

Contribution to the Study of Canine Heartworm in Tiaret (Algeria)

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Abstract: Canine heartworm disease also called cardiopulmonary filariasis is a non-contagious helminthosis, particularly affecting canines, due to the presence in the right heart and the pulmonary artery of a worm called: heartworm, this is a serious disease that causes overall heart failure, highly hampers the dogs who become unable to make the expected services and most often leads to death. The aim of this study was to determine the prevalence of heartworm in dogs in the wilaya of Tiaret, a town in Western Algeria. Between February 2010 and December 2015, 1300, blood samples were taken from dogs of various age, each sample was analyzed using various laboratory techniques (direct microscopic evaluation of a sample of fresh blood, MGG staining, rapid test immunomigration “*Dirofilaria immitis* witness”). Then, autopsy of dead dogs and/or euthanized. A prevalence of 0% was found on a sample of 1300 dogs. This study shows for the first time the prevalence of heartworm in Tiaret region of Algeria and emphasizes the importance of routine screening for the disease to establish any possible emergence of pathology.

Key words: Dogs, heartworm, microscopic observation, prevalence, Tiaret, witness test

INTRODUCTION

Heartworm is a nematode parasite that causes heartworm cardiopulmonary canine and feline. It is also the causative agent of human lung heartworm; a zoonotic parasitic disease is found mainly in temperate regions, tropical and subtropical world (Montoya *et al.*, 2010; Vieira *et al.*, 2014).

Different species of *Culex* mosquito species belonging to the genera *Aedes*, *Anopheles* were involved in the transmission of this parasite which allows the intermediate stage to complete its life cycle (Cancrini and Kramer, 2001; Morchon *et al.*, 2012). The weather is a critical factor because of climatic requirements (high relative humidity and 15°C higher average temperature) of intermediate hosts. Heartworm is a serious and potentially deadly disease. The pathophysiological response to heartworm infection is mainly due to the presence of adult worms heartworm in the pulmonary arteries and the right ventricle of the heart (Cringoli *et al.*, 2001), the number of worm, the immune response of the host, the duration of infection and exercise levels determine the severity of cardiopulmonary pathology (Hoch and Strickland, 2008; Montoya *et al.*, 1998) clinical manifestations in dogs include coughing, shortness of breath, weight loss, exercise intolerance, weakness, anemia, cyanosis and congestive heart failure (Cardoso *et al.*, 2012) in the human host, *Dirofilaria immitis* is the causative agent of pulmonary heartworm disease

and in many cases producing benign pulmonary nodules usually otherwise identified chest radiography in asymptomatic patients what wrong can first be identified as malignant tumors (Echeverri *et al.*, 1999; Muro *et al.*, 1999; Theis *et al.*, 2001). *Dirofilaria immitis* worms have also been found in the crane, liver intraocular and adipose tissue mesenteric, testicular arteries (McCall *et al.*, 2008; Theis *et al.*, 2001). The clinical significance of human heartworm disease has been associated almost exclusively derived from iatrogenic surgery to remove lung nodules.

The epidemiological situation of heartworm disease depends on several factors such as global warming, changes in the seasonal dynamics of the vector population, the movement of animals between countries, worm burden, age and immune response host which can play a role in the current geographical spread of the disease (Dimri *et al.*, 2012; Morchon *et al.*, 2012). In this research, we will study the current prevalence and seroprevalence of heartworm in dogs living in the Tiaret region (Algeria).

MATERIALS AND METHODS

The study was achieved in the Tiaret region, town located to the west of Algeria is characterized by a continental climate (concerning remote areas of the coast is characterized by high annual temperature range (over 20°C)), the severe winter and summer is hot and dry, it receives 300-400 mm³ of rainfall a year on

average. Climate and socio-economic life in the region with farming do not offer very adequate conditions for the development of mosquitoes, heartworm vectors.

This study was executed from February 2010 to December 2015 and 1300 dogs (768 male and 532 female) were sampled the dogs were taken to the veterinary clinic for a routine health examination and others came several veterinary offices and a few farms in the wilaya, their clinical signs and a detailed history (gender, age, weight, race, use and geographic origin) were obtained with a thorough physical examination to look for the presence of cardiopulmonary signs, auscultation, pulse measurement, capillary refill, jugular vein distention and abdominal palpation. At the clinic carnivores in the Institute of Veterinary Science; at least 5 mL of blood was collected from the cephalic vein of dogs the 1300. From this volume of blood, at least 3 mL were collected in tubes with an anticoagulant and at least 2 mL in dry tubes and then, forwarded to the parasitological laboratory.

Microscopic examination: Blood samples were treated on the day of collection. First, fresh blood from each sample undergoes a blood count of formula (NSF); then examined under a microscope to determine the presence of microfilariae by the thick film technique, the blood was centrifuged at 1500 r m^{-1} for 5 min, the supernatant was then decanted and the pellet was stained with a drop of methylene blue in 0.1% (Pannabecker *et al.*, 1995) and undergoes MGG staining.

Antigen detection: Dog blood samples were tested for the detection of circulating antigens *Dirofilaria immitis* using a test kit “witness *Dirofilaria*”, based on a fast immunomigration (Watier *et al.*, 2011).

Survey: The 1,300 dogs used in this study, 59, 07% were male and 40.92% were female; divided into 14 races with a predominance of the German Shepherd breed 35.61%. For their use, 69.23% were kept as pets. According to their inhabitants, 68.84% live in urban areas and 31.15% lived in rural areas, 98.84% were owned and 1.15% unowned.

With regard to their ages, the sample was divided into seven age groups whose highest percentage is represented by older dogs between 2-3 year (28.63%).

RESULTS AND DISCUSSION

The equipment and methods used and the results obtained in our serological survey require comments and reviews.

Animal material: Our study included 1300 dogs selected randomly in the Tiaret region (Algeria); considering the size of our sample and the sampling method, we may question on:

- The representativeness of the sample
- The total number of dogs that includes the Tiaret region

Indeed, there is no official statistic on the number of dogs in the region; censuses were never concerned dog populations which limit the estimate of the total number of dogs in the area. Hence, the random selections of the number of animals up our sampling. So, we can not say with accuracy whether sampling is low or high. However, this number is relatively large and adapted to laboratory equipment at our disposal namely kits and reagents.

Compared to the work done by others on heartworm, our sample seems representative. In Portugal a study is made on 696 dogs for 3 successive year 2011-2013 (Alho *et al.*, 2014).

In Spain researchers provide their comments through a study of 823 dogs during 2002, 2003 (Montoya *et al.*, 2006). In Brazil 2002 a prevalence of 2% was the result of a study on canine heartworm on a sampling of 2556 dogs (Labarthe *et al.*, 2002).

In our study, we show for the first time the absence of canine heartworm infection in Tiaret (Algeria) 1300 dog samples analyzed. No positive cases either by microscopic observation by either the kit (witness test). Corroborated cases by autopsy.

This prevalence could be explained by the fact that microfilariae detection techniques are not able to detect occult infection (amicrofilarique infections). These infections could occur due to several causes: low parasite loads, prepatent infections by young adults infection by an aging adult female with impaired fertility. Antiparasitic treatment is free at the institute.

This prevalence is found in other studies citing: In Germany (Baden-Wurtemberg) is one of the hottest regions, 44 hunting dogs were tested in June 2007, using the Knott test, heartworm antigen was not detected in any some samples; another sample of 288 dogs considerable of the upper Rhine central region tested negative for the antigen of heart worm between February and August 2007 (Pantchev *et al.*, 2009a).

Argentina investigate the canine heartworm made on different region of the country revealed a prevalence ranging from 0% in areas up to 71% in other regions (Vezzani *et al.*, 2006).

In the Czech Republic, 110 dogs are the subject of a study on canine heartworm; a prevalence of 0% was found in dogs that have never traveled, a positive imported dog and a prevalence of 30.5% among dog imported with the presence of circulating antibodies and no worms (Svobodova and Misonova, 2005).

In France in aim to determine the prevalence of heartworm disease; the blood of 1050 dogs was collected, analyzed, tested, a prevalence of 0.22% was found (Pantchev *et al.*, 2009b).

In Montana over a period of 3 years a total blood test of 3490 dogs by ELISA revealed 24 dogs heartworm positive representing a prevalence of 0.6% (Knapp *et al.*, 1993).

In Italy the analysis of blood of 351 dogs revealed that carrier were 0.6 D and D immitis repens (Cringoli *et al.*, 2001).

Many studies have brought that commercial serological kits have low sensitivity to parasitic load (one to five heartworm adult females), when worms show low fertility, when the presence of microfilariae persists for 1-3 years after death adult females, the prepatent infection or only male infection (McCall *et al.*, 2008a, b).

In 2012, a study showed a prevalence of 3.6% of portuguese heartworm disease in healthy dogs. In this study, the central region had a prevalence of 0.9% in healthy dogs (Cardoso *et al.*, 2012).

No significant difference was observed in the results throughout the year. Although, the transmission period is limited to a period of the year because of the relationship of the life of the vector with time, a long period of development of the parasite in dogs (7-9 months) and that the most infected dogs are asymptomatic for months or years, it is difficult to draw a curve during the year for prevalence (McCall *et al.*, 2008a, b).

In France, Corsica, Camargue region Hyeres, Dombes, Languedoc-Roussillon (dirofilarioses parasitological prevalence of 0.7% on 5000 samples in 1986) (Gauchard, 2005).

Heartworm is zoonotic but, human infections are rare. Man may be infected but by vectors and not directly by the dog (Geffray, 2001; Trees and Shaw, 1999). The diagnosis of heartworm disease by ELISA test; false negative reactions may occur:

- In low infestation (<5 wired)
- In the presence of immature parasites (prepatent period)
- In the presence of male parasites. Circulating soluble antigens emanate mainly from the female genital tract (Atkins, 2003)

In Australia, both studies had the objective of determining the prevalence of heartworm canine, the first shifted to 230 dogs, a dog was positive, a prevalence of 0.4%. The second on 1428 dogs found the prevalence EST1% (Copland *et al.*, 1992).

This study provides evidence that dogs in Tiaret (Algeria), do not have a high risk of infection with heartworm. The epidemiological knowledge of human heartworm disease is still rare.

CONCLUSION

Canine heartworm disease is widely distributed around the world but especially interested wetlands, warm and temperate in both hemispheres.

The causative agent: a wired heartworm is responsible for a severe cardiopulmonary disease. In all regions where it occurs, the disease progresses in very localized outbreaks, according to the habitats of mosquitoes, the adult heartworms are localized in the body at the heart and adjacent blood vessels. They reproduce by giving birth to microfilariae that travel in the bloodstream and ensure the sustainability of the infestation. The highlighting of these embryos is problematic and requires the experimental diagnosis. It is made possible through simple or more elaborate methods.

The most immediate is microscopic examination of a drop of fresh blood between slide and cover slip. However, in a number of cases the disease is not accompanied by microfilarial excretions. So, we will use other means of investigation. Serological screening is the one. It is to isolate and quantify the witnesses of the infestation (Ab or Ag). The search for these antibodies or antigens uses several techniques reserved for well-equipped laboratories. It is the precipitation hypersensitivity test agar, agglutination, complement fixation, immunoelectrophoresis indirect immunofluorescence, ELISA and of rapid immunomigration kits such as "heartworm witness".

The aim of our study was to determine in the Tiaret region the prevalence of heartworm disease in dogs. However, this study allows us to say that heartworm disease does not exist in this region and to maintain the allowance of the region we plan to create continuous training for veterinarians and develop this awareness to prevent the emergence and spread of this parasitic disease in the region of Tiaret (Algeria) for example, improving measures for diagnosis and prevention against the vectors. This is especially true in view of the possible lack of symptoms in animals microfilarémiques and long incubation periods during which animals are capable of infecting mosquitoes. The discovery of asymptomatic positive animals microfilarémiques highlights the potential that exists.

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