor for *D. immitis* maturation to infective third-stage larvae (L3) in the mosquito (McCall *et al.*, 2004). In Riyadh, the population of mosquito species is present throughout the year, however, the highest abundance are recorded in June, followed by a considerable decline towards September. In October, the mosquito population starts to increase towards January (temperature and humidity were optimum) and they start to decrease again in January (winter season) and reach the minimum in March (Al-Khreji, 2005).

The prevalence of 1.58 % of *D. immitis* in cats by use of Ag ELISA is the first report of the heartworm in cats in Saudi Arabia. The heartworm has been reported from cats in other countries and occurs at variably low rates such as 0.26% to 1.9% (Kalkstein *et al.*, 2000; Tolbert and Tolbert, 2004; Montoya-Alonso *et al.*, 2014). Higher rates of 3.0% to 4.8% were also reported from some countries (Sukhumavasi *et al.*, 2012; Maia *et al.*, 2015; Cong *et al.*, 2016). The sensitivity of antigen ELISA testing is relatively low in cats, because this test only detect antigen from adult female worms. A negative result does not rule out an infection from male worms or pre-adult worms, most of which are common in cats. Hence, it is recommended to carry out both antigen and antibodies tests in cats and this is what has been performed during the present investigation. Antibodies were detected in 2.11% of the cats tested which is far much lower than what has been reported earlier by several investigators such as 15% and 33% (Vieira *et al.*, 2015; Montoya-Alonso *et al.*, 2011). There was no effect of age, sex and rearing system on the seroprevalence of heartworm in cats in Saudi Arabia.

The results of the present study demonstrate for the first time the evidence of *D. immitis* antigen in domestic dogs and cats reared in Riyadh, Saudi Arabia. These animals lived in Saudi Arabia and it is expected that the infection was acquired while animals are in the country. Further

studies are required to investigate the associated clinical and laboratory findings in dogs and cats presenting with clinical signs that could be related to heartworm disease. Furthermore, the administration of preventive medications against heartworm should be considered in the light of the results of this study.

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Conflict of interest

The authors declare that they have no competing interests.

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