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Prevalence and factors associated with *Dirofilaria immitis* infection in dogs in Sertão Paraibano, Northeast Brazil[1]

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ABSTRACT.- Soares L.A., Matias I.C., Silva C.G., Oliveira Filho H.S., Alves P.M.M., Sousa H.G.F., Brasil A.W.L., Vilela V.L.R., Galiza G.J.N. & Maia L.A. 2022. **Prevalence and factors associated with *Dirofilaria immitis* infection in dogs in Sertão Paraibano, Northeast Brazil.** *Pesquisa Veterinária Brasileira 42:e07041, 2022*. Laboratório de Patologia Animal, Hospital Veterinário, Centro de Saúde e Tecnologia Rural, Universidade Federal de Campina Grande, Campus de Patos, Avenida Universitária s/n, Bairro Santa Cecília, Patos, PB 58708-110, Brazil. E-mail: laynaslanabreu@gmail.com

Dirofilaria immitis is a heart and large vessel parasite that mainly affects domestic dogs and has shown a re-emerging zoonosis in recent years. The objective of this study was to determine the prevalence, associated factors, and geographic areas of *D. immitis* in dogs in the city of Sousa, Paraíba, Northeast Brazil. A total of 320 dogs were selected and evaluated, 160 domiciled and 160 wandering, from the 32 districts of the city. Clinical examination, blood collection, and epidemiological data retrieval were performed for each animal. The sanitation conditions of the environment were visually observed at the time of evaluation. Three methods were used to investigate the morphometric diagnosis of microfilariae: capillary blood smear (ESC), peripheral blood smear (ESP), and modified Knott test. The data were subjected to univariate and multivariate statistics for the observation of risk factors and qualitative assessment of the examinations. Of the 17.5% (56/320) of animals testing positive for *D. immitis,* 25% (40/160) were wandering and 10% (16/160) were domiciled dogs. Positive dogs were found in 24 of the 32 neighborhoods evaluated, with Angelim and Doutor Zezé having the highest percentages of 1.56% (5/320) each. Only the categories of cardiac alterations (OR 6.231 [1,539-25,236]) and stray dogs (OR 2.463 [1,281-4,735]) demonstrated potential risk factors for infection. Of the 56 positive animals, 28 were positive in the three tests, and another 28 showed variance between methods and/or between prepared slides. No other filaridae were observed. The city of Sousa is considered to have a significant prevalence of infection by *D. immitis*, and forms of control and prophylaxis are required to reduce the risks of transmission to animals, as well as to humans.

INDEX TERMS: *Dirofilaria immitis*, infection, dogs, Brazil, arrhythmia, parasite, wandering, zoonosis.

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RESUMO.- [**Prevalência e fatores associados à infecção por *Dirofilaria immitis* em cães no Sertão Paraibano, Nordeste do Brasil.**] *Dirofilaria immitis* é um parasito de coração e de grandes vasos que acomete principalmente cães domésticos, também causador de uma zoonose reemergente nos últimos anos. O objetivo deste trabalho foi determinar a prevalência, fatores associados e áreas geográficas de *D. immitis* em cães na cidade de Sousa, Paraíba, Nordeste do Brasil. Para isso, foram selecionados e avaliados 320 cães, sendo 160 domiciliados e 160 errantes, provenientes dos 32 bairros da cidade. Foi realizado o exame clínico, coleta de sangue e dados epidemiológicos de cada animal. O ambiente foi observado visualmente quanto as condições de saneamento no momento de avaliação. Para pesquisa de microfilárias, foram realizados três métodos: esfregaço sanguíneo capilar (ESC), periférico (ESP) e teste de Knott-modificado, associado ao diagnóstico morfométrico das microfilárias. Os dados foram submetidos a estatísticas uni e multivariadas para observação de fatores de risco e avaliação qualitativa dos exames. Obteve-se prevalência de 17,5% (56/320) de animais positivos para *D. immitis*, sendo 25% (40/160) errantes e 10% (16/160) domiciliados. Dos 32 bairros avaliados, em 24 foram encontrados cães positivos, sendo Angelim e Doutor Zezé, os bairros com maior percentual 1,56% (5/320) cada. Somente as categorias de alterações cardíacas (OR 6,231 [1.539-25.236]) e cães errantes (OR 2,463 [1.281-4.735]) demonstraram potencial fator de risco para infecção. Dos 56 animais positivos, 28 apresentaram positividade nos três testes, e outros 28 demonstraram variância entre métodos e/ou entre lâminas confeccionadas. Não foram observados outros filarídeos. Considerou-se que a cidade de Sousa apresenta prevalência significativa de infecção por *D. immitis*, sendo necessário estabelecer formas de controle e profilaxia, para reduzir os riscos da transmissão para animais, como também para humanos.

TERMOS DE INDEXAÇÃO: Infecção, *Dirofilaria immitis*, cães, caninos, arritmia, errante, parasito, zoonose.

INTRODUCTION

Dirofilaria immitis (Leidy, 1856) is a filarioid that is generally transmitted by Culicidae and mainly affects domestic dogs and wild canids (Silva et al. 2008, Taylor et al. 2017), and to a lesser extent, felids (Alberigi et al. 2020) and humans (Velev et al. 2019, Gabrielli et al. 2021). It is responsible for causing canine filariasis, also known as heartworm disease in dogs (Nelson et al. 2014). Carrier dogs act as reservoirs of the parasite, serving as a source of infection for other animals and humans, which is a relevant factor for human health (Silva & Langoni 2009). Pre-adult parasites reach the human pulmonary artery and trigger an inflammatory response with the formation of pulmonary nodules (Simón et al. 2012), which can be mistakenly diagnosed as neoplasms (Fontes-Sousa et al. 2019).

D. immitis infection in dogs is of cosmopolitan occurrence (Montoya-Alonso et al. 2015, Kamyingkird et al. 2017, Donnett et al. 2018) that is reported mainly in coastal areas, where the hot and humid climate provides favorable conditions for the proliferation of intermediate hosts, the Culicidae. However, in recent years, this infection has re-emerged in places where it previously existed and appeared in locations where is has not yet been reported (Labarthe 2014, Rodrigues et al. 2019).

In Brazil, *D. immitis* infection is endemic, and studies on the prevalence of this parasite have been conducted in the south (Leite et al. 2007, Kannenberg et al. 2019), southeast (Trancoso et al. 2020, Mendes-de-Almeida et al. al. 2021), north (Ogawa et al. 2013, Moreira et al. 2019) and midwest (Fernandes et al. 2000). In the Northeast region, studies on the prevalence of *D. immitis* in dogs have been conducted mainly in coastal regions, including Alagoas 1.3% (15/1097) (Brito et al. 2001), Bahia 0% (00/200) (Carlos et al. 2007), Maranhão 15% (224/1,495) (Ahid et al. 1999), Pernambuco 11.54% (12/104) (Ramos et al. 2016), and Rio Grande do Norte 47.7% (51/107) states (Ferreira et al. 2004). Variations in the prevalence of *D. immitis* infection at specific locations are mainly related to the types of diagnostic tests used. In Paraíba, the occurrence of *D. immitis* in dogs has been reported in the municipalities of Sousa (Matias et al. 2016) and Patos (Rodrigues et al. 2019). However, despite the presence of parasites in the sertão of Paraíba, no studies have reported *D. immitis* in this non-coastal area.

A lack of information exists on the actual parasitemia levels in the municipality of Sousa in relation to its pathogenic importance. The objective of this study was to determine the prevalence and factors associated with *D. immitis* infection in dogs in Sertão Paraibano, and to identify epidemiological and demographic factors that favor the occurrence of this parasitosis.

MATERIALS AND METHODS

Study area. This study was conducted in the municipality of Sousa (6°45'39" S, 38°13'51" W), located in the Sertão mesoregion, state of Paraíba, Northeast Brazil, from April to December 2018. The city of Sousa is 373.63km from the coastal region João Pessoa, the capital of the state of Paraíba. It has a territorial area of 728,492km^2, consisting of 32 neighborhoods, an estimated population of 69,723 inhabitants, and a population density of 89.10inhab/km^2 (IBGE 2016). It contains a basic sanitation structure consisting of an open sewage network that centrally crosses the entire municipality and flows into the banks of Rio do Peixe, a sub-basin of the Piranhas-Açu River that partially surrounds the north of the city. To the northeast, there are rural areas with irrigated grass plantations. Garbage collection occurs in all neighborhoods on alternate days and there is no sewage supply and treatment. Based on the Köppen-Geiger climate classification, it has a hot semi-arid climate of the "BSh" type, and in 2018 the average annual temperature was 27°C with rainfall of 900mm (INMET 2019).

Dog population and sampling. The size of the human population in the municipality of Sousa was used to define the number of animals that participated in the study. The proportion recommended by Canatto et al. (2012), of 4.3 people per animal, returned a population of 16,175 dogs. To determine the minimum number of animals to be evaluated, simple random sampling was performed (Formula 1) with adjustment for finite populations from the result obtained (Formula 2) (Thrusfield 2004).

$$n = \frac{Z^2 \times P(1-P)}{d^2}$$

Where n = number of animals sampled, Z = normal distribution value for the CI 95%, P = expected prevalence 23.1% (Labarthe et al. 2014), d = 5% absolute error.

$$Najus = \frac{N \times n}{N + n}$$

Where n_{ajus} = adjusted sample size, N = total population size, n = initial sample size.

The minimum number of animals to be evaluated was 268. However, as the municipality of Sousa has 32 neighborhoods, 10 animals were collected from each neighborhood, totaling 320 animals, of which 160 dogs were domiciled and 160 were strays. For each neighborhood, five stray dogs and five domiciled dogs from the same area were chosen at random and according to the availability of owners to participate in the research, respectively. The dogs were aged nine months or older.

The neighborhoods were evaluated in terms of location and separated into four zones, the North Zone (Alto do Cruzeiro, Angelim, Areia, Bancários, Boa Vista, Centro, Guanabara, and Várzea da Cruz), South Zone (Conjunto Nossa Senhora de Fátima, Frei Damião, Jardim Bela Vista, José Lins do Rêgo, Sorrilândia 1, Sorrilândia 2, Sorrilândia 3, and Sousa 1), West Zone (Alto Capanema, André Gadelha, Conjunto Augusto Braga, Jardim Brasília, Jardim Iracema, Jardim Santana, Lagoa dos Patos, and Projeto Mariz) and East Zone (Doutor Zezé, Estação, Estreito, Gato Preto, Jardins, Raquel Gadelha, São José, and Zú Silva).

Animal and environmental epidemiological survey. Epidemiological data of the domiciled animals and the environment were recorded through interviews with the owners of each animal. Information was collected on sex, age, breed, coat color, coat length, vaccination, anthelmintic treatment, previous illnesses and treatments, travel history, food, contact with other animals, environmental health status, and mosquito control. For stray animals, data were collected through observation of phenotypic factors and the environment in which they were found.

Evaluation and collection of samples. The animals were subjected to a general clinical evaluation for nutritional status analysis, mucosal color, lymph node palpation, hydration level, peripheral pulse, and cardiac and respiratory auscultation. Subsequently, mechanical restraint was performed, and 3mL of blood was collected from the cephalic or jugular vein and placed in previously identified sterile tubes containing the anticoagulant EDTA (sodium salt of ethylenediaminetetraacetic acid). Blood samples were collected between 08:00 and 18:00, when microfilaremia was higher, due to the adaptation of the filaria to the feeding habits of the vector (Acha & Szyfres 2003). The samples were packaged and sent to the Laboratory of Veterinary Parasitology (LPV) of the HV-IFPB for peripheral blood smear (ESP) and modified Knott tests. Capillary blood smears (ESC) were obtained from ear tips. Blood smear slides were fixed and stained using Panótico Rápido®, identified, and forwarded to the LPV for microscopic visualization.

Circulating microfilaria detection tests. Three methods were used to investigate circulating microfilariae: capillary blood smear (ESC), peripheral blood smear (ESP), and the modified Knott test (Newton & Wright 1956). Two slides were prepared for each procedure. The modified Knott test was performed according to Bowman (2010). The method consisted of mixing 1mL of blood with 10mL of 2% formaldehyde in a Falcon tube, which was then homogenized by inversion and agitation to lyse the erythrocytes and conserve the microfilariae in a distended format. The sample tube was then centrifuged at 1,500rpm for 5 min. After centrifugation, the supernatant was discarded by inverting the tube, and a drop of 0.1% methylene blue was added to the decanted part to homogenize the mixture. Subsequently, with the aid of a micropipette, part of the sediment was placed on a slide and covered with a coverslip for visualization under an optical microscope of distended microfilariae stained in blue.

Morphometric diagnosis of the microfilariae of the positive animals was performed in association with the modified Knott test. The features described by the CVBD (2006) were used to differentiate *D. immitis* microfilariae from other filaridae. According to CVBD (2006), the microfilariae of *D. immitis* are larger, measuring 205-283µm in length and 5-6.5µm in width, and they have conical heads and straight tails, while other filarids, such as *Acanthocheilonema* (*Dipetalonema*) *reconditum*, are smaller (213-240µm long, 4-5µm wide), with a blunt head and a bent hook-shaped tail.

Statistical analysis. Based on the epidemiological questionnaires, an analysis was performed to verify a possible association between the questionnaire data (independent variable) and test results (dependent variable). The variables evaluated were: living situation, sex, age, breed, coat color, coat length, vaccination, anthelmintic treatment, clinical signs of disease, previous illnesses and treatments, travel history, contact with other animals, dumps/rubble, sewers, presence of vegetation, mosquito control, neighborhood assessment, and collection time.

Initially, a univariate exploratory analysis was performed to select variables that presented $P \leq 0.2$ using the chi-square or Fisher's exact test. Second, the selected variables were subjected to multivariate analysis through multiple logistic regression at a significance level of 5% (Hosmer & Lemeshow 2000).

The final model was adjusted using the coefficient of Hosmer and Lemeshow to best fit the value to $P \geq 0.05$. The collinearity of the independent variables was determined by correlation analysis, and when the correlation coefficient was <0.9, one of the variables was eliminated according to biological plausibility (Dohoo et al. 1997). Data analyses were performed using SPSS 23.0 for Windows software. The results shown in Figure 1 were obtained using ArcGIS 9.1.

Ethics Committee on the Use of Animals (CEUA). This research was evaluated and approved by the CEUA under registration No. 01.0462.2017 and registration No. 23000.000549.2018-37 after adaptation to the opinion delivered to the committee on 05/25/2018.

RESULTS

The prevalence of *Dirofilaria immitis* in dogs in the municipality of Sousa in at least one of the tests evaluated was 17.5% (56/320, 95% [CI 13.3-21.7]), of which 71.43% were strays and 28.57% were domiciled. The levels according to category were 25% (40/160, 95% [CI 59.6-833.3]) in stray dogs and 10% (16/160, 95% [CI 16.7-40.4]) in domestic animals, with statistical differences ($P<0.0013$). Of the 32 neighborhoods evaluated, dogs presenting *D. immitis* were found in 24 in at least one of the tests performed. The numbers of positive stray and domiciled dogs in each neighborhood are listed in Table 1 and Figure 1, respectively.

The neighborhoods of Angelim (North Zone) and Doutor Zezé (East Zone) each showed the higher percentage of 1.56% (5/320). Angelim is located on the outskirts of the northern part of the municipality of Sousa, delimited by the Rio do Peixe and containing irrigated grass plantation areas. In this neighborhood, 0.63% (2/320) of domiciled animals and 0.94 % (3/320) of stray animals were positive for the parasite. The Doutor Zezé neighborhood is near the sewage channel that crosses centrally to the eastern side of the city, with 5/320 positive stray dogs of unknown origin.

The characteristics of the animals and the demographic aspects of the environments evaluated in the epidemiological

questionnaires were statistically analyzed in relation to the presence of *D. immitis*. The variables of living situation, breed, age, anthelmintic treatment, and clinical examination showed values of $P<0.2$ by the chi-square test and were selected for multivariate analysis (Table 2). Additionally, only cardiac alterations (clinical examination) and stray dogs (living situation) were potential risk factors for infection in the multivariate analysis. Cardiac alterations (odds ratio 6.231 - [CI 1.539-25.236]) and stray animals (OR 2.463 - [CI 1.281-4.735]) were identified as risk factors in the logistic regression analysis (Table 3).

The circulating microfilariae research tests in the 56 positive dogs revealed that 28 (50%) were positive in the three tests, and another 28 (50%) showed variance between methods and/or prepared slides. The modified Knott method yielded the highest number of positive samples (56 dogs). However, 51 dogs (91.07%) were positive on two slides, while five (8.93%) were positive on only one slide. The ESP showed 36 (64.29%) with microfilaria on two slides, 12 (21.43%) on a single slide, and eight (14.28%) with no microfilaria on either slide. The ESC revealed that 32 (57.14%) dogs were positive on both slides, 12 (21.43%) on one slide, and 12 (21.43%) were negative (Table 4).

In the positive blood smears, *D. immitis* specimens were characterized by basophilically stained vermiform structures. The microfilariae identified in the modified Knott test were among the values mentioned by CVBD (2006) for *D. immitis*. No other filaridae were observed.

DISCUSSION

The prevalence of *Dirofilaria immitis* in dogs in Sertão da Paraíba as determined in this study is important to obtain an initial view of the occurrence and distribution of this parasitosis in non-coastal regions, which may present epidemiological conditions that favor the transmission of the parasite. According to Alho et al. (2014), certain factors have bolstered the increase in the occurrence of infection by *D. immitis* in endemic and interior regions, including globalization, the emergence of new species of vector mosquitoes, insecticide resistance, and a lack of sentinel host control. Additionally, artificial climate and ecological changes, such as rising temperatures, rainfall, and the formation of "heat islands" through urban sprawl have been promoting adaptation, development, and multiplication of vectors, thus favoring the transmission of this parasite. Although the demographic conditions studied were not statistically significant, it is believed that the municipality of Sousa presents demographic epidemiological factors that contribute to the proliferation and maintenance of mosquitoes responsible for the transmission of the disease, including the hot semi-arid climate, improper sanitation, and the presence of an open sewage channel intersecting some districts of the municipality.

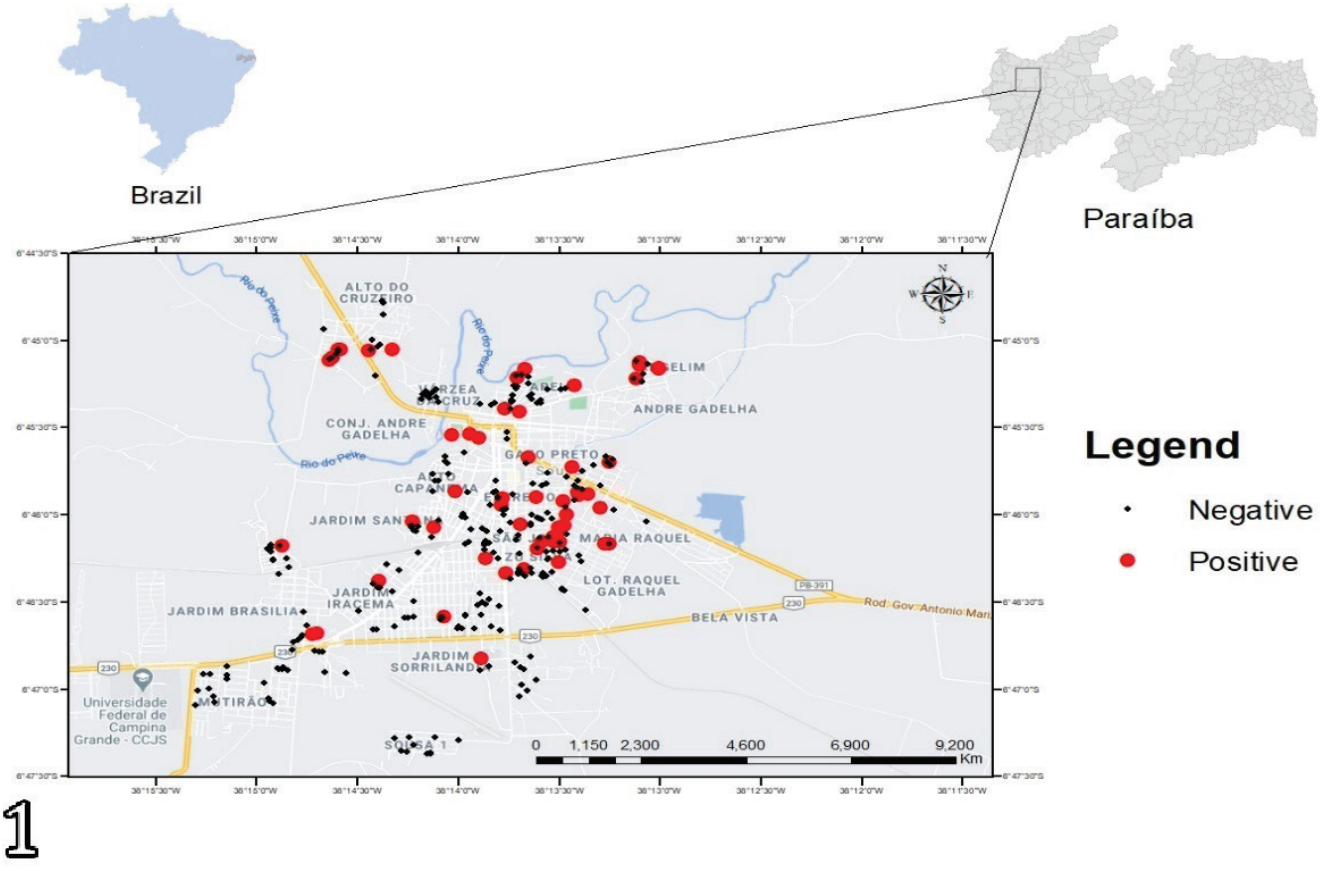

Fig.1. Map with spatial distribution of positive and negative animals for *Dirofilaria immitis* distributed in all neighborhoods of the municipality of Sousa, Paraíba, from April to December 2018.

Table 1. Number of domiciled and stray dogs positive for *Dirofilaria immitis* distributed by neighborhoods in the municipality of Sousa, Paraíba, from April to December 2018

Positive neighborhoods	Number of positive dogs domiciled	Number of stray positive dogs	Total number of positive dogs (domiciled + strays)
		North Zone	
Alto do Cruzeiro	1 (0.31%)	1 (0.31%)	2 (0.63%)
Angelim	2 (0.63%)	3 (0.94%)	5 (1.56%)
Areias	0 (0%)	1 (0.31%)	1 (0.31%)
Bancários	1 (0.31%)	2 (0.63%)	3 (0.94%)
Boa Vista	2 (0.63%)	2 (0.63%)	4 (1.26%)
Centro	0 (0%)	3 (0.94%)	3 (0.94%)
Guanabara	1 (0.31%)	0 (0%)	1 (0.31%)
TOTAL	7 (2.17%)	12 (3.72%)	19/320 (5.89%)
		South Zone	
Frei Damião	0 (0%)	2 (0.63%)	2 (0.63%)
José Lins do Rêgo	0 (0%)	1 (0.31%)	1 (0.31%)
Sorrilândia 1	0 (0%)	1 (0.31%)	1 (0.31%)
TOTAL	0 (0%)	4 (1.24%)	4/320 (1.24%)
		West Zone	
Alto Capanema	0 (0%)	1 (0.31%)	1 (0.31%)
André Gadelha	1 (0.31%)	3 (0.94%)	4 (1.26%)
Conjunto Augusto Braga	1 (0.31%)	0 (0%)	1 (0.31%)
Jardim Brasília	0 (0%)	2 (0.63%)	2 (0.63%)
Jardim Iracema	0 (0%)	1 (0.31%)	1 (0.31%)
Jardim Santana	0 (0%)	2 (0.63%)	2 (0.63%)
Lagoa dos Patos	0 (0%)	1 (0.31%)	1 (0.31%)
TOTAL	2 (0.63%)	10 (3.10%)	12/320 (3.72%)
		East Zone	
Doutor Zezé	0 (0%)	5 (1.56%)	5 (1.56%)
Estreito	0 (0%)	2 (0.63%)	2 (0.63%)
Gato Preto	2 (0.63%)	1 (0.31%)	3 (0.94%)
Jardins	1 (0.31%)	1 (0.31%)	2 (0.63%)
Raquel Gadelha	2 (0.63%)	2 (0.63%)	4 (1.26%)
São José	1 (0.31%)	1 (0.31%)	2 (0.63%)
Zú Silva	1 (0.31%)	2 (0.63%)	3 (0.94%)
TOTAL	7 (2.17%)	14 (4.34%)	21 (6.51%)
Total	16 (5%)	40 (12.5%)	56/320 (17.50%)

Table 2. Univariate analysis of risk factors associated with *Dirofilaria immitis* infection in dogs in the municipality of Sousa, Paraíba, from April to December 2018 (*P*≤0.2)

Variable	Category	Total number of animals	Number of positive animals (%)	P	OR	CI
Living situation	Domiciled	160	16 (10.0)	<0.0001	3.00	[1.600 – 5.264]
	Wanderer	160	40 (25.0)			
Breed	NDB	261	53 (20.3)	0.005	0.210	[0.063 – 0.698]
	With breed	59	3 (5.1)			
Age	Up to 3 months	16	0 (0)			
	Up to 12 months	40	3 (7.5)	0.075	-	-
	More than 12 months	280	53 (18.9)			
Anthelmintic treatment	No	262	52 (19.8)			
	Yes, 4 months ago or less	34	2 (5.9)	0.062	-	-
	Yes, more than 4 months ago	24	2 (8.3)			
Clinical signs of disease	Without changes	213	33 (15.5)			
	Heart changes	35	14 (40.0)	0.002	-	-
	Signs of Leishmaniasis	42	6 (14.3)			
	Other changes	30	3 (10.0)			

NDB = no defined breed, OR = odds ratio, CI = confidence interval.

The East (21/320) and North (19/320) Zones had the highest occurrence of positive dogs, with the Angelim and Doutor Zezé districts presenting the greatest number of animals. In the North Zone, demographic conditions were observed that likely influenced the occurrence of *D. immitis*, such as the proximity to the Rio do Peixe, and the areas where irrigated grass was planted. In the East Zone, the five positive dogs from the Doutor Zezé neighborhood were all strays, suggesting that the infection may have occurred in more favorable places for contamination and that these animals subsequently migrated to this neighborhood. However, the open-air sewage network that crosses the neighborhoods of this area was a possible factor influencing the transmission of the parasite in the East Zone.

The variable "living situation" was considered a risk factor for *D. immitis* infection, demonstrating a higher prevalence of parasitosis in stray dogs than in domiciled dogs. Similar results were observed by Labarthe et al. (2014), who compared the risk of infection between domiciled and stray animals and observed a positivity rate of 39.3% for stray dogs and 30.2% for domiciled dogs. It is believed that domiciled dogs are likely to have less exposure to transmitting vectors because they live indoors and have a greater likelihood of receiving anthelmintic treatments that prevent the development of the parasite (Manev 2020).

Leite et al. (2007) suggested that stray dogs are at greater risk of infection, as they have free access to localities in the municipality and are not monitored by public agencies that control their populations. In addition, stray dogs are more susceptible to other pathogens because of their outdoor lifestyle and greater exposure to vectors (Diakou et al. 2016, Guven et al. 2017). It is believed that stray dogs carrying *D. immitis* act as reservoirs with free access to neighborhoods in the city of Sousa without control or treatment, and they infect other dogs and possibly other animals and humans.

Features of the hosts, such as sex, age, and breed, along with extrinsic factors, can influence the occurrence of infection (Almeida et al. 2001). It was observed that 20.3% of the positive animals were of mixed-breed, which corroborates the results of Almeida et al. (2001) and Moreira et al. (2019), who also found no statistically significant difference between the prevalence of infection and race. Although the results indicate a greater chance of infection in mixed-breed animals, there were a larger number of these canines evaluated and there may have been a higher breeding rate of mixed-breed than pure-bred dogs. Age was not considered a risk factor; however, it presented a statistical difference in the univariate analysis, where 53 of the positive dogs were considered over 12 months old, which is an ideal condition for the pre-patent period of microfilariae development (Bowman 2010), corroborating the findings of Trancoso et al. (2020).

Therapeutic and prophylactic anthelmintics had been administered in 16.9% (54/320) of the animals, according to the results of the questionnaire: "yes, for 4 months or less" and "yes, for more than 4 months," and all dogs in this group tested negative for *D. immitis*. Diethylcarbamazine-based dewormers and agents from the macrocyclic lactone family are used for the treatment and prevention of *D. immitis*, which are effective enough to interrupt the larval stage two months after infection and cause few adverse effects in animals (Meireles et al. 2014). However, four animals had positive results, presenting a history of recent anthelmintic treatment, which questions the veracity of their use by the owners of these animals, as well the effectiveness of the anthelmintic against the worm or high parasite load of these animals during the period of application.

Cardiac arrhythmia (13/320) was the clinical sign observed in the largest number of positive animals; however, none of the dogs presented a clinical picture of heartworm disease. It

Table 3. Risk factors of the multivariate analysis for infection by *Dirofilaria immitis* in dogs in the municipality of Sousa, Paraíba, from April to December 2018 ($P \leq 0.05$)

Variable	OR	CI 95%	P
Living situation: wandering dog	2.463	[1.281-4.735]	0.007
Clinical examination: cardiac changes	6.231	[1.539-25.236]	0.010

OR = odds ratio, CI = confidence interval.

Table 4. Variation of positivity in blood tests for circulating microfilariae in 28 (50%) dogs positive for *Dirofilaria immitis* in the municipality of Sousa, Paraíba, from April to December 2018

Dog	Blood tests						Dog	Blood tests					
	KNOTT		ESP		ESF			KNOTT		ESP		ESF	
01	+	-	+	-	-	-	15	+	+	+	-	+	+
02	+	+	+	-	-	+	16	+	+	+	+	+	-
03	+	+	+	-	-	+	17	+	-	-	-	-	-
04	+	+	-	-	-	+	18	+	+	-	-	-	+
05	+	+	+	-	+	+	19	+	+	+	-	+	+
06	+	+	+	-	+	+	20	+	+	+	-	+	+
07	+	+	+	-	+	+	21	+	-	-	-	-	-
08	+	-	-	-	-	-	22	+	+	+	-	-	-
09	+	+	+	+	+	-	23	+	+	-	-	+	-
10	+	-	-	-	-	-	24	+	+	+	-	+	-
11	+	+	-	-	+	-	25	+	+	+	-	+	+
12	+	+	+	+	+	-	26	+	+	-	-	-	-
13	+	+	+	+	+	-	27	+	+	-	-	-	-
14	+	+	+	-	+	+	28	+	+	-	-	+	+

KNOTT = modified Knott test, ESP = peripheral blood smear, ESF = fresh blood smear.

is believed that these animals had mild to moderate infection, since dogs with a high parasite load can sustain right ventricular failure due to stenosis, blockage, and vascular lesions causing hypertrophy, which can eventually progress to right heart failure over time (Zachary 2018). As the case progresses, nonspecific clinical signs are manifested by the animal, such as cough, exercise intolerance, dyspnea, and heart and lung sounds, characterizing heartworm disease (Nelson & Couto 2015). Heart murmur is an important clinical sign in animals with heartworm congestive heart failure (Ferreira 2015). The dog that presented a heart murmur (1/320), manifested during clinical evaluation, also displayed apathy, weight loss, and respiratory noises. However, it was not possible to diagnose this as heartworm disease, and a thorough clinical examination associated with the use of imaging tests was required to exclude other diseases with similar symptoms.

The identification of parameters and risk factors for infection by this parasite, stimulates practical protocols for the control and prophylaxis of this zoonotic parasite. Areas with the highest occurrence of *D. immitis* in dogs favor a greater risk of infection in humans (Simón et al. 2012). It has been suggested that the city of Sousa has epidemiological factors that contribute to infection in humans, which are probably underdiagnosed and erroneously related to neoplasms. According to Cavallazzi et al. (2002), the frequency and distribution of human heartworm disease are mainly influenced by the size of the dog population, prevalence of canine heartworm disease, density of mosquitoes, and exposure of these animals to vector bites, which are factors considered significant in this study.

In areas where this parasitosis occurs in dogs, clinical and epidemiological investigations of transmission to humans are necessary, due to the difficulty of diagnosis through serological and molecular methods; the use of imaging and histopathological examinations is recommended (Gabrielli et al. 2021). It is suggested that due to the significant prevalence of *D. immitis* infection in dogs in the Sertão region and the close relationship between these animals and humans, there are possible transmissions, underdiagnosed cases, and erroneous diagnoses as neoplasms.

Four dogs that showed positive results in any of the tests had negative blood smears (4/28); however, they showed positivity on only one slide in the modified Knott test. This result was expected since the concentration of red blood cells and microfilariae obtained in the Knott test facilitates their research and identification (Newton & Wright 1956). For the identification of microfilariae through a blood smear, infections above 20 to 50 microfilariae per mL are required (Dillon 2007, Silva & Langoni 2009), suggesting a lower sensitivity compared to that of a concentration test. The low circulating parasite load at the time of collection, use of anthelmintics, and/or recent infection may also contribute to the variation in results between tests.

For the most effective diagnosis of *D. immitis* infection, it is important to associate circulating microfilariae screening tests with antibody or antigen detection tests, such as enzyme-linked immunosorbent assay (ELISA) and/or rapid immunochromatographic tests that demonstrate high sensitivity (Nelson et al. 2014). Antibody detection tests provide more security in the diagnosis of this parasitosis than the modified Knott test, because it does not present positivity in cases of dogs parasitized by adult worms of the same sex, recent infections, and animals with a high immune response that masks the microfilaremia (Fernandes et al. 2000). Therefore, it is believed that the prevalence of this parasitosis in Sertão da Paraíba should be based on the evaluation of these tests, which mirror the rate of infection in these animals.

Additionally, the sampling method for each neighborhood should be properly planned in future research, as some neighborhoods have a greater population, which corresponds to more animals, and a proportional sampling should be evaluated, which could present a variation to the prevalence found in this study. The occurrence rate of *D. immitis* in dogs in this study was not compatible with the majority of information found in the literature, representing unique and important research for the region.

CONCLUSIONS

The prevalence of *Dirofilaria immitis* in dogs in the municipality of Sousa was relevant to Northeast Brazil, with the first report of this parasitosis in the non-coastal region of Paraíba.

Stray animals and arrhythmia were considered as risk factors for *D. immitis* parasitemia.

The modified Knott test showed the higher positivity results for infection compared to blood smears.

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