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# SEROLOGICAL EVIDENCE OF CANINE VECTOR-BORNE DISEASES CAUSED BY *ANAPLASMA* SPP., *BORRELIA BURGDORFERI, EHRLICHIA CANIS* AND *DIROFILARIA IMMITIS* IN DOGS FROM GOVERNADOR ISLAND, RIO DE JANEIRO, BRAZIL

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**ABSTRACT**

The study aimed to detect the serological positivity rates and spatial distribution of *Anaplasma* spp, *Borrelia burgdorferi*, *Dirofilaria immitis*, and *Ehrlichia* spp. among client-owned dogs in Governador Island, Rio de Janeiro, using the in-clinic SNAP® 4Dx® Plus test. Among resident dogs, 48.08% scored positive for one or more of the four pathogens. The specific seroprevalence was highest for *Ehrlichia* (25.19%), followed by *D. immitis* (14.5%) and *Anaplasma sp.* (8.39%), while *B. burgdorferi* was not detected. Combined infections were detected in almost one-tenth of the positive samples. No sex difference was observed, and infections were more common in dogs aged 3 to 10 years. This study is the first survey of canine vector-borne diseases on the Island. Given the results, client education, treatment of positive cases, and popularization of prophylactic measures are recommended to protect dogs and humans residing in the island region.

**Key words***:* *Anaplasma* spp., *Borrelia burgdorferi*, Canine vector-borne diseases, *Dirofilaria immitis*, *Ehrlichia canis*.

## Introduction

Canine vector-borne diseases (CVBDs) caused by several pathogens including viruses, bacteria, and helminths pose significant concerns to veterinary medicine and public health (Maggi and Krämer, 2019).

The disease epidemiology has been undergoing worldwide modification over time and is substantially influenced by globalization, climate change, urbanization, deforestation, the growing human population and their pets, increased individual and pet mobility, and distribution and density of competent arthropod vectors across territories, facilitating the dissemination of these pathogens (Angelou et al., 2019). Several arthropod vectors, such as ticks, mosquitoes, fleas, lice, and triatomine bugs transmit these pathogens to dogs, and eventually to other hosts (Dantas-Torres.,2008).

An important group of CVBDs affecting dogs in Brazil comprises anaplasmosis, borreliosis, heartworm disease, and ehrlichiosis, which present wide geographic distribution and varying degrees of prevalence (Dantas-Torres. 2008).

CVDBs are common in tropical regions, especially in Brazil, and recent studies reported the current risk burden, treatment options, and prevention (Dantas-Torres et al. 2 020). In relation to Rio

de Janeiro metropolitan area, a recent publication highlighted the epidemiologic situation of these diseases, revealing a prevalence of 7% for *Dirofilaria immitis*, 27.1% for *Ehrlichia* spp., 9.8% for *Anaplasma spp*., while *Borrelia burgdorferi* was not detected (Mendes-de-Almeida et al., 2021). However, there is a paucity of epidemiological information for Governador Island and most of these diseases have been sporadically diagnosed by the local clinicians. Therefore, this study aimed to identify the serological evidence of infection caused by *D. immitis*, *Ehrlichia spp.*, *Anaplasma spp.*, and *B. burgdorferi* among domestic-owned dogs residing on the island.

***Anaplasmosis***

Anaplasmosis in dogs and cats is caused by *Anaplasma phagocytophilum* and *Anaplasma platys*, the agents of canine granulocytic anaplasmosis and cyclic thrombocytopenia, respectively. They occur in Brazil and other tropical and subtropical areas, with variable prevalence rates (Mendes-de-Almeida et al., 2021).

***Borreliosis***

It is also known as Lyme borreliosis and is caused by *B. burgdorferi*, a cosmopolitan zoonotic agent first identified in Brazil in the 1980s, affecting people from the Rio de Janeiro metropolitan area (Figueira et al., 1989). The first evidence of the infection among dogs in Rio de Janeiro was reported in the late 1990s, by using serology (Soares, 1999). Lyme borreliosis is a potentially serious disease, with systemic involvement, and may affect several animal species and humans. Serological surveys conducted in southeast states of Brazil demonstrated prevalence in up to 20% of domestic dogs (Dantas-Torres, 2008).

***Ehrlichiosis***

*Ehrlichia canis* is a Gram-negative obligate intracellular bacterium infects mammal leukocytes, causing canine monocytic Ehrlichiosis, and is found enzootically in Brazil, with high prevalence rates among dogs referred to veterinary clinics (Dantas-Torres, 2008).

***Heartworm disease***

Heartworm disease (HWD), is caused by the large-sized nematode *D. immitis* (Leidy, 1856), considered the most pathogenic and widespread geographically filarial nematode of domestic and wild canids, and is capable to cause serious pathomorphological lesions, especially in the heart, lungs, and kidneys (Dantas-Torres et al., 2022, Rafailov, 2022). It also can harm several other mammalian species, including black bears, cats, ferrets, lions, otters, ocelots, and pinnipeds (Cardoso et al., 2012). In Brazil, the heartworm parasite is transmitted by several species of mosquitoes, especially of genera *Culex*, *Aedes*, *Anopheles*, and *Ochlerotatus* (Mendes-de-Almeida et al., 2021). It occurs endemically in most regions of the country, especially in coastal territories, and may show prevalence rates higher than 60% in some endemic foci (Dantas-Torres, 2010). In Rio de Janeiro, heartworm is frequently identified in dogs attending veterinary clinics, while it is detected in only 1.2% of domestic cats (Alberigi et al., 2020). A previous investigation showed 7.69% of military dogs positive for heartworms on serological examination in Governador Island (Almeida et al., 2016). Recently, the first clinical case of heartworm affecting a domestic cat from the same region was reported (Alberigi et al., 2020).

## Materials and methods

Study area – Geographic characterization: The study was conducted on Governador Island (Ilha do Governador), an island with an area of 36,12 km$^2$ in Guanabara Bay (Virgilio et al, 2011), in Atlantic Ocean, which is a part of the metropolitan area of Rio de Janeiro (Figures 1 and 2).

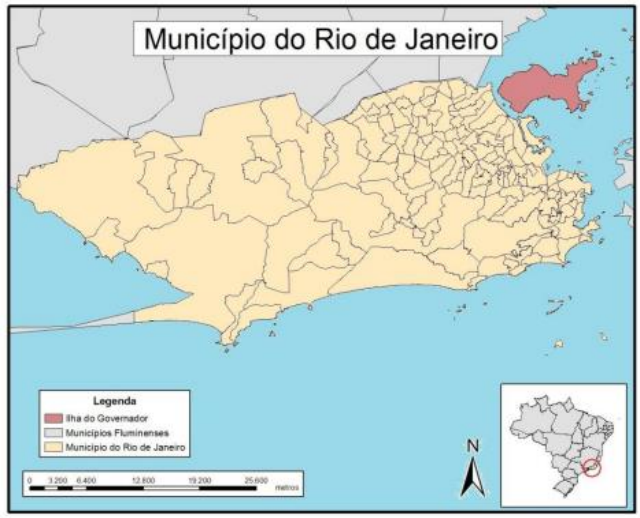


**Figure 1: Rio de Janeiro Municipality (Virgilio et al., 2011)**

**Figure 2: Governador Island. 2016 Hodoyoshi 1 satellite Picture. Hidden categories: Extracted images. CC-BY-SA-4.0. This file is licensed under the Creative Commons Attribution-Share Alike 4.0. International license. Free Use.**

The latitude and longitude are 2º46'13''; 22º 50' 04'' and 43º 09'27''; 43º 16'17', respectively. A regular movement of individuals and their pets is observed between Governador Island and the mainland of Rio de Janeiro municipality, where CVBDs have been found commonly in domestic dogs. In Governador Island is located the Galeão International Airport, where the movement of national and international passengers, their pets, and goods occurs. The area is located in the intertropical belt, between the mountains and sea in the Southeast region, and has a humid tropical climate,

with two well-defined seasons (summer–wet and winter–dry). The maximum and minimum temperature are 26.7ºC and 20.4ºC, respectively, and the average annual rainfall is 1,107 mm, with an average frequency of 124 rainy days per year (Fialho, 2010).

Sample collection and examination: The research was conducted from May 2020 to May 2021. The study population comprised 131 dogs and was based on convenience sampling, without consideration of the patient's present complaint, after obtaining verbal consent from owners. All dogs were living in different neighborhoods of the island and had no history of treatment or use of macrocyclic lactones for heartworm in the past 6 months. Blood samples were collected from saphenous or cephalic venipuncture using disposable syringes and placed in Eppendorf tubes containing EDTA, stored at 2–8°C, and examined within 12 h from the collection. We used the rapid ELISA blood test (SNAP® 4Dx Plus Test Kit, IDEXX Laboratories, USA) for the detection of antibodies to *Anaplasma platys/A phagocytophilum, Ehrlichia spp. (E. canis/E. ewingii), B. burgdorferi,* and antigen of *D. immitis*. All samples were tested according to the manufacturer's instructions. The reported sensitivity and specificity of the in-clinic ELISA were > 89% for the detection of antibodies against *A. phagocytophilum, A. platys, B. burgdorferi, E. canis,* and *E. ewingii*. The sensitivity of the assay to detect *D.immitis* antigen was 98.9%, while the specificity was 99.3% (Stillman et al., 2014).

***Statistical analysis***

Obtained data were recorded in a Microsoft Excel spreadsheet and imported into Graph Pad Instate software, Version 3.1. Descriptive statistics were used to calculate the relative and absolute frequencies. Categorical variables were expressed as percentages and numbers while continuous variables were presented as mean. A chi-square test was performed to compare proportions for the qualitative variables. Differences were considered significant when $p<0.05$. The age groups defined for age prevalence were as follows: young adult (1–2 years), adult (3–10 years), and senior (>10 years), as proposed previously (Mendes-de-Almeida et al., 2021).

## Results

The screened dogs were of both sex (52% female), aged 1–15 years, with mean of 7.96+/-4.36 years (95% CI= 7.19–8.73). Twenty-five breeds were included in the study, in which purebreds were predominant (58.7%) compared to mongrels. There was no diference of seroreactivity by sex for the pathogens (Table 1). Overall, 63 (48.08%) dogs scored positive for at least one of the four pathogens. The most frequent agent detected was *Ehrlichia* spp. in 33 (25.19%), followed by *D. immitis* in 19 (14.5%); and *Anaplasma* spp. in 11 (8.39%). *B. burgdorferi* was not detected. For combined infections, 12 (9.16%) and one dog (0.76%) were simultaneously exposed to two and three pathogens, respectively (Table 2). The infections were predominant in adults aged 3–10 years, compared to young adults and seniors ($p<0.05$). The mean age of the positive versus negative dogs for any of the pathogens was 8.6 and 7.6 years (Table 2 & 3), respectively, without significant difference ($p=0.419$).

**Table 1: Seroreactivity by sex**

| Sex | *Ehrlichia* | *D. immitis* | *Anaplasma* | (n) | (%) |
|---|---|---|---|---|---|
| Male | 14 | 11 | 5 | 30 | 48% |
| Female | 19 | 8 | 6 | 33 | 52% |
| Total | 33 | 19 | 11 | 63 | 100% |

**Table 2: Seroreactivity to single and combined canine vector-borne diseases (CVBDs) in 131 dogs**

| Pathogen | Positive (n) | Positivity % |
|---|---|---|
| Overall | 63 | 48.08 |
| *Anaplasma spp.* | 11 | 8.39 |
| *Dirofilaria immitis* | 19 | 14.50 |
| *Ehrlichia spp.* | 33 | 25.19 |
| *Borrelia burgdorferi* | – | - |
| Co-infection | 12 | 9.16 |
| *Anaplasma* + *Ehrlichia* | 7 | 5.35 |
| *Dirofilaria* + *Ehrlichia* | 4 | 3.05 |
| *Anaplasma* + *Dirofilaria* + *Ehrlichia* | 1 | 0.76 |

**Table 3: Seroreactivity by age groups**

| | Samples positivity | | | | |
|---|---|---|---|---|---|
| **Age group** | ***Ehrlichia*** | ***D.immitis*** | ***Anaplasma*** | **(n)** | **(%)** |
| Young adult | 2 | 3 | 0 | 5 | 8% |
| Adult | 20 | 12 | 7 | 39 | 62% |
| Senior | 11 | 4 | 4 | 19 | 30% |
| Total | 33 | 19 | 11 | 63 | 100% |

The largest number of positive samples was from animals residing in highly deforested and densely urbanized areas (Figure 3).

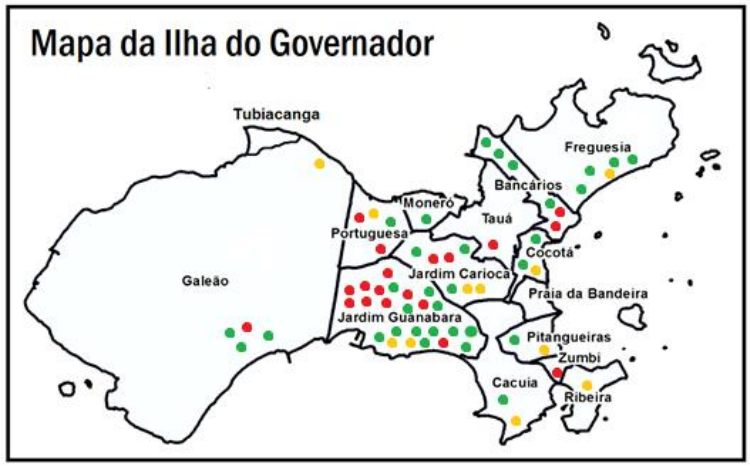


**Figure 3: Geographic distribution of positive samples (Adapted from: https://www.facebook.com/IlhadoGovernadorFatos/)**

## Discussion

CVBDs can significantly impact canine health since they can cause serious clinical changes. Dogs can be sequentially or simultaneously infected with more than one agent; from arthropods infected with a single pathogen to vectors concurrently infected with different organisms (Cardoso et al., 2012). In fact, co-infection appears to be relatively common since it was detected in almost one-tenth of our samples.

There was no significant sex difference for any of the pathogens, wich is similar to findings in Spain (Pérez et al., 2021), and contrary to others that found male predominance (Suh et al.,2017) in Korea, or female predominance in Barranquilla and Puerto-Colombia, Colombia (Otalora et al.2022), and Santa Catarina, Brazil (Zuchi et al., 2020).

The most recent survey on CVBDs conducted in the metropolitan area of Rio de Janeiro demonstrated prevalence of heartworm in 7%, Ehrlichia in 27.1%, and Anaplasma in 9.8% of dogs, respectivelly (Mendes-de-Almeida et al., 2021), while in our study the prevalence was 14.5 % for heartworm, 25.19% for *Ehrlichia spp.*, and 8.39% for *Anaplasma*, respectivelly. The overal prevalence of 48.08% suggests that Governador Island constitutes a "hot spot" of CVBDs.

Age appeared to be an important factor for infections, since we found a higher prevalence in the adult group when compared with young adults (p=0.0001) and the senior group (p=0.0007), wich is parallel with the findings in Colombia (Otalora et al., 2022).

For *B.burgdorferi*, specific antibodies were not detected, which is in line with the absence or very low seroprevalence reported for other regions of Brazil (Mendes-de-Almeida et al., 2021; Montandon et al., 2022).

## Conclusion

This study revealed high occurrence and wide distribution of CVBDs among dogs living on Governador Island, posing a significant risk of infection to the local canine population. Considering the impact of these infections on the dog's health, knowledge regarding their epidemiology must be enhanced by routine screening for CVBDs. Our findings reinforce the need to popularize diagnostic and preventive measures to reduce the burden of these diseases on the canine population and the chance of human infection since some of the pathogens are of zoonotic concern.

## Declaration of conflicting interests

The author(s) declared no potential conflicts of interest in relation to the research, authorship, and/or publication of this article.

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