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Evaluation of Calcium, Phosphorus and Alkaline Phosphatase in *Dirofilaria immitis* Infection in Dogs

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Abstract: Filariasis is one of the most important parasitic diseases caused by the filaroid nematodes with a worldwide distribution and affects man, animals and birds. Heartworm disease, caused by the filarial nematode, Dirofilaria immitis, is a major, potentially life-threatening disease of dogs, with worldwide distribution and global significance. It is not only veterinary important but it also has zoonotic potential in many regions. Heartworm disease is a serious but preventable mosquito-borne parasitic disease that primarily affects dogs and cats. The current study was conducted on 80, 3-5 years old mix breed dogs suspected of dirofilariasis in the Faculty of Veterinary Medicine, Islamic Azad University of Tabriz and rural around Tabriz. Direct method and the modified knott's method were used for diagnosis of the parasitic microfilaria. Some parameters including Calcium, Phosphorus and Alkaline phosphatase were determined. The results of the present study indicated that 20 of the 80 examined dogs were infected by Dirofilaria immitis microfiler and the prevalence of dirofilariasis was 25%. The mean levels of ALP, total Bilirubin and indirect Bilirubin in infected dogs were significantly higher than the uninfected dogs ($p<0.05$). The mean levels of Ca, P and direct bilirubin in infected dogs didn't show significant change in comparison to the uninfected dogs ($P>0/05$). As a result, the parasite has side effects in liver, bile ducts and red blood cells. Some changes were detected in biochemical parameters in dogs with dirofilariasis so attention must be paid to them in diagnosis and treatment.

Key words: Dirofilariasis · Calcium · Phosphorus · Alkaline Phosphatase · Bilirubin

INTRODUCTION

Filariasis is one of the most important parasitic diseases caused by the filaroid nematodes with a worldwide distribution and affects man, animals and birds. Heartworm disease, caused by the filarial nematode, Dirofilaria immitis, is a major, potentially life-threatening disease of dogs, with worldwide distribution and global significance. It is not only veterinary important but it also has zoonotic potential in many regions [1, 2]. Heartworm disease is a serious but preventable mosquito-borne parasitic disease that primarily affects dogs and cats. Dogs are usually asymptomatic until maturation of heartworm larvae in the right atrium and pulmonary artery causes cardiopulmonary effects [3]. Typically, maturation of the larvae takes about six months. The chances of contracting heartworm disease without prophylaxis depend on exposure to mosquitoes. Dogs are more likely to become infected if they live in the Southeast, Gulf Coast, or Mississippi River Valley near fresh or saltwater, housed outside and are not receiving heartworm preventives. Dogs can survive a heartworm infection by receiving appropriate treatment. There is a risk of thromboembolism and death following adult heartworm treatment when worms break loose from their location in the right atrium in addition to side effects of the drugs that are commonly used to treat adult heartworms [4]. Many organs such as lungs, heart, liver and kidneys may be affected by the infection [5]. The most important damage is seen in the pulmonary arteries, the right ventricle of the heart and the kidneys [5-8]. The lifecycle of canine heartworm (Dirofilaria immitis) begins when a mosquito bites a dog that has an infection. Adult female worms in the dog produce first-stage larvae called microfilariae. These larvae (L1) are picked up by mosquitoes that take a blood meal from the dog. Once L1

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larvae are inside the mosquito they must undergo two molts in order to be infectious to another dog. The L3 larvae in the mosquito can be transferred to another dog, starting a new infection. Dogs can develop adult heartworms (and therefore heartworm disease) 6 months after being inoculated with infective larvae. In dogs with *D. immitis*, probable alterations in alkaline phosphatase (ALP) have been reported [9, 10]. Total body calcium in normal adults is 1 to 2 kg. 99% calcium is in the skeleton. Physiologic roles of calcium are maintaining the structural integrity of the skeleton and for cellular processes (it is also an intracellular second messenger for many hormones, paracrine factors and neurotransmitters). Extracellular calcium in plasma found in: a) ionized calcium (~ 50%) b) protein-bound calcium (~40%) c) calcium that is complexed to bicarbonate, citrate and phosphate etc (~ 10%). Calcium absorption occurs principally in the duodenum and the jejunum by an active transport process. The main determinant of intestinal absorption of calcium is 1, 25-(OH)2D. In the adult, phosphorus constitutes 10 to 13 g/kg of body weight. 80 - 85% Phosphorus is in the skeleton and 10% is intracellular. Normal plasma inorganic phosphate (P) concentration is 0.8 to 1.4 mmol/l. P is 85% free and 15% protein bound. P absorption is directly proportional to dietary P intake. Most plasma phosphate is filtered by the glomerulus, after which 80 - 90% is actively reabsorbed.

One of the most important activities of liver is excretion of toxins and waste disposals and also production of important metabolic substances through digestion and absorption process that is bile [11]. Through the bile, toxins, pigment, acids and bile salts and conjugated bilirubin can be excreted [10]. There are conjugated bilirubin, acids and bile salts within the bile that after excretion into the intestine can interfere in the absorption of lipids by forming micelle till lipase secreted from the pancreas to be able to influence the lipid easily. Bilirubin is a product of hemoglobin metabolism. Old red blood cells or some red blood cells that don't have normal function are removed by reticuloendothelial system such as spleen. After lysis of red blood cells, hemoglobin is released from it, then iron is segregated from hemoglobin structure and remained hemoglobin is converted to biliverdin and then to non-conjugated bilirubin that is transferred to liver with albumin and in the liver, after entering to smooth reticulo endoplasmic system is converted to conjugated bilirubin through glocoronyl transferase (UDP) and finally, bilirubin will excreted through the bile. After excretion to intestine, by glocoronidase +bacteria, glocronic acid is segregated from bilirubin structure and is revived to three substance that two of them are feces pigments and the most important of them is urobilinogen. 80% of urobilinogen is excreted through the feces and 20% is reabsorbed and 95-98% of reabsorbed urobilinogen is excreted again through the bile and 2-5 % will be excreted through urine [11]. For treatment, 7.3 mg of ivermectin was 100% effective in preventing experimental infection with Dirofilaria immitis larvae and resulted in negative results for heartworm antigen in a field trial [12]. The objective of this study was to study the Calcium, Phosphorus, Alkaline Phosphatase and Bilirubin levels in dogs with dirofilariosis.

MATERIALS AND METHODS

Animals: This study was conducted on 80, 3-5 year old mixed breed dogs suspected of dirofilariasis in the Faculty of Veterinary Medicine, Islamic Azad University, Tabriz Brunch. The direct method and modified knott method were used for diagnosis of the parasitic microfilaria [13]. Identification of adult *D. immitis* was performed as defined by previous literatures [14-16]. Calcium, phosphorus, bilirubin (total & indirect) and alkaline phosphatase were measured by commercial kits (ziest chem) and spectrophotometer.

Statistical Analysis: Data were analyzed using SPSS Ver.14, under windows XP. To compare averages of biochemical parameters in the groups, we used statistical test, ANOVA and statistical software. All data were analyzed by t-test and on the basis of comparing their averages with the control group. It demonstrated meaningful statistical differences ($P<0.05$).

RESULTS

The results of this study indicated that 20 of the 80 examined dogs were infected by Dirofilaria immitis micro filer and the prevalence of dirofilariasis in this study was 25% (Table 1). The mean levels of Ca, P and direct bilirubin in infected dog didn't show significant change in comparison to the un-infected dogs ($P>0.05$). But the mean level of ALP, total Bilirubin and indirect Bilirubin were increased significantly in comparison to the un-infected dogs ($P<0.05$) (Table 2).

DISCUSSION

D. immitis is an important parasite seriously affecting an animal's health and locating within, especially the pulmonary artery and the right ventricle of dogs. Although the risk of infection has been recorded to

Table 1: Number and percent of infected and un-infected dogs

	Infected dogs	Un-infected dogs
Dog	20	60
Percent	25%	75%

Table 2: Biochemical findings of Ca, P, ALP, Bilirubin (TB, DB, IB) of infected and un-infected dogs (IU/L)

Parameters	Infected dogs	Un-infected dogs
Ca(mg/dl)	8/58±0/36[a]	8/86±0/50[a]
P (mg/dl)	3/89±0/80[a]	3/80±0/11[a]
ALP (IU/L)	130/77±13/87[a]	40/51±2/91[b]
Total Bilirubin	0/60±0/08[a]	0/10±0/05[b]
Direct Bilirubin	0/10±0/00[a]	0/02±0/00[a]
Indirect Bilirubin	0/51±0/04[a]	0/08±0/07[b]

The different letters in each line shows significant difference between dogs ($p<0.05$) (Mean values ± SD)

display variations because of age, it has been stated to be prevalent between the ages 3 and 7 [17, 18]. Encountering *D. immitis* infection between 4- and 7-year-old dogs, except a 2-year-old one, was found to be consistent with the literature. The results of the present study indicated that 20 of the 80 examined dogs were infected by Dirofilaria immitis microfiler and the prevalence of dirofilariasis in the current study was 25%. In this study mean of ALP has increased that may be due to dirofilaria damage on liver especially bile ducts. It reported by Sevimli [18]. Niwetpathomwat determined an increase in ALP and BUN values [1]. In the present study, most of serumal enzymes are related to liver. The number of microfilaria were seen in blood samples and can be localized in liver. Adult parasites or microfilaria may be localized in this organ and damage it. The mean levels of calcium and phosphorus didn't show statistical significant change in infected dogs. In this study May be the filler of adult parasite didn't have effect on kidney function, bone function or metabolic of these parameters. It reported that infection intensity was because of the number and localization of adult parasites [7] and the number of microfilaria seen in blood samples was not in relation to the number of adult parasites [16]. In previous study, increase calcium and decrease phosphorus levels were reported in canine filariasis [20]. The hyperbilirubinemia (total & indirect) may be attributed to hemolytic anemia with resultant hemolytic jaundice and damage the liver by parasite or filers. The obtained results were in harmony with earlier findings [21, 22]. As a result, the parasite can cause side effects in liver, bile ducts and Red blood cells. So that some changes were detected in biochemical parameter in dogs with dirofilariosis. It must be considered in diagnosis and treatment.

REFERENCES

1. Niwetpathomwat, A. Kaewthamasorn, M. Tiawsirisup, S. Techangamsuwan and S. Suvarnvibhaja, 2007. A retrospective study of the clinical hematology and the serum biochemistry tests made on canine dirofilariasis cases in an animal hospital population in Bangkok, Thailand. Research in Veterinary Sci., 82: 364-369.
2. Anuchai, N., A. Sukullaya, T. Somporn, S. Siram and K. Morakot, 2006. Canine dirofilariasis and concurrent tick-borne transmitted diseases in Bangkok, Thailand. J. Comparative Clinical Pathol., 15: 249-253.
3. Neafie, R.C., 1976. Dirofilariasis. In: C.H. Binford and D.H. Connor, (eds) Pathology of tropical and extraordinary disease. Armed Forces Institute of Pathology, Washington, pp: 391-396.
4. Knight, D.W., 1999. Guidelines for the Diagnosis, Prevention and Management of Heartworm (*Dirofilaria Immitis*) Infection in Dogs. Web posting of the American Heartworm Society.
5. Lombard, C.W., 1987. Heartworm disease. In: J.D. Bonagura, (ed) Cardiology. Churchill Livingstone, New York, pp: 275-299.
6. Kaiser, L. and F.W. Jeffrey, 2004. Dirofilaria immitis: worm burden and pulmonary artery proliferation in dogs from Michigan (United States). Veterinary Parasitol., 124: 125-129.
7. Rawlings, C.A., 1986. Other heartworm syndromes. In: C.A. Rawlings, (ed) Heartworm disease in dogs and cats. Saunders, Philadelphia, pp: 175-207.
8. Rawlings, C.A. and C.A. Calvert, 1989. Heartworm disease. In: S.J. Ettinger, (ed) Textbook of veterinary internal medicine: disease of dogs and cats, 3rd edn. Saunders, Philadelphia, pp: 1163-1184.
9. Sodikoff, C.H., 1995. Laboratory profiles of small animal diseases. A guide to laboratory diagnosis, 2nd edn. Mosby, New York.
10. Willard, M.D., H. Tvedten and G.H. Turnwald, 1994. Gastrointestinal, pancreatic and hepatic disorders. In: M.D. Willard, H. Tvedten and G.H. Turnwald, (eds) Small animal clinical diagnosis by laboratory methods, 2nd edn. Saunders, Philadelphia, pp: 179-218.
11. Mojabi, A., 1999. Veterinary Clinical Biochemistry. Tehran: Publication of Tehran University, Second Edition.

12. Khayatnouri, M.H. and Y. Garedaghi, 2012. Efficacy of Ivermectin Pour-On Administration Against Natural Toxascaris Leonina Infestation in Native Dogs World J. Zool., 7(1): 12-16.
13. Euzeby, J., 1981. Diagnostic Expérimental des Helminthoses Animales (Animaux Domestiques, Animaux de Laboratoire, Primate), Informations Techniques des Services Vétérinaires, Paris, France, Travaux pratiques d'helminthologie vétérinaire. Livre 1 Généralités. Diagnostic Ante Mortem. pp: 277-312.
14. Barriga, O.O., 1982. Dirofilariasis. In: J.H. Steele and M.G. Schultz, (eds) Handbook series in zoonoses, section C; parasitic zoonoses, Vol. II. CRC, Florida, pp: 93-110.
15. Rommel, J.E., W. Korting and T. Schnieder, 2000. Veterinarmedizinische Parasitologie. 5th ed. Auflage. Blackwell Wissenschafts-Verlag, Berlin, pp: 609-622.
16. Soulsby, E.J.L., 1982. Helminths, artropods and protozoa of domesticated animals. Baillere Tindall, London, pp: 307-311.
17. Montaya, J.A.M., M. Ferree, O. Moliba and J. Corbera, 1998. The prevalence of Dirofilaria immitis in Gran Canaria, Canary Islands, Spain (1994-1996). Veterinary Parasitol., 75: 221-226.
18. Perez-Sanchez, R., M. Gomez-Bautista and A.E. Grandes, 1989. Canine filariasis in Salamanca (Northwest Spain). Annals of Tropical Medicine and Parasitol., 83: 143-150.
19. Sevimli, F., K. Kozan, E. Bülbül, A. Birdane, F.M. Köse and A. Sevimli, 2007. Dirofilaria immitis infection in dogs: unusually located. and unusual findings. Parasitology Res., 101: 1487-1494.
20. Hashem, M. and A. Badawy, 2008. Hematological and biochemical studies on filariasis of dogs. The Internet J. Veterinary Medicine, 4(2).
21. Anuchai, N., K. Morakot, T. Sonthaya, T. Somporn and S.A. Siram, 2007. Retrospective study of the clinical hematology and the serum biochemistry tests made on canine dirofilariasis cases in an animal hospital population in Bangkok, Thailand. Research in Veterinary Sci., 82: 364-369.
22. Shafqaat, A., A.A. Butt, G. Muhammad and M. Athar, 2004. Khan Haemato-biochemical studies on the haemoparasitized camels. International J. Agric. and Biology (IJAB), 6: 331-334.