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Incidence and Hematological Changes in Dogs Infected with *Dirofilaria immitis* in Thailand

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ABSTRACT

Dirofilaria immitis is responsible for heartworm disease in dogs. Clinical signs are non-specific, ranging from asymptomatic to severe symptoms. The most common symptoms include coughing, emaciation, dyspnoea, and sudden loss of consciousness. Therefore, diagnosing heartworm infection in dogs requires a combination of methods, such as hematology and serology. This study was conducted on dogs with clinical signs, including anorexia, coughing, panting, and hind legs weakness, that was referred accidentally to a pet clinic in Thonburi district, Bangkok Province, Thailand, during 2020-2022. The examination was performed using a rapid enzyme immunoassay test and a thin blood smear. The total number of dogs admitted to a pet clinic during that period was 980. The result indicated infection of 21 (12 male and 9 female) dogs with heartworm (2.14%). The mean age of dogs was 5.62 ± 2.48 years. All infected dogs were classified under an open husbandry system that did not consistently use heartworm prevention products such as the macrocyclic lactone group. In the groups that received topical ectoparasites products, 10 dogs were detected with heartworm infection. The hematological changes in the infected dogs consisted of leucocytosis and increased levels of ALT, BUN, and creatinine. The study results can guide owners in choosing products that can prevent heartworm. Anti-mosquito nets should be deployed in areas where pets live, and always keep the environment clean.

Keywords: Dog, Heartworm, Hematology, Serum biochemistry

INTRODUCTION

Heartworm disease is caused by *Dirofilaria immitis*, a parasite carried by mosquitoes, such as *Culex theileri* and *Anopheles maculipennis* (Ferreira et al., 2015). Infection can be found in many animals, including dogs, cats, ferrets, and humans (McCall et al., 2008; Hoch and Strickland, 2008). Presently, heartworm infection in dogs has been reported worldwide, including in Italy (Magi et al., 2012), Brazil (Alves et al., 1999), and Korea (Lee et al., 1996). Heartworm is a zoonosis through which humans are accidentally infected by mosquito bites from dogs that carry heartworms. Human clinical signs are mostly respiratory symptoms (McCall et al., 2008; Polak et al., 2014; Little et al., 2018).

The infected dogs are usually asymptomatic; however, they might show symptoms when there is a large amount of heartworm and a disturbance of the blood vessels (Hoch and Strickland, 2008). Clinical signs in infected dogs include emaciation, weight loss, inability to exercise for long periods, coughing, dyspnoea, panting, and sudden loss of consciousness (Hoch and Strickland, 2008; Lu et al., 2017). As the symptoms of infected dogs are not specific, detecting heartworm infection requires a combination of several examination methods, such as Enzyme-Linked Immunosorbent Assay (ELISA), radiography, echocardiography, and molecular detection (Kamyingkird et al., 2017; Lu et al., 2017; Kim et al., 2020).

Dogs infected with heartworm have been reported to suffer hematologic changes, such as anemia, thrombocytopenia, leucocytosis, and increased liver and renal enzymes (Niwetpathomwat et al., 2007; Kim et al., 2020). Therefore, hematological tests could be helpful in monitoring or evaluating subclinical heartworm infection or assessing the severity of the occurrence (Kim et al., 2020). There is still a lack of evidence about hematologic changes in heartworm-infected dogs, factors affecting infection, and infection incidence. Therefore, the current study aimed to survey the incidence of heartworm infection and hematological changes in dogs referred to a pet clinic in Thonburi district, Bangkok, Thailand, during 2020-2022.

MATERIALS AND METHODS

Ethical approval

The current study followed the Institutional Animal Care and Use Committee (IACUC) of Suan Sunandha Rajabhat University (SSRU), Bangkok, Thailand. The researchers were trained in using animals for research under the training code U1-08960-2563. Information about animals has been disclosed with consent from the owners.

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Database collection

The dogs (n = 980) came to an animal clinic in Thonburi district, Bangkok Province, Thailand (establishment license 01-957/2562, latitude 13.707529, longitude 100.478054) from 2020 to 2022. Twenty-one dogs were found with heartworm infection. Diagnosis of heartworm infection in the dogs using blood smear and rapid enzyme immunoassay testing (IDEXX SNAP® 4Dx®, United States (was made following the manufacturer's instructions. Clinical signs of infected dogs included anorexia, depression, coughing, panting, hind legs weakness, and vomiting. Physical examination of the dogs revealed fever (39.4 ± 0.27° Celsius, normal dogs temperature is 38 ± 0.88° Celsius, Cichocki et al., 2017), increased heart and lung sounds, emaciation, and dehydration. A vet specialist in small animal internal medicine collected the data. Data included gender, age, breed, rectal temperature, close-open husbandry pattern, ectoparasites prevention program, such as topical (*Frontline*®, *France*), chewing (NexGard®, Brazil), or injection (Baymec®, Korea), and heartworm prevention program. The dog's history data comes from the history taken from the dog's owner.

Clinical hematology and serum biochemistry collection

Blood was collected from the cephalic vein (1.5 ml) in an EDTA (Ethylenediaminetetraacetic acid, China) tube for hematology, blood smear, and rapid enzyme immunoassay testing. Moreover, 1.5 ml of blood was collected in a heparin tube for serum biochemistry testing. Hematology used automatic hematology cell counter (MS 4, Melet Schloesing laboratories, Cergy-Pontoise Cedex, France) evaluation included White Blood Cells (WBC), Hematocrits (Hct), and Platelets (PLT). Serum biochemistry used an automatic analyzer machine (BT 2000, Biotechnica Instruments, Rome, Italy) evaluation consisted of Alanine Aminotransferase (ALT), Blood Urea Nitrogen (BUN), and creatinine. Hematology, serum biochemistry, and blood smears were examined at a standard laboratory (Laboratory of Vet Clinical Center, Bangkok, Thailand). The blood samples were collected aseptically following the study conducted by Sirois (2014).

Statistical analysis

Descriptive analysis was used for the study of database collection, clinical hematology, and serum biochemistry recording using IBM SPSS statistics, version 29 (USA).

RESULTS

From 980 dogs referred to a pet clinic, dogs infected with heartworm involved 21 mixed-breed dogs (2.14%), comprising 12 males (57.14%) and 9 females (42.86%). The average age and rectal temperature of the dogs were 5.62 ± 2.48 years and 39.4 ± 0.27° Celsius, respectively. The husbandry system indicated that all dogs infected with heartworm were classified as an open system (100%). Of the investigated dogs, 10 (47.62%) with heartworm infections were regularly administered with a product to prevent ectoparasites. Moreover, 16 dogs (76.19%) were found to receive the heartworm prevention medication (Ivermectin 6 µg/kg, Heartgard Plus™, Venco et al., 2004) for more than 4 months, while 5 dogs (23.81%) had never received such medication (such as the macrocyclic lactone group, Table 1).

The thin blood smear and the rapid enzyme immunoassay test indicated positive dogs (Figure 1). The average total WBC count in the infected dogs was 17.16 ± 5.65 ($\times 10^3$ cells/µL). The Hct and PLT values were found to be 39.45 ± 7.27% and 301.14 ± 84.90 ($\times 10^3$ cells/µL) in the infected dogs, respectively. The serum biochemistry of infected dogs, ALT, BUN, and creatinine were recorded as 182.33 ± 198.78 IU/L, 71.19 ± 23.83 mg/dL, and 2.09 ± 0.82 mg/dL, respectively (Table 2).

Table 1. Associated factors with heartworm infection in infected dogs of Thonburi district, Bangkok, Thailand, during 2020-2022

Criteria	Dogs infected with *Dirofilaria immitis*	No.	Percentage
Breed	Mixed-breed dogs	21	2.14
	Gender		
	Male	12	57.14
	Female	9	42.86
Age (years)	2	1	4.76
	3	5	23.81
	4	1	4.76
	5	4	19.05
	6	4	19.05
	7	1	4.76
	8	2	9.52
	10	3	14.29
Husbandry system	Close	0	0
	Open	21	100
	Prevention ectoparasites		
	Consistent	10	47.62
	Never/Sometimes	11	53.38
	Prevention heartworm		
	Consistent	0	0
	Never	5	23.81
	Sometimes	16	76.19

Data collection (n=980), No: Number of dogs, Never: Dogs never used preventive products, Sometimes: Dogs use preventive products sometimes but not regularly, Prevention ectoparasites: Dogs use effective drugs against fleas or ticks, Prevention heartworm: Dogs use effective drugs to eliminate heartworm larvae.

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Table 2. Clinical hematology and serum biochemistry of dogs infected with *Dirofilaria immitis* in Thonburi district, Bangkok Province, Thailand during 2020-2022

Parameters	Infected dogs (n = 21)		Normal Range	
	Mean	Observation*	Mean	Range
WBC ($\times 10^3$ cells/µL)	17.16	8.79-32.40	12.05	5.00-14.10
Hct (%)	39.45	21.40-46.60	63.50	35.00-57.00
PLT ($\times 10^3$ cells/µL)	301.14	61.00-497.00	416	211.00-621.00
ALT (IU/L)	182.33	30.00-984.00	64.50	10.00-109.00
BUN (mg/dL)	71.19	35.00-120.00	18	8.00-28.00
Creatinine (mg/dL)	2.09	1.16-4.96	1.1	0.50-1.70

*Observation= range of data observed from raw data. Normal range References (Cyntia, 2011).

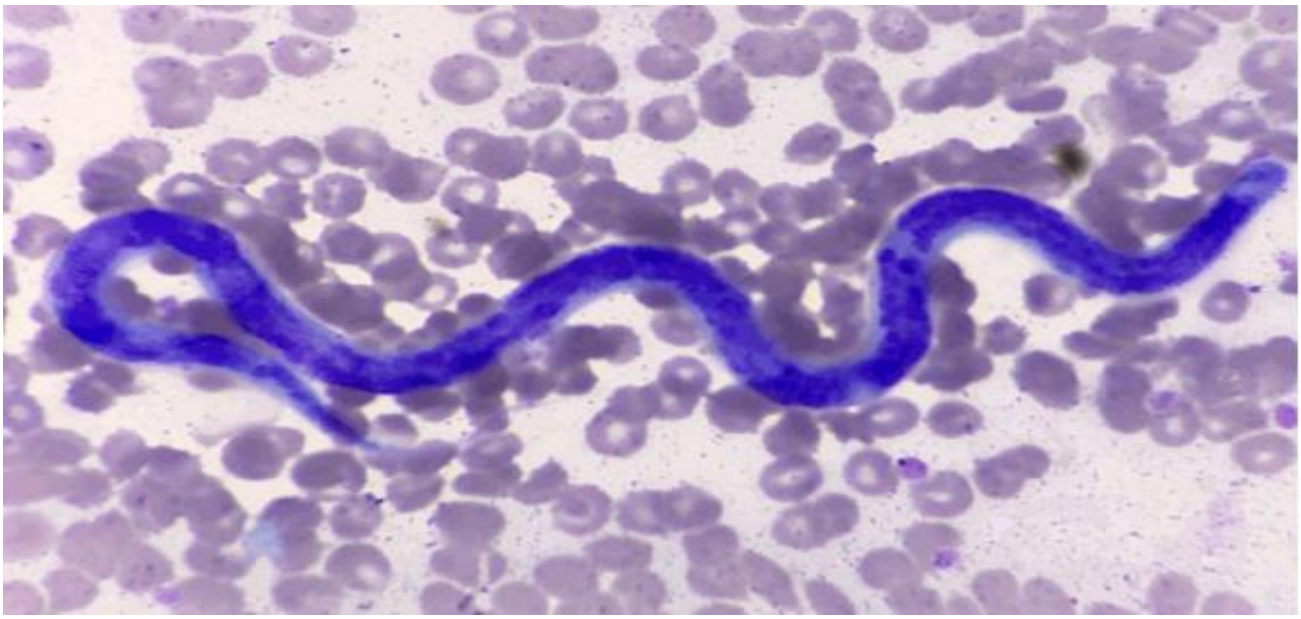

Figure 1. Microfilaria of *Dirofilaria immitis* in an infected dog detected by thin blood smear method under Giemsa staining, 1000× magnification in Thonburi district, Bangkok Province, Thailand

DISCUSSION

The detection of heartworm in dogs has been reported in many countries, such as Italy (Little et al., 2018), the United States (Little et al., 2018), and Thailand (Niwetpathomwat et al., 2007; Kamyingkird et al., 2017). In the current study, all dogs infected with heartworm were found to be mixed breeds. According to a previous report, heartworms can be found in all breeds of dogs (Vieira et al., 2014). The infected dogs were dominantly male dogs (n = 12). However, Boonyapakorn et al. (2008) report indicated no gender difference in heartworm infection in dogs. The average age of the heartworm-infected dogs was 5.62 ± 2.48 years, ranging from 2-10 years. In previous studies, the infected dogs aged were between 2- 6years and over 10 years of age (Boonyapakorn et al., 2008).

Regarding the husbandry system, it was found that all infected dogs (n = 21) were in an open system, which is consistent with previous studies, indicating that infected dogs were often located outside the home and were at greater risk of being bitten by mosquitoes (Borthakur et al., 2015). No differences were between the groups of dogs who consistently used prevention ectoparasites products and those who never, or only occasionally, used prevention ectoparasites products. The reason can be the chosen product was ineffective or inadequate in preventing heartworm, for instance, using only fipronil to control ectoparasites. Therefore, prevention should include other drugs, such as the macrocyclic lactone group (Noack et al., 2021). Previous studies have reported that using moxidectin in combination with doxycycline effectively eliminates dogs' larvae and adults of heartworms (Genchi et al., 2019).

Regarding the history, it was found that five infected dogs that had never been administered the heartworm prevention product were strays brought in by compassionate people. The remaining 16infected dogs had owners to take care of them. The results are consistent with previous reports that dogs without heartworm prevention products were at greater risk of infection (Boonyapakorn et al., 2008).

The hematology and serum biochemistry changes results indicated that the average WBC count was higher in the infected dogs than in the reference range (17.16 $\times 10^3$ cells/µL). The current study result showed all dogs' mean Hct were in the normal range (39.45%), and these data disagree with Kim et al. (2020), reporting anemia and found that

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anisocytosis in dogs with severe status resulted from hemolysis and red blood cell destruction from passing through the worm (Kim et al., 2020). In this study, infected dogs have no sign of severe anemia may be due to a low number of heartworms in infected dogs. The PLT data revealed that infected dogs averaged within the normal range, compared to the reference values. The mean ALT in the dogs infected in the current studywas higher than the reference value (182.33 IU/L). This is related to previous studies by Niwetpathomwat et al. (2007) that have also found elevated ALT in heartworm-infected dogs, which may be associated with intracellular damage, leading to enzyme release (Niwetpathomwat et al., 2007). The BUN and creatinine mean values were higher than the reference values (71.19 and 2.09 mg/dL). The increase in these two values is commonly associated with renal dysfunction, dietary intake, and dehydration (Niwetpathomwat et al., 2007). Previous studies have found that heartworm-infected dogs have elevated BUN and creatinine values, which may be related to immune-mediated glomerulonephropathy (Rawlings and Calvert, 1989; Niwetpathomwat et al., 2007).

Heartworm infection in dogs by carrier mosquitoes can indicate the quality of life in dogs. Infection means that dogs have been subjected to improper handling of vector defenses and inappropriate animal welfare standards (Merck, 2012). Mosquito control, a clean environment, and a lack of stagnant water can improve the environment where dogs are raised; moreover, the living area of pets should have anti-mosquito nets, using chemical eliminates, such as organophosphate (Benelli, 2015). Future studies are needed to explore strategies to educate dog owners about the severity and importance of heartworm prevention to attain better animal welfare management.

CONCLUSION

The study found that heartworm-infected dogs in the Thonburi district, Bangkok province, Thailand, were male and female mixed-breed dogs aged 2 to 10 years. All infected dogs were classified as open husbandry systems and did not consistently use heartworm prevention products. However, despite the use of ectoparasites products, heartworm-infected dogs can still be detected. Heartworm detected in dogs with regular administration of ectoparasites products may result from the inefficacy of the preventative products that do not eliminate heartworm larvae in the bloodstream. The hematological changes in infected dogs included leucocytosis as well as increased levels of ALT, BUN, and creatinine. Owners should keep the dog's area clean, install mosquito nets, and employ regular heartworm prevention strategies.

DECLARATIONS

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Competing interests
The authors declare no competing interests.

Ethical consideration
The authors extensively considered the ethical concerns, including plagiarism, fabrication, falsification, double publication or submission, and consent to publication.

Authors' contribution
Narong Kulnides suggested research guidelines and data analysis, presented information and wrote a manuscript. Athip Lorsirigool conducts research data collection, analyzes data, presents data, and wrote a manuscript. Natapol Pumipuntu recommends and revised content in a manuscript. Chaikamon Chantrarasmee conducted research data collection. Nopparuj Janthong performed data collection. All authors read and approved the final manuscripts.

Availability of data and materials
The authors confirm that the data supporting the findings of this study are available.

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