

Full Length Research Paper

Prevalence of canine *Dirofilaria immitis* infection in the city of Algiers, Algeria

Ben-Mahdi Meriem-Hind* and Madani Mohamed

Veterinary National Superior School of Algiers, P. O. Box 161, Hacène Badi, El Harrach, Algiers, Algeria.

Accepted 7 September, 2009

Heartworm disease is caused by *Dirofilaria immitis* a zoonotic, vector-borne parasite and one of the most pathogenic nematode parasites of the dog. The epidemiological status of this parasitosis in Algeria stays to this day unknown even though this country has the ideal biotope to the development of this disease. The aim of this study was to investigate the prevalence of *D. immitis* infection in dogs from Algiers, in the north of Algeria. Blood samples were collected from 184 dogs from November 2006 to September 2007 and were examined by the modified Knott method to detect circulating microfilaria. A total of 34 subjects were microfilaria positive, giving a prevalence value of 18.48%. All the samples were further tested by antigen-ELISA in order to detect dogs with prepatent or occult heartworm infections. The prevalence rose to 24.46% (with 45 seropositives dogs). More male (14.13%) than female (10.33%) dogs were affected in this study, although there was no significant difference between both groups. With regard to general health status, our data showed a high prevalence of 18.48% of asymptomatic carriers of the parasite *D. immitis*, only 5.98% of the positive samples were from unhealthy dogs. The difference was statistically significant ($p < 0.05$). This first report on the status of *D. Immitis* infection in domestic dogs in Algiers, suggests that heartworm treatment and adequate prophylaxis should be undertaken to control *D. Immitis* infection in the canine population of Algiers city in Algeria.

Keywords: *Dirofilaria immitis*, prevalence, ELISA, sexe distribution, dogs, Algiers.

INTRODUCTION

Cardiopulmonary dirofilariosis commonly named heartworm disease is a non contagious parasitic disease, caused by a filarial nematode *Dirofilaria immitis* which is one of the most pathogenic nematode parasites for the dog. Dogs are considered the definitive host for *D. immitis*, however, it may infect more than 30 animal species (e.g., coyotes, foxes, wolves and other wild canids, domestic cats and wild felids, ferrets, etc.) and humans as well, it is a zoonosis transmitted by mosquitos bite (*Aedes*, *Anopheles* and *Culex*) (McCall et al., 2008).

Clinically canine dirofilariosis is characterised by a pulmonary hypertension that progressively develops into a right sided heart failure. In the case of massive infestation this disease evolves into a very serious haemolytic syndrome or a lethal obstruction of the vein cava called the vein cava syndrome (Atkins et al., 1988).

For humans the affection is primarily pulmonary, confined in a coin sized inflammatory granuloma within the lung, which can be taken for a carcinoma or pulmonary cancerous metastasis, implying the realization of a surgical thoracotomy and partial excision of the pulmonary lung in order to establish a definitive diagnosis which can be very costly and highly invasive for the patient (Shah, 1999; Bielawski et al., 2001; Foroulis et al., 2005; Theis, 2005).

Canine dirofilariosis is widely distributed throughout the world and is endemic in temperate, tropical and subtropical climatic regions particularly in the south east of the United States of America, in many countries of south America, in Australia, in Asia and in the south of Europe around the Mediterranean sea (Bidgood and Collins, 1996; Genchi et al., 2005; McCall et al., 2008)

No studies have been published regarding the prevalence of canine dirofilariosis in the north of Africa. Furthermore, the epidemiological status of this parasitosis in Algeria is practically unknown. Therefore, the aim of the current study was to determine the prevalence of

*Corresponding author. E-mail: mhbenmahdi@yahoo.fr. Fax: +213 21 638 198.

Table 1. Prevalence of *D. immitis* infection using modified Knott test and ELISA kit.

Technique	No. of examined dogs	No. of positive	Positive rate (%)
Modified Knott technique	184	30	16.30
ELISA kit (Petcheck)	184	45	24.46

Table 2. Prevalence of *D.immitis* correlated with sex and general health status.

	No. of examined dogs	No. of positive	Positive rate (%)	P
Sex				
Females	81	19	10.33	0.78
Males	103	26	14.13	
General health status				
Good	158	34	18.48	0.02
Poor	26	11	5.98	
Total	184	45	24.46	

Difference was statically significant when $P < 0.05$.

D. immitis in dogs in Algiers city.

MATERIALS AND METHODS

Study area

The study was conducted in Algiers city, the capital of Algeria. Algiers is located in the north center of Algeria at 36°46'N latitude and 3°03'E longitude. The city is situated at an elevation of 167 m above sea level with a south Mediterranean climate: summers are hot and humid while winters are mild and rainy.

Animals

A total of 184 cross breed male and female dogs, were randomly selected from November 2006 to September 2007. The animals were clinically examined and differentiated by their general health status (poor, good). The age range was from 0.5 to three years. Dogs younger than 6 months were excluded from the study considering the life cycle of *D. immitis* and none had received heartworm chemoprophylaxis or chemotherapy.

Blood samples

Whole blood was drawn from the cephalic vein of each dog. The blood samples were collected in a tube containing EDTA as anticoagulant, stored in a cooler box at 4°C and processed within 24 h.

Detection of *D. immitis* infection

Two different methods were used to identify heartworm infection. The first method was the modified Knott's technique (Newton and Wright, 1956). One milliliter (1 ml) of fresh blood was gently mixed with 9 ml of formaline at 2%, to break the erythrocytes and fix the leukocytes and the microfilariae. After 2 – 3 min, the material was centrifuged for 5 min, at 3000 rpm and the supernatant was discarded. Thirty-five microliters (35 µl) of the sediment was smear-

ed on each of three clean glasses slides, that were subsequently stained by a methylene blue solution (2‰) for 2 to 3 min and examined to determine the presence of *D. immitis* microfilariae, under 100X and 400X magnification. All the samples were subsequently examined for circulating antigens of *D. immitis* using a commercial ELISA kit (PetCheck®, IDEXX Laboratories, USA), according to the manufacturer's instructions (read at 640 nm). PetChek Canine Heartworm Antigen Test is an enzyme immunoassay designed to detect the presence of circulating antigen from adult *D. immitis* in serum or plasma.

STATISTICAL ANALYSIS

Statistical analysis of the prevalence was carried out with a χ^2 test of significance in order to correlate it with the variable considered (sex and health status). The difference was significant for the value of $P < 0.05$.

RESULTS

From the 184 dogs included in this study, 81 (44%) were females and 103 (56%) were males. One hundred and fifty eight dogs (85.9%) presented a good general health status and only 26 (14.1%) a poor general health status, all remained in outdoors.

Tables 1 and 2 show the results obtained for the 184 blood samples processed:

Of a total of 184 dogs tested, 34 (18.48%) were positive for *D. immitis* infection with the Knott's modified technique. Meanwhile, when testing the same samples for adult antigen of *D. immitis* by ELISA test, forty five animals were positive which brings the seroprevalence to 24.46%.

More male (14.13%) than female (10.33%) dogs were affected as shown in the Table 2. But this difference was not statistically significant ($p > 0.05$).

Among the 45 seropositive dogs only 11 dogs presented

a bad general health which means that 24.44% of the seropositive dogs were unhealthy, meanwhile 34 seropositive dogs were in good general health which means that 75.56% of the positive dogs were asymptomatic carriers of the parasite *D. immitis*. Our results showed a high prevalence of 18.48% of asymptomatic carriers of the parasite *D. immitis*, only 5.98% of the positive samples were from unhealthy dogs. The difference was statically significant ($p < 0.05$).

DISCUSSION

The epidemiological status of this parasitosis in Algeria stays to this day unknown even though this country has the ideal biotope to the development of this disease. The present study is the first report on the status of *D. immitis* infection in dogs in Algiers to date. Few studies have been done, even less published and rare report issued for the African continent (Matola, 1991; Schwan and Durand, 2002; Davoust et al., 2008).

The prevalence values of *D. immitis* were 18.48 and 24.46% respectively by modified Knott technique and by PetChek ELISA test. This difference can be explained by the fact that dogs with prepatent or occult heartworm infections can be amicrofilaremic (Hoover et al., 1996). Detecting circulating antigens from adult female *D. immitis* by monoclonal antibodies offers better sensitivity and specificity for diagnosis of *D. immitis* infection (Sisson et al., 1985; Weil et al., 1985; Brunner et al., 1988). Furthermore, the antigen detecting ELISA methods allowed the detection of cases with a lower number of worms than haematological tests (Martini et al., 1996). The antigen test used in this study (PetCheck, IDEEX Laboratories) has been reported as having a sensitivity of approximately 80% (Snyder et al., 2000). Moreover, PetCheck was found by Courtney and Zeng, more sensitive when compared to five other diagnostic tests for the detection of antigen in *D. immitis* infection (Courtney and Zeng, 2001).

The prevalence of heartworm infection in dogs in the current study was lower than that reported in Greece (34%) (Founta et al., 1999) and in Italy along the Po river (more than 50%); higher than in the nearest Sardinia island (17%) (Rossi et al., 1996; Scala et al., 2005) and in Barcelona region in Spain (12.8%) (Aranda et al., 1998) but close to the one registered in the endemic zone of the Tenerife islands (21%) (Montoya et al., 2006) and was inferior to the one reported in Las Palmas (36%) (Guerrero et al., 1989). Weather is a critical factor in the prevalence of the disease. Transmission depends on the intermediate host, a mosquito which requires high relative humidity and an average temperature higher than 15°C, the *D. immitis* larva, once ingested by mosquitoes, do not develop further unless at a regular temperature higher than 14°C (McCall et al., 2008).

Algiers offers the ideal biotope to the mosquito vector proliferation and for the development of larvae of *D.*

immitis (hot weather with suitable temperature: annual average temperature higher than 19°C), the city is also surrounded by many stagnant waters areas.

Selby et al. (1980) and Montoya et al. (1998) reported that male are more susceptible to infection by *D. immitis* (Selby et al., 1980; Montoya et al., 1998). In this present study no significant differences in prevalence were observed between male and female dogs ($P > 0.05$). This result is in accordance with the data provided by other authors (Oge et al., 2003; Song et al., 2003; Duran-Struuck et al., 2005).

When evaluating the prevalence of *D. immitis* by general health status of the animals, the obtained results showed that only 24.44% of positive dogs were in bad general health, the others were in good general health and did not show any particular sign of disease. This implies that a great majority of positive dogs (75.56%) were asymptomatic carriers. Which brings the overall prevalence for asymptomatic carriers to a 16.89%. This high percentage is well better explained by the proper pathological pattern of the canine dirofilariosis. In fact, the parasite takes up to 6 months to fully mature and the evolution of the disease could take years before any clinical manifestations appear. Thereby, clinicians should systematically test screen every animal that is not on preventive medication at the time of regular consultations. Because of the prognostic ineluctably dark of this affection, without any proper and effective medical treatment and the endemic evolution of this parasitosis within a canine population could have heavy medical and economical consequences particularly for all institutions using work dogs. Furthermore, the slow and insidious evolution of this disease considerably lessens the quality of life and notably reduces the performances of these animals (McCall et al., 2008).

In conclusion *D. immitis* infection in dogs was confirmed in Algiers city. The relative high prevalence of asymptomatic carriers found justifies the routinely systematic dog testing for *D. immitis* and long life chemoprophylaxis against this pathogenic agent. Further studies in other regions of Algeria have to be carried out. Furthermore, a precise identification of the vector in the Algiers's region is necessary to improve the efficacy of the disease fighting strategy.

ACKNOWLEDGMENTS

The authors wish to thank Mrs Zenia for her kind assistance in the statistical analysis of the results.

REFERENCES

- Aranda C, Panyella O, Eritja R, Castella J (1998). Canine filariasis. Importance and transmission in the Baix Llobregat area, Barcelona (Spain). *Vet. Parasitol.* 77(4): 267-275.
- Atkins CE, Keene BW, McGuirk SM (1988). Investigation of caval syndrome in dogs experimentally infected with *Dirofilaria immitis*. *J. Vet. Intern. Med.* 2(1): 36-40.

- Bidgood A, Collins GH (1996). The prevalence of *Dirofilaria immitis* in dogs in Sydney. *Austr. Vet. J.* 73(3): 103-104.
- Bielawski BC, Harrington D, Joseph E (2001). A solitary pulmonary nodule with zoonotic implications. *Chest.* 119(4): 1250-1252.
- Brunner CJ, Hendrix CM, Blagburn BL, Hanrahan LA (1988). Comparison of serologic tests for detection of antigen in canine heartworm infections. *J. Am. Vet. Med. Assoc.* 192(10): 1423-1427.
- Courtney CH, Zeng Q (2001). Comparison of heartworm antigen test kit performance in dogs having low heartworm burdens. *Vet. Parasitol.* 96(4): 317-322.
- Davoust B, Normand T, Bourry O, Dang H, Leroy E, Bourdoiseau G (2008). Epidemiological survey on gastro-intestinal and blood-borne helminths of dogs in north-east Gabon. *Onderstepoort. J. Vet. Res.* 75(4): 359-364.
- Duran-Struuck R, Jost C, Hernandez AH (2005). *Dirofilaria immitis* prevalence in a canine population in the Samana Peninsula (Dominican Republic) - June 2001. *Vet. Parasitol.* 133(4): 323-327.
- Forouli CN, Khaldi L, Desimonas N, Kalafati G (2005). Pulmonary dirofilariasis mimicking lung tumor with chest wall and mediastinal invasion. *Thorac Cardiovasc Surg.* 53(3): 173-175.
- Founta A, Theodorides Y, Frydas S, Chliounakis S (1999). The presence of filarial parasites of dogs in Serrae Province. *Bull. Hellenic. Vet. Med. Soc.* 50: 315-320.
- Genchi C, Rinaldi L, Cascone C, Mortarino M, Cringoli G (2005). Is heartworm disease really spreading in Europe? *Vet. Parasitol.* 133(2-3): 137-148.
- Guerrero J, Genchi C, Vezzoni A, de Lahitte JD, Bussieras J, Rojo FA, Ortega LM, Rodenas A, Bulman GM, Larson MH, Labarthe N, Charles T, Bordin E (1989). Distribution of *Dirofilaria immitis* in selected area of Europe and South America. Heartworm of Symposium 89. American Heartworm Society, pp. 13-18.
- Hoover JP, Campbell GA, Fox JC, Claypool PL, Mullins S.B. (1996). Comparison of eight diagnostic blood tests for heartworm infection in dogs. *Canine. Pract.* 21: 11-19.
- Martini M, Capelli G, Poglayen G, Bertotti F, Turilli C (1996). The validity of some haematological and ELISA methods for the diagnosis of canine heartworm disease. *Vet. Res. Commun.* 20(4): 331-339.
- Matola YG (1991). Periodicity of *Dirofilaria immitis* microfilariae in a dog from Muheza district, Tanzania. *J. Helminthol.* 65(1): 76-78.
- McCall JW, Genchi C, Kramer LH, Guerrero J, Venco L (2008). Heartworm disease in animals and humans. *Adv. Parasitol.* 66: 193-285.
- Montoya JA, Morales M, Ferrer O, Molina JM, Corbera JA (1998). The prevalence of *Dirofilaria immitis* in Gran Canaria, Canary Islands, Spain (1994-1996). *Vet. Parasitol.* 75(2-3): 221-226.
- Montoya JA, Morales M, Juste MC, Banares A, Simon F, Genchi C (2006). Seroprevalence of canine heartworm disease (*Dirofilaria immitis*) on Tenerife Island: an epidemiological update. *Parasitol. Res.* 100(1): 103-105.
- Newton WL, Wright WH (1956). The occurrence of a dog filariid other than *Dirofilaria immitis* in the United States. *J. Parasitol.* 42(3): 246-258.
- Oge H, Doganay A, Oge S, Yildirim A (2003). Prevalence and distribution of *Dirofilaria immitis* in domestic dogs from Ankara and vicinity in Turkey. *Dtsch Tierarztl Wochenschr.* 110(2): 69-72.
- Rossi L, Pollono F, Meneguz PG, Gribaudo L, Balbo T (1996). An epidemiological study of canine filarioses in north-west Italy: what has changed in 25 years? *Vet. Res. Commun.* 20(4): 308-315.
- Scala A, Atzori F, Varcasia A, Genchi C, Cappelli G (2005). Epidemiology of dog filariosis in Sardinia island, Italy. *Proceedings of the 20th International Conference of the World Association for the Advancement of Veterinary Parasitology.* Christchurch, New Zealand, p246.
- Schwan EV, Durand DT (2002). Canine filariosis caused by *Dirofilaria immitis* in Mozambique: a small survey based on the identification of microfilariae. *J. S. Afr. Vet. Assoc.* 73(3): 124-126.
- Selby LA, Corwin RM, Hayes HM, Jr (1980). Risk factors associated with canine heartworm infection. *J. Am. Vet. Med. Assoc.* 176(1): 33-35.
- Shah MK (1999). Human pulmonary dirofilariasis: review of the literature. *South. Med. J.* 92(3): 276-279.
- Sisson D, Dilling G, Wong MM, Thomas WP (1985). Sensitivity and specificity of the indirect-fluorescent antibody test and two enzyme-linked immunosorbent assays in canine dirofilariasis. *Am. J. Vet. Res.* 46(7): 1529-1533.
- Snyder PS, Levy JK, Salute ME, Gorman SP, Kubilis PS, Smail PW, George LL (2000). Performance of serologic tests used to detect heartworm infection in cats. *J. Am. Vet. Med. Assoc.* 216(5): 693-700.
- Song KH, Lee SE, Hayasaki M, Shiramizu K, Kim DH, Cho KW (2003). Seroprevalence of canine dirofilariasis in South Korea. *Vet. Parasitol.* 114(3): 231-236.
- Theis JH (2005). Public health aspects of dirofilariasis in the United States. *Vet. Parasitol.* 133(2-3): 157-180.
- Weil GJ, Malane MS, Powers KG, Blair LS (1985). Monoclonal antibodies to parasite antigens found in the serum of *Dirofilaria immitis*-infected dogs. *J. Immunol.* 134(2): 1185-1191.