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# Updated canine infection rates for *Dirofilaria immitis* in areas of Brazil previously identified as having a high incidence of heartworm-infected dogs

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## Abstract

**Background:** Canine heartworm infections were frequently diagnosed in Brazil before the new millennium. After the year 2000, the frequency of diagnosis showed a sharp decline; however, a few years later, new evidence indicated that the parasite was still present and that canine infection rates seemed to be increasing. Therefore, an updated survey of canine heartworm prevalence was conducted in several locations in south, southeast, and northeast Brazil.

**Methods:** Dogs from 15 locations having previously reported a high prevalence of heartworm infection were included in the survey according to defined criteria, including the absence of treatment with a macrocyclic lactone for at least 1 year. Blood samples from 1531 dogs were evaluated by an in-clinic immunochromatography test kit (Witness® Heartworm, Zoetis, USA) for detection of *Dirofilaria immitis* antigen. At each location, epidemiologic data, including physical characteristics and clinical signs reported by owners or observed by veterinarians, were recorded on prepared forms for tabulation of results by location, clinical signs, and physical characteristics.

**Results:** The overall prevalence of canine heartworm infection was 23.1%, with evidence of heartworm-infected dogs detected in all 15 locations studied. There was a tendency for higher prevalence rates in environmentally protected areas, despite some locations having less-than-ideal environmental temperatures for survival of vector mosquitoes. Among physical characteristics, it was noted that dogs with predominantly white hair coats and residing in areas with a high (≥20%) prevalence of heartworm were less likely to have heartworm infection detected by a commercial heartworm antigen test kit than were dogs with other coat colors. In general, dogs older than 2 years were more frequently positive for *D. immitis* antigen than were younger dogs. Clinical signs of heartworm infections were rare or owners were unable to detect them, and could not be used for reliable prediction of the presence of heartworm.

**Conclusions:** These results indicate that the prevalence of *D. immitis* has increased in these areas of Brazil over the past few years. Small animal practitioners in these areas should include routine screening tests for heartworm infections in every dog's annual evaluation protocol and make sure to have uninfected dogs on prevention.

**Keywords:** Canine antigen tests, Canine heartworm, Clinical signs, *Dirofilaria immitis*

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## Background

*Dirofilaria immitis* (Leidy, 1856) Raillet & Henry, 1911, is a mosquito-borne parasite species distributed throughout all continents although in different prevalences. The prevalence of the parasite in Brazil was reported to be 8% during the 1980s [1], with hotspots that could be as high as 45% in the coastal lowlands of the state of São Paulo [2] and in the eastern lowland section of the state of Rio de Janeiro [3]. Several canine heartworm hotspots were identified in coastal areas such as Florianópolis (12%), state of Santa Catarina [4]; Guaratuba (6%) and Guaraqueçaba (21%), state of Paraná [5]; Bertioga (45%) and Guarujá (14%), state of São Paulo [2], região dos lagos (52%) and Niterói (37%), state of Rio de Janeiro [3]; Recife (12%) [6] and Itamaracá (29%) [7], state of Pernambuco; and Salvador (5.4%) and Lauro de Freitas (23.3%), state of Bahia [8]. As awareness of the disease increased following introduction of chemoprophylatic drugs in Brazil and with the increased treatment of canine tick-borne diseases, the number of heartworm-infected dogs declined. At the beginning of the new millennium (2001), the reported national prevalence of heartworm infection in Brazil was 2%, while *Ehrlichia canis* seroprevalence was 30% [9]. The reasons for the downward trend in *D. immitis* infection in Brazil included appropriate use of chemoprophylaxis, widespread use of off-label injectable ivermectin, and increased use of tetracycline (or derivatives) to control ehrlichiosis [10] that may affect the *Wolbachia* endosymbiont as well as the survival and reproduction of adult heartworm [11].

Following the observed decline in the prevalence of heartworm in the early years of the new millennium, the first report of a Brazilian outbreak was in the state of Rio de Janeiro, at the eastern lowland section [12]. Subsequent to this report, small animal practitioners from different areas of the country began to detect heartworm infections in dogs in their clinics during routine blood work [13]. Despite the apparent increase in reports of infected dogs, there are no updated systematic surveys conducted in Brazil. Therefore, the need for an update on canine heartworm prevalence in Brazil is unquestionable.

The damage promoted over time to pulmonary arteries, right ventricle, and to all vascular structures near the lungs by the adult worms often leads to severe disease [14], generally recognized by clinical signs, such as coughing, dyspnea, and exercise intolerance [15,16]. Although the disease is well known in the literature, many times it is difficult to be identified by owners and veterinarians; thus, infected dogs may not receive prompt treatment for heartworm infection. Therefore, there is an urgent need to convince small animal practitioners to include heartworm testing as a routine examination for their dogs even when owners report the absence of any clinical signs that could suggest *D. immitis* infection.

Considering that updated data regarding the prevalence of *D. immitis* in areas of Brazil is warranted and that reliable clinical signs of heartworm infection in dogs are missed or underestimated by owners, the present article reports the prevalence of canine heartworm infection at different sites in the coastal area of Brazil as well as dog-owners' perceptions regarding clinical signs of the presence of heartworm infection. These data are expected to aid veterinary practitioners for better and earlier diagnosis of canine heartworm infection in their practice.

## Methods

The study was designed to include approximately 1600 dogs from areas previously identified as having high rates of heartworm infection. Dogs were selected for inclusion in the study according to the following criteria: i) dogs should have lived at the location for at least 1 year; ii) if possible, there should be no more than three dogs kept in the home; iii) dogs could have not received any treatments with macrocyclic lactones for at least 12 months; and iv) a formal consent must be signed by owners. The protocol was approved by the committee of animal use (CEUA) of the Universidade Federal Rural do Rio de Janeiro.

Along the Brazilian coast, the states Santa Catarina, Paraná, São Paulo, Rio de Janeiro, Bahia, and Pernambuco were included in the survey (Figures 1, 2 and 3). These states had previously been reported to have high rates of heartworm infection. The minimum number of samples to be obtained in each state was calculated with Epi Info 2000 (Centers for Disease Control and Prevention, Atlanta, Georgia) for determining 90% confidence intervals, considering the canine population to be 15% of the human population [17] and the estimated heartworm prevalence to be as reported previously [2,4-8].

Blood samples were obtained from dogs presented for rabies vaccination or spay and neuter campaigns. At places where no such campaigns were available, active search for homes with dogs was used, avoiding buildings that were multiple-housing units. At each locale, epidemiologic data, including each dog's characteristics and clinical signs, were recorded on prepared survey forms. Blood samples were processed to obtain plasma to be tested by an in-clinic immunochromatography test kit (Witness® HW (heartworm) antigen test kit, Zoetis, USA) for detection of *D. immitis* antigen.

Test results were compiled by locale, clinical signs, and dog characteristics for determination of statistical significance by chi square or Fisher exact tests.

## Results

A total of 1531 blood samples were obtained from September 2013 through March 2014, reaching the

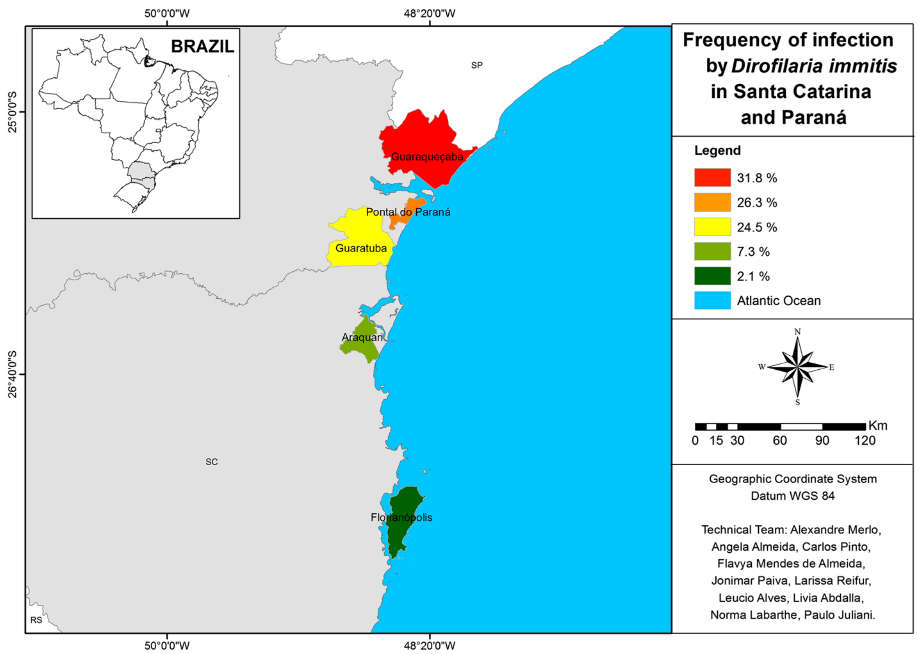


**Figure 1** Map showing the percentage of canine positive test results for heartworm antigen in southern Brazil.

minimum sample size for a 90% confidence interval. Overall, 23.1% of the samples examined were positive for heartworm antigen (Table 1). Heartworm-infected dogs were diagnosed in all locales.

The lowest area rate (13.2%) was found in the southern states, which have a milder climate. Nonetheless, even in the southern area, in less urbanized locales with better preserved natural resources, some states had rates higher than the overall prevalence in the survey (Table 1, Figure 1). Anthropization and climate seem to have had the greatest influence in the southeast, in which 26.3% of dogs tested positive. In Guarujá, a locale with mild

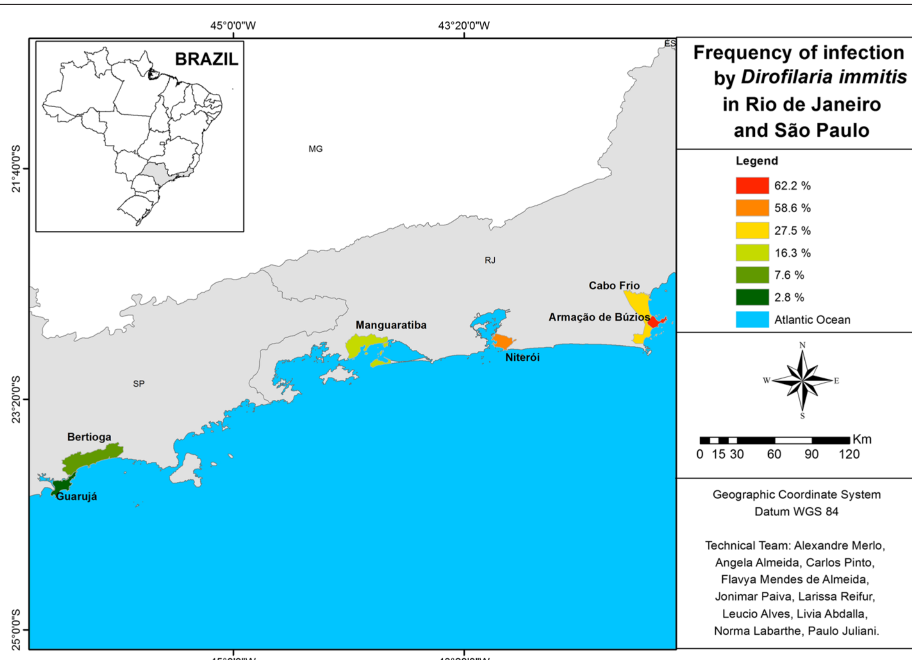


**Figure 2** Map showing the percentage of canine positive test results for heartworm antigen in southeastern Brazil.

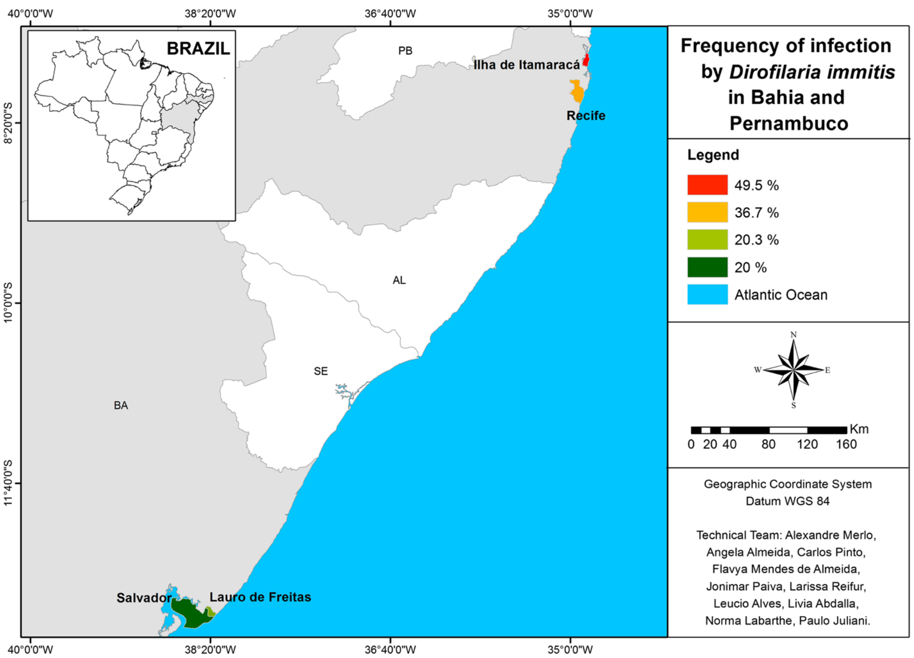


**Figure 3 Map showing the percentage of canine positive test results for heartworm antigen in northeastern Brazil.**

temperatures (10.5° to 34.5°C) and which is highly impacted by industrial activities, 2.8% of the samples tested positive. In Niterói, where nature is better preserved, despite being located in a metropolitan area and temperature range from 10.1° to 41°C, 58.6% of the samples tested positive; and at Armação de Búzios, a well-preserved summer resort, 62.2% of samples tested positive (Table 1, Figure 2). The collective rate of dogs positive for heartworm in the southeast area was 26.3%. The overall regional rate of positive tests in the northeast was 29.7%, with the two biggest cities of the region (Salvador and Recife) displaying high rates (20% and 36.7%, respectively) in areas that are less anthropized (Table 1, Figure 3).

### Results in areas with low prevalence

In areas where a low prevalence of heartworm infection was detected (<20%), owner perception of the density of mosquitoes and the majority of individual canine characteristics were not associated with positive test results (Table 2). Two individual characteristics that could be associated with positive results were gray coat color versus other coat colors ($\chi^2 = 17.93$; df = 4; $P = 0.001$) and size (weight). Small dogs (<5 kg), medium-large dogs, and large dogs (>25 kg) were less associated with positive test results than were medium-small dogs (5–15 kg) ($\chi^2 = 19.37$; df = 3; $P < 0.001$) (Table 2).

### Results in areas with high prevalence

At locales where positive test results were high (≥20%), owner perception of mosquito density also showed no association with canine heartworm infection ($\chi^2 = 1.23$; df = 2; $P = 0.540$). However, hair coat length, predominant coat color, life style, age, and size all demonstrated an effect on prevalence of heartworm. Short-haired dogs were positive for heartworm antigen more often than dogs with medium coat length ($\chi^2 = 5.46$; df = 1; $P = 0.027$) or dogs with long hair ($\chi^2 = 5.58$; df = 1; $P = 0.024$). Dogs with white hair coats tested negative for heartworm antigen more often than dogs with black ($\chi^2 = 11.5$; df = 1; $P < 0.001$), golden ($\chi^2 = 17.34$; df = 1; $P < 0.001$), or brown coats ($\chi^2 = 5.391$; df = 1; $P = 0.020$). Outdoor animals showed higher probability of testing positive than those kept indoors most of the time ($\chi^2 = 7.103$; df = 1; $P < 0.008$). Younger dogs (1–2 years) were less likely to test positive than were dogs of other (older) age groups ($\chi^2 = 8.23$; df = 3; $P = 0.042$). The percentage of large dogs (>25 kg) that tested positive was statistically similar to that for medium-large dogs (15–25 kg) ($\chi^2 = 2.61$; df = 1; $P = 0.106$), and dogs in both of these two size (weight) groups tested positive significantly more often than medium-small or small dogs ($\chi^2 = 19.46$; df = 1; $P < 0.001$) (Table 2). Medium-small dogs and small dogs had similar percentages of positive tests ($\chi^2 = 1.18$; df = 1; $P = 0.277$).

### Clinical signs and physical categories

Physical variables that had a significant influence on test results included age group ($\chi^2 = 31.1$; df = 3; $P < 0.001$); size ($\chi^2 = 21.3$; df = 3; $P < 0.001$), and breed ($\chi^2 = 22.7$; df = 2; $P < 0.001$) (Table 3).

**Table 1 Detection of antigens of *Dirofilaria immitis*[a] in canine blood samples from different areas of Brazil**

| Region/locales | Antigens of *D. immitis* | |
|---|---|---|
| | Positive/total | % |
| **South** | | |
| Florianópolis, SC | 3/146 | 2.1 |
| Araquari, SC | 11/150 | 7.3 |
| Guaratuba, PR | 12/49 | 24.5 |
| Guaraqueçaba, PR | 7/22 | 31.8 |
| Pontal do Paraná, PR | 31/118 | 26.3 |
| Combined | 64/485 | 13.2 |
| **Southeast** | | |
| Guarujá, SP | 4/142 | 2.8 |
| Bertioga, SP | 7/92 | 7.6 |
| Mangaratiba, RJ | 23/141 | 16.3 |
| Niterói, RJ | 92/157 | 58.6 |
| Cabo Frio, RJ | 11/40 | 27.5 |
| Armação de Búzios, RJ | 23/37 | 62.2 |
| Combined | 160/609 | 26.3 |
| **Northeast** | | |
| Lauro de Freitas, BA | 30/148 | 20.3 |
| Salvador, BA | 24/120 | 20.0 |
| Recife, PE | 22/60 | 36.7 |
| Itamaracá, PE | 54/109 | 49.5 |
| Combined | 130/437 | 29.7 |
| **Overall Total** | **354/1531** | **23.1** |

[a]Tested with Witness® HW (heartworm) antigen test kit, Zoetis, USA.

**Table 2 Number and percentage positive for antigens of *Dirofilaria immitis*[a] in canine blood samples according to the relative prevalence in areas sampled**

| Categories | *D. immitis* antigens | | | |
|---|---|---|---|---|
| | Areas with <20% prevalence | | Areas with ≥20% prevalence | |
| | Positive/total | % | Positive/total | % |
| Hair coat length | | | | |
| Short | 35/430 | 8.1 | 217/555 | 39.1† |
| Medium | 9/185 | 4.9 | 77/252 | 30.6* |
| Long | 4/56 | 7.1 | 12/53 | 22.6* |
| Predominant hair color | | | | |
| White | 19/226 | 8.4*† | 35/159 | 22.0* |
| Black | 13/205 | 6.3† | 116/305 | 38.0† |
| Golden | 9/150 | 6.0† | 109/260 | 41.9† |
| Brown | 3/75 | 4.0† | 37/103 | 35.9† |
| Gray | 4/15 | 26.7* | 9/33 | 27.3†* |
| Life style | | | | |
| Outdoors | 38/583 | 6.5 | 200/509 | 39.3* |
| Indoors | 10/88 | 11.4 | 106/351 | 30.2† |
| Age (yr) | | | | |
| 1–2 | 23/330 | 7.0 | 57/204 | 27.9* |
| >2–4 | 8/111 | 7.2 | 95/268 | 35.5† |
| >4–6 | 9/126 | 7.1 | 71/174 | 40.8† |
| >6 | 8/104 | 7.7 | 83/214 | 38.89† |
| Length of time at locale (yr)[b] | | | | |
| 1–2 | 15/205 | 7.3 | 78/245 | 31.8 |
| >2–5 | 23/266 | 8.6 | 135/364 | 37.1 |
| >5 | 10/200 | 5.0 | 93/250 | 37.2 |
| Travel[b] | | | | |
| No | 45/650 | 6.9 | 296/813 | 36.4 |
| Yes | 3/20 | 15.0 | 10/47 | 21.3 |
| Weight (kg) | | | | |
| <5 | 9/185 | 4.9† | 33/126 | 26.2† |
| 5–15 kg | 33/266 | 12.4* | 145/457 | 31.7† |
| >15–25 kg | 2/116 | 1.7† | 83/194 | 42.8* |
| >25 kg | 4/104 | 3.9† | 45/83 | 54.2* |
| Hemoparasites[c] | | | | |
| Never | 44/638 | 6.9 | 245/704 | 34.8 |
| Yes (previously) | 1/18 | 5.6 | 33/70 | 47.1 |
| Received doxycycline[d] | | | | |
| No | 47/636 | 7.4 | 243/706 | 34.4 |
| Yes | 1/32 | 3.1 | 31/79 | 39.2 |

[a]Witness® HW (heartworm) antigen test kit, Zoetis, USA; [b]Data not provided for one animal; [c]Data not provided for 101 animals; [d]Data not provided for 78 animals. Different symbols (* or †) within columns indicate significant difference ($P < 0.05$).

Overall, presence of clinical signs reported by owners was not predictive of positive tests results for *D. immitis* (Table 3). However, when associating clinical signs with age or size, without regard for the test result, some correlation could be detected (Table 3): Age was correlated with frequency of coughing ($\chi^2 = 52.2$; df = 3; $P < 0.001$), exercise intolerance ($\chi^2 = 60.5$; df = 3; $P < 0.001$), and weight loss ($\chi^2 = 33.3$; df = 3; $P < 0.001$). Size of the animals was predictive of the presence of coughing ($\chi^2 = 8.33$; df = 3; $P = 0.040$) and weight loss ($\chi^2 = 10.1$; df = 3; $P = 0.018$); however, it was not predictive of exercise intolerance.

## Discussion

The presence of *D. immitis* was detected at all locales in the survey, demonstrating that the reduction, or in some areas, the supposed disappearance of heartworm observed by small animal practitioners early in the new millennium [13] has been replaced by an observed increase in the presence of the parasite in these areas of Brazil. We found the overall percentage of positive *D. immitis* antigen test results, despite differences in

**Table 3 Percentage of positive canine tests for heartworm antigens[a] within physical category and according to clinical signs**

| Category | | | | Coughing | | Syncope | | Dyspnea | | Exercise intolerance | | Weight loss | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. Pos | Total | % | No. Pos | No. Neg | No. Pos | No. Neg | No. Pos | No. Neg | No. Pos | No. Neg | No. Pos | No. Neg |
| **Age (yr)** | | | | | | | | | | | | | |
| 1–2 | 80 | 534 | 15.0* | 6 (7.5) | 21 (4.6) | 1 (1.3) | 1 (0.2) | 2 (2.5) | 7 (1.5) | 3 (3.8) | 20 (4.4) | 5 (6.3) | 22 (4.8) |
| >2–4 | 103 | 379 | 27.2[†] | 11 (10.7) | 36 (13.0) | 0 (0.0) | 2 (0.7) | 3 (2.9) | 7 (2.5) | 9 (8.7) | 22 (8.0) | 8 (7.8) | 26 (9.4) |
| >4–6 | 80 | 300 | 26.7[†] | 13 (16.3) | 23 (10.6) | 0 (0.0) | 0 (0.0) | 6 (7.5) | 7 (3.2) | 7 (8.8) | 23 (10.6) | 8 (10.0) | 13 (6.0) |
| >6 | 91 | 318 | 28.6[†] | 20 (22.0) | 48 (21.1) | 3 (3.3) | 9 (4.0) | 4 (4.4) | 15 (6.6) | 17 (18.7) | 48 (21.1) | 12 (13.2) | 40 (17.6) |
| **Weight (kg)** | | | | | | | | | | | | | |
| <5 | 42 | 311 | 13.5* | 8 (19.0) | 35 (13.0) | 0 (0.0) | 3 (1.1) | 1 (2.4) | 9 (3.3) | 6 (14.3) | 22 (8.2) | 9 (21.4) | 23 (8.6) |
| 5–15 | 178 | 723 | 24.6[†] | 24 (13.5) | 70 (12.8) | 2 (1.1) | 7 (1.3) | 7 (3.9) | 20 (3.7) | 16 (9.0) | 55 (10.1) | 9 (5.1) | 39 (7.2) |
| >15–25 | 85 | 310 | 27.4[†] | 9 (10.6) | 17 (7.6) | 0 (0.0) | 1 (0.4) | 2 (2.4) | 4 (1.8) | 7 (8.2) | 22 (9.8) | 8 (9.4) | 21 (9.3) |
| >25 | 49 | 187 | 26.2[†] | 9 (18.4) | 6 (4.3) | 2 (4.1) | 1 (0.7) | 5 (10.2) | 3 (2.2) | 7 (14.3) | 14 (10.1) | 7 (14.3) | 18 (13.0) |
| **Breed** | | | | | | | | | | | | | |
| Mongrel | 245 | 924 | 26.5[†] | 31 (12.7) | 67 (9.9) | 3 (1.2) | 8 (1.2) | 5 (2.0) | 18 (2.7) | 19 (7.6) | 56 (8.3) | 18 (7.3) | 54 (8.0) |
| Mixed | 45 | 330 | 13.6* | 11 (24.4) | 23 (8.1) | 0 (0.0) | 0 (0.0) | 3 (6.7) | 6 (2.1) | 5 (11.1) | 22 (7.7) | 5 (11.1) | 20 (7.0) |
| Purebred | 64 | 277 | 23.1[†] | 8 (12.5) | 37 (17.4) | 1 (1.6) | 4 (1.9) | 7 (10.9) | 11 (5.2) | 12 (18.8) | 34 (16.0) | 10 (15.6) | 27 (12.7) |
| **Sex** | | | | | | | | | | | | | |
| Female | 196 | 838 | 23.4 | 21 (10.7) | 56 (8.7) | 1 (0.5) | 4 (0.6) | 4 (2.0) | 18 (2.8) | 18 (9.2) | 59 (9.2) | 15 (7.7) | 46 (7.2) |
| Male | 158 | 693 | 22.8 | 29 (18.4) | 72 (13.5) | 3 (1.9) | 8 (1.5) | 11 (7.0) | 18 (3.4) | 18 (11.4) | 54 (10.1) | 18 (11.4) | 55 (10.3) |
| **Data missing** | | | | 3 | 9 | 2 | 5 | 4 | 11 | 3 | 7 | 1 | 10 |

[a]Tested with Witness® HW (heartworm) antigen test kit, Zoetis, USA. Different symbols (* or †) within a column indicate difference among category variables ($P < 0.01$).

diagnostic methodologies and inclusion criteria, was higher in all three Brazilian regions compared with findings in previous studies and when the pooled prevalence (3.9%) of the three surveyed regions observed previously [9] is compared with the current result (23.1%), it is irrefutable that there is a recrudescence of heartworm in these areas.

Notwithstanding all locales being coastal, differences in landscape due to human action seem to have influenced test results in some areas. Guarujá (state of São Paulo) is a locale where, in the past, 14.2% of examined dogs were positive for heartworm infection [2] and in the current study, prevalence of heartworm was only 2.8%. Also, in Florianópolis (state of Santa Catarina), prevalence decreased from 12% reported in 1992 [4] to 2.1% in the current study. Both cities are structured. Guarujá is affected by industrial pollution from neighboring cities, and Florianópolis experienced a human population boom during the past decades. Although human densification enhances the canine population, which in turn facilitates the transmission of heartworm [18], it transforms the environment, usually making it inhospitable to the majority of mosquito-vector species [19,20], thereby disturbing transmission.

On the other hand, at conserved estuaries, such as in the lowlands of Paraná, where the local economy is based on tourism or artisanal fishing, even though their annual mean temperatures generally range from 14° to 22°C [21], the prevalence of heartworm-infected dogs was higher than that observed in the more urbanized cities of Salvador or Lauro de Freitas, where the average temperature is much warmer (20°–28°C) [21]. Therefore, if on one hand, temperature is directly related to the number of mosquito generations produced [22-24] and with the speed of parasite development in the mosquito vectors [25], the level of environmental conservation seems to play a crucial role in maintaining dense mosquito populations.

The perception of dog owners regarding the presence of mosquitoes was not associated with the prevalence of heartworm detected by testing for *D. immitis* antigen with a reliable commercial antigen test kit. This lack of perception suggests that inhabitants in these areas are accustomed to the presence of vectors and, therefore, are unwilling to control mosquitoes, which likely contribute to enhancing *D. immitis* transmission.

In areas where the prevalence of infected dogs was lower than 20%, a higher percentage of dogs with gray hair coat tested positive; however, the sample size for this hair color was small (15/1531) and may have biased the results. At areas with a prevalence 20% or higher, the majority of dogs with a white hair coat tested negative for heartworm. The prevalence of heartworm infection in these dogs with a white hair coat was significantly lower than among dogs with black, golden, or brown hair color. If mosquitoes can perceive different colors, as suggested by Maranhão [26], it is possible that the white hair coat may play a protective role in areas where heartworm transmission challenge is high. In addition to hair coat color, the length of the hair coat (long), and life style (living primarily indoors) appeared to be associated with reduced heartworm infections, presumably because these characteristics interfere with the vectors' ability to locate hosts and obtain blood meals [27]. Considering that short hair coat presumably provides mosquitoes with better access to a dog's skin [28-30] and that dogs that stay primarily outdoors are exposed to the sylvatic and the more efficient mosquito vectors that are hemi-synanthropic and exophylic [29,30], these characteristics also may have played a key role in increasing the percentage of positive test results.

Despite these findings of statistical association of certain physical characteristics, these results suggest that the relationship of canine individual characteristics (hair coat color or length, and outdoors life style) to test results is minor, mainly because it could only be detected where challenge was high. In previously surveyed areas, where the prevalence of heartworm infection was lower (10.4% and 15%), interference by these factors could not be detected [27,31].

The fact that the length of time the dogs lived at enzootic locales did not increase the percentage of positive test results suggests that infections occur soon after the animal is introduced to a location. Also, traveling experiences did not influence the results, suggesting that dogs in the areas surveyed most likely became infected in the home region; however, it would be expected that these animals could eventually spread the infection if and when they traveled abroad, as suggested before [32].

The prevalence of heartworm infection among younger dogs (1–2 years) may have been smaller than for the older age groups due to the long prepatent period of the infection (6–7 months) [14], especially because the time dogs lived at the enzootic areas did not demonstrate an effect on test results. An association between the small size (i.e. dogs weighing <5 or 5–15 kg) and indoor life style may have been the reason for smaller animals to have fewer positive test results as compared with the prevalence among larger dogs (i.e. dogs weighing 15–25 or >25 kg), as observed previously [33,34].

Breeds have been previously compared as purebred versus mongrels, with most reports showing no difference among different breed categories [35,36], although one report showed purebred dogs to have more positive test results [37]. Therefore, it is difficult to interpret the lower percentage of positive test results in mixed-breed dogs compared with results in mongrels or purebred dogs, and the current results may have occurred by chance alone.

Independent of *D. immitis* antigen test results, testing of various characteristic variables against the reported clinical signs reported by owners indicated that older dogs presented a higher prevalence of exercise intolerance, cough, and weight loss than younger dogs. With regard to size, smaller dogs were more inclined to have a cough and lose weight more frequently, possibly due to heart or respiratory diseases associated with other etiologies [38,39].

## Conclusions

Canine *D. immitis* infection was detected in every region surveyed, with a tendency to have higher percentage of positive test results where nature is better conserved. In areas where heartworm was highly prevalent, there were significantly more cases of *D. immitis* infection in large dogs, outdoor dogs, and dogs with short hair coats. Clinical signs observed by owners did not correlate with positive test results, suggesting that clinical signs of heartworm infections are rare or, at best, subtle, and call for other methods to be used for detection of heartworm infection, particularly for light or early infections. Therefore, small animal practitioners must include heartworm routine screening tests in every Brazilian dog's annual evaluation protocol and make sure to have uninfected dogs on prevention.

#### Competing interests

Norma Labarthe is a consultant for Bayer Animal Health, Idexx Laboratories, and Zoetis in Brazil.
Flavya Mendes-de-Almeida is a consultant for Bayer Animal Health and Idexx Laboratories.
Jonimar Paiva is a consultant for Zoetis in Brazil.
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#### Authors' contributions

NVL conceived of the study and participated in coordination of the study, the acquisition of data, interpretation of results, and helped to draft the manuscript. JPP participated in the study coordination and performed the statistical analysis. FMA participated in the study coordination, acquisition of data, laboratory analysis and helped to draft the manuscript. LR, CJCP, PSJ, MAO de A, and LCA participated in the study coordination, acquisition of data, and laboratory analysis. AM participated in the study design and coordination, interpretation of results, and helped to draft the manuscript. All authors read and approved the final manuscript.

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