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Posted Date: 28 June 2024

doi: 10.20944/preprints202406.2004.v1

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Article

Dogs as Sentinels for Emergent Pathogens: Serological Evidence in a Preserved Area in Brazil

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Abstract: For many tick-borne organisms, dogs may be utilized as sentinel hosts to estimate the risk of human infection. The detection of antibodies in these animals indicates the circulation of pathogens in each location for a set period of time. The standard protocol for the surveillance of tick-borne diseases established by the Brazilian National Reference Laboratory for Rickettsiosis at the Ezequiel Dias Foundation (FUNED) includes testing the seropositivity of dogs for *Rickettsia* spp. and *Ehrlichia canis*. Dog serum samples were collected during FUNED's surveillance protocol in a preserved area in Brazil. Despite *Anaplasma phagocytophilum* being endemic in Brazil, this agent is not included in FUNED's standard protocol. To determine if *A. phagocytophilum* should be included in the standard testing protocol, a collaboration with Fuller Laboratories was established and the seropositivity for *A. phagocytophilum* was tested. The estimated prevalence for singular infections: 25% *Rickettsia* spp.; 52.3% *E. canis*; and 70.5% *A. phagocytophilum*. The estimated prevalence for co-infections: 17% *Rickettsia* spp. and *E. canis*; 18.2% *Rickettsia* spp. and *A. phagocytophilum*; 39.8% *E. canis* and *A. phagocytophilum*; and 13.6% *Rickettsia* spp., *E. canis*, and *A. phagocytophilum*. These results confirmed a significant presence of A. phagocytophilum and establishes a potential threat upon the public health.

Keywords: *Rickettsia* spp.; *Ehrlichia canis*; *Anaplasma phagocytophilum*; dog; sentinel; sentinel host; human; epidemiological surveillance; public health

1. Introduction

The (re)emergence of zoonoses has been linked to significant ecological changes driven by factors such as globalization, habitat modification, urbanization, and climate change, among others [1,2]. Climate change generates both direct and indirect impacts on human health. Indirectly, it causes imbalances in ecosystems, biodiversity, and hydrological/biogeochemical cycles, thereby influencing the morbidity and mortality profiles of diseases, such as those transmitted by vectors. This raises significant concerns in the global health sector, posing a substantial threat to public health as well as to animal health [3–5].

Following insects, ticks are the second most capable vectors for transmitting pathogens to humans. They can carry and transmit a diverse range of etiological agents, including bacteria, viruses, protozoa, and helminths, during their blood meals on vertebrate hosts [6–8]. This remarkable ability to disseminate pathogens, combined with their indirect impacts, has led to an increase in Tick-Borne Diseases (TBD), which are now recognized as the second leading cause of health issues in both human

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and veterinary health [9]. Additionally, these diseases have been spreading globally, even reaching regions previously free of such pathogens. This growing prevalence underscores the global relevance of TBD within the framework of One Health, emphasizing the interconnectedness of human, animal, plant, and ecosystem health [10–15].

In Brazil, the main zoonosis transmitted by ticks is the Spotted Fever (SF), which can range from mild forms, when associated with *Rickettsia parkeri* Mata Atlântica strain, to severe forms with a high lethality rate when the agent is the bacterium *Rickettsia rickettsii*, a fact related to epidemiological scenarios that are quite variable [16,17]. And due to the increase in the number of human cases, SF surveillance in Brazil was markedly influenced by four health actions: I) Brazilian Ministry of Health (BMH) started to consider it as a notifiable disease; II) SF became part of the Notifiable Diseases Information System (SINAN), an agency that aims to collect, gather and disseminate data among the health surveillance network; III) formation of the National Network for Environmental Surveillance for Spotted Fever and other rickettsiosis, initiating training in environmental surveillance of rickettsiosis and IV) SF and other rickettsiosis became part of the list of diseases of immediate compulsory notification, and must be notified within 24 hours [16].

Due to the above, SF surveillance in Brazil encompasses both epidemiological and environmental monitoring, with the following objectives: A) early detection and treatment of suspected cases to reduce lethality; B) investigate and control of outbreaks through the implementation of control measures; C) understand the distribution of the disease by location, time, and affected individuals; D) identify and investigate probable sites of infection (IPLs); and E) recommend the adoption of control and prevention strategies [16].

Additionally, the BMH evaluates the occurrence of another TBD: ehrlichiosis. This disease is caused by bacteria of the genus Ehrlichia sp., which has already been recognized in cats, wild animals, humans and dogs, and is widely detected throughout the country [18,19]. The main species of *Ehrlichia* in dogs is *Ehrlichia canis*. *E. canis* is highly endemic in many regions of Brazil [20] due to the geographic distribution of tick vectors, which contributes directly to the higher prevalence of ehrlichiosis that has been observed [21].

Despite the reports of people affected by E. canis [22,23], the main species of human importance is *Ehrlichia chaffeensis*, which causes human monocytic ehrlichiosis (HME), a pathogen already identified in animals in Brazil, especially cervids [24]. Due to its zoonotic character, surveillance of circulation in animals becomes relevant in the context of single health.

Another TBD caused by bacteria circulating in the country belongs to the genus *Anaplasma* sp.. Granulocytic Anaplasmosis is a disease that affects dogs, horses, cats, ruminants and humans, which can be infected by the emerging intracellular bioagent *Anaplasma phagocytophilum* [25–28]. The occurrence of anaplasmosis in dogs has been geographically associated with Human Granulocytic Anaplasmosis (HGA) [29], as well as the occurrence of the presence of ixodid ticks in the circulation of *A. phagocytophilum* in Brazil, despite the lack of identification of the vector responsible for transmission in the tropical region [30]. Although *A. phagocytophilum* has already been detected in several studies in Brazil [26–31], this bioagent is not currently included in the standard surveillance protocol in Brazil.

As the tick *Rhipicephalus sanguineus* sensu lato is involved in the transmission of *Ehrlichia* sp. and some species of *Anaplasma* sp. [32,33], co-infection of ehrlichiosis and anaplasmosis in routine small animal clinical testing is not uncommon, and this co-infection is reported with a prevalence of 9.1% in symptomatic dogs through rapid routine testing [34].

Given the persistent and growing challenges in epidemiology, alongside the knowledge of the interaction between hosts, pathogens and vectors, which is at the heart of the study of TBD, a surveillance method is needed. Within this paradigm, seroepidemiological surveys in sentinel hosts – which have been used as an effective tool and play a crucial role, since sentinels do not pose a threat of direct transmission of diseases to humans – offer valuable insights, which are aligned with the objectives of epidemiological and environmental surveillance in the country [35–38].

The use of dogs as sentinel hosts for TBD is a well-established surveillance strategy in the scientific literature due to the fact that dogs are susceptible to TBD infections; survive these infections;

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exhibit detectable and measurable immune responses; and most importantly they closely interact with humans [35]. This strategy has been employed in numerous serological surveys worldwide [39–41]. In Brazil, dogs are chosen as sentinel species due to their involvement in infection scenarios, both directly and indirectly, within epidemic and enzootic cycles [36,42–44]. Consequently, they serve as bioindicators of the epidemiological status; the detection of antibodies in these animals indicates the circulation of pathogens in specific localities and time periods, aiding in the tracking of TBD risks and monitoring infection trends [45–47].

As previously noted, although *A. phagocytophilum* has been detected in several studies in Brazil [26,27,30,31], this bioagent is not currently included in the standard surveillance protocol of the National Reference Laboratory for Spotted Fever and other Rickettsiosis of the Ezequiel Dias Foundation (FUNED). This study, a collaboration between FUNED and Fuller Laboratories in California, aimed to provide scientifically based information to support the implementation of strategic public health actions. As such, blood samples were obtained from dogs in Serra do Cipó National Park (SCNP) in Minas Gerais, Brazil, and were analyzed serologically for *Rickettsia* spp., *E. canis*, and *A. phagocytophilum*.

2. Materials and Methods

2.1. Fields of Study

This cross-sectional study took place in SCNP, within the state of Minas Gerais, about 100 km from Belo Horizonte (the capital of Minas Gerais) [48], and in the surrounding Retiro and Açude communities, with access to the Areias and Alto Palácio entrances (Figure 1). The SCNP is a Brazilian conservation unit and known as "Jardim do Brasil", a title given by the landscape architect Burle Marx. With a total area of 33,800 hectares, the unit currently protects several endangered species of Brazilian fauna and flora in the Cerrado biome. The rugged topography, with altitudes ranging from 700 to 1,670 meters in altitude, is located in the southern portion of the Serra do Espinhaço, an important divider between two major Brazilian hydrographic basins: the São Francisco and the Doce River [48]. The SCNP makes it possible to carry out scientific research, recreation and ecological tourism; and has seen 342,476 visitors within the last 5 years [48]. In addition to the purpose of environmental conservation, many traditional communities that have direct contact with preserved environmental aspects are found in the SCNP region, an important factor considering the public health measures expanded to the reality of possible pathogens circulating in the region.

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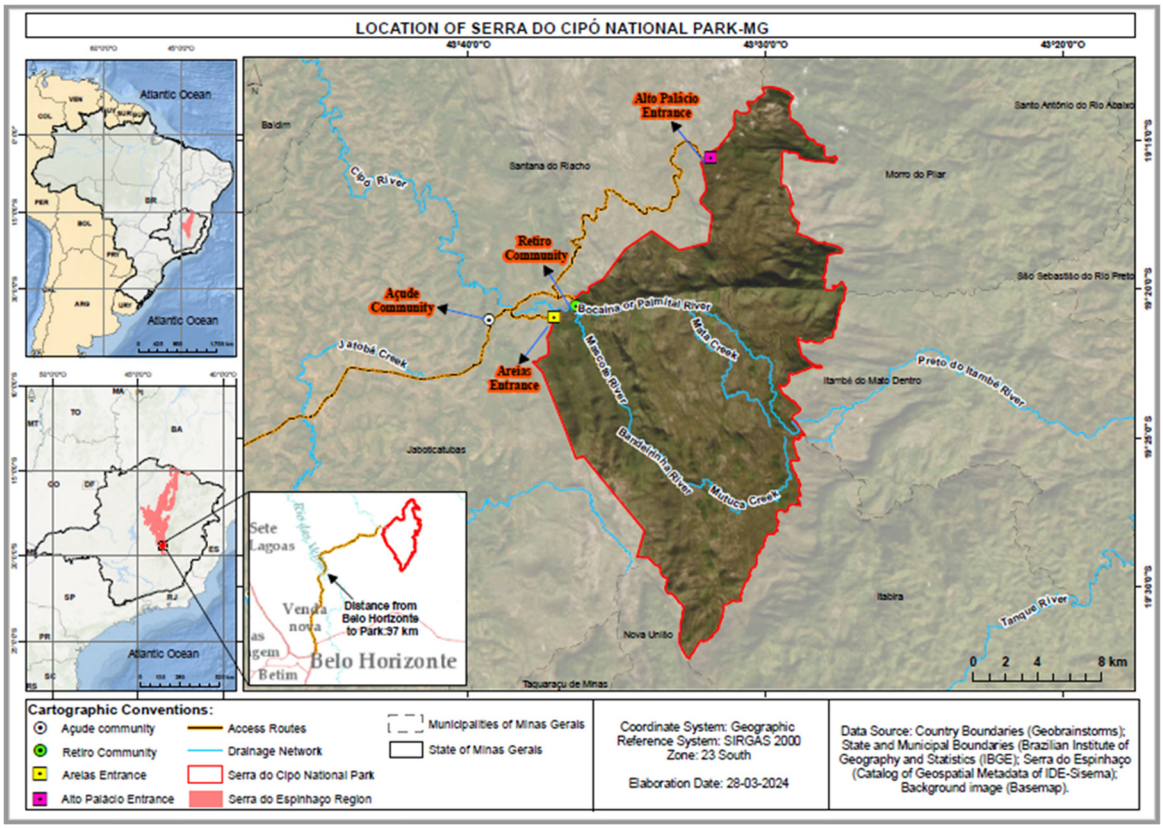

Figure 1. Blood samples from sentinel animals (both domiciled and stray dogs) were collected at the Serra do Cipó National Park (SCNP), which is located about 100 km from the capital of Belo Horizonte in the state of Minas Gerais. Blood samples were also obtained from the surrounding areas of the Retiro and Açude Communities, with access to the Areias and Alto Palácio gates.

2.2. Obtaining and Processing Samples

Six routine field collection campaigns were carried out by professionals from the municipality between March 2021 and February 2022, resulting in 88 blood samples from dogs (domiciled, semi-domiciled, and stray). These samples were sent to the National Reference Laboratory for Spotted Fever and other Rickettsiosis of FUNED, for antibody research utilizing the Indirect Immunofluorescence Assay (IFA), which is the gold standard methodology according to BMH [16]. IFA analysis for *Rickettsia* spp., and *E. canis* followed FUNED's routine protocol. IFA analysis for *A. phagocytophilum* followed the instructions of the kit manufacturer: Fuller Laboratories, California, United States of America [49].

The samples were received, sorted, catalogued and stored in a freezer at -80°C until the beginning of the analysis procedures. The samples were thawed, homogenized and diluted in phosphate-buffered saline solution (PBS) pH 7.4, in individualized microtubes with the respective dilutions for *Rickettsia* spp. 1:64, *E. canis* 1:40 and *A. phagocytophilum* 1:80. Then, 25 µl of diluted serum was added to the wells of the respective slides for *Rickettsia* spp., *E. canis* and *A. phagocytophilum*. The slides were incubated at 37ºC for 30 minutes, washed twice in PBS for 5 minutes, and dried at room temperature. Then, 25 µl of anti-IgG antibody specific for each animal species, labelled with fluorescein, prepared at a 1:100 dilution, was added to each well; incubating again for 30 minutes at 37ºC. Next, the slides were washed twice for 5 minutes with PBS and mounted with buffered glycerin and coverslips; then examined under a UV microscope at 400X magnification with fluorescein filters. Antibody titer of 1:64 or higher was considered reactive for *Rickettsia* spp., 1:40 or higher was considered reactive for *E. canis*, and 1:80 or higher was considered reactive for *A. phagocytophilum*.

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2.3. Statistical Analysis

It was calculated based on the report of the occurrence of the frequency of serological findings (prevalence with a 95% confidence interval), as well as from the analysis by means of specific tests from the statistical program R, Core Team (2024). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/ (https://www.R-project.org/) [50]. A bubble chart generated to illustrate the distribution of the prevalence of simple infections and co-infections is seen in Figure 4. A chi-square test of independence was performed to examine the relation between co-infections of *A. phagocytophilum* and *E. canis*, or between co-infections of *E. canis* and *Rickettsia* spp., or between co-infections of *A. phagocytophilum* and *Rickettsia* spp.

3. Results

Analysis by IFA demonstrated that there was a substantial percentage of sero-reactivity toward the bioagents that was observed in the sentinel hosts within the SCNP (Figure 1).

Of the 88 dogs tested, 83% (73/88 samples) tested positive for infections with one or more of the three infectious agents: *Rickettsia* spp., *E. canis*, and/or *A. phagocytophilum*, and 17% (15/88 samples) tested negative for infections with any of the three infectious agents (Figure 2A). Within the subset of the positive samples seen in Figure 2A, 30% (22/73 samples) tested positive for having *Rickettsia* spp. present, 56% (41/73 samples) tested positive for having *E. canis* present, and 85% (62/73 samples) tested positive for having *A. phagocytophilum* present (Figure 2B).

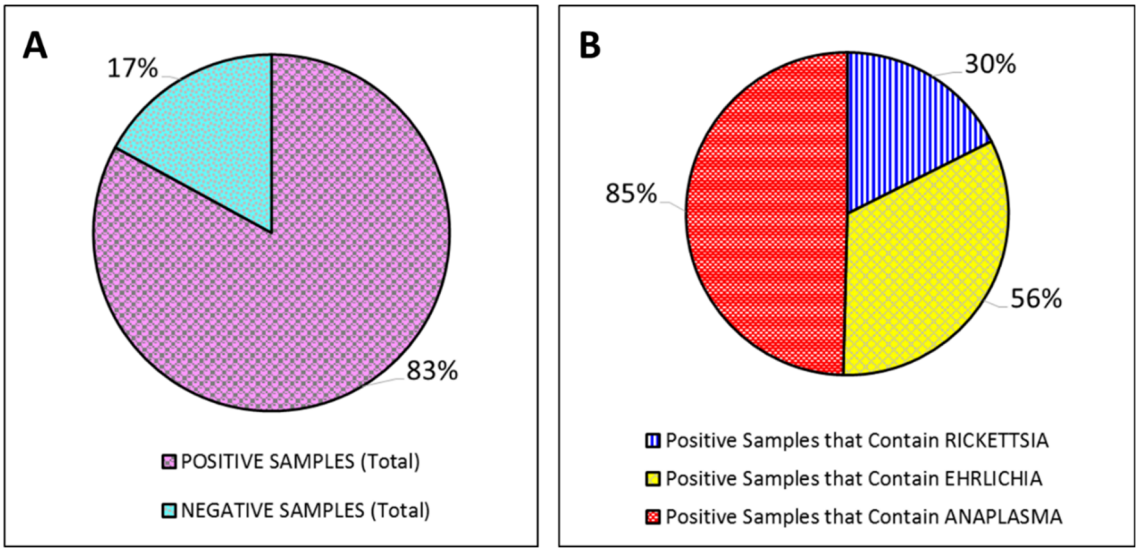

Figure 2. Six fieldwork campaigns were carried out between March 2021 and February 2022 at the Serra do Cipó National Park (SCNP), resulting in a total of 88 blood samples collected from dogs (domiciled and stray). 2A) The total percentages of samples that tested positive (83% [73/88 samples]) for infections with one or more of the three infectious agents: *Rickettsia* spp., *Ehrlichia canis*, and/or *Anaplasma phagocytophilum*; as well as the total percentage of samples that tested negative (17% [15/88 samples]) for infections with any of three infectious agents. 2B) Within the subset of the positive samples seen in Figure 2A, 30% (22/73 samples) tested positive for having *Rickettsia* spp. present, 56% (41/73 samples) tested positive for having *E. canis* present, and 85% (62/73 samples) tested positive for having *A. phagocytophilum* present.

The percentage of positive samples with either single infections or co-infections of *Rickettsia* spp., *E. canis*, and/or *A. phagocytophilum* is demonstrated in Figure 3. Within the subset of the positive samples seen in Figure 2A, 33% (24/73 samples) tested positive for infections with *A. phagocytophilum* only, 30% (22/73 samples) tested positive for co-infection with *E. canis*, and *A. phagocytophilum*, 7% (5/73 samples) tested positive for an infection with *E. canis* only, 5% (4/73 samples) tested positive for

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co-infection with *Rickettsia* spp., and *A. phagocytophilum*, 3% (2/73 samples) tested positive for co-infection with *Rickettsia* spp., and *E. canis*, 16% (12/73 samples) tested positive for co-infection with *Rickettsia* spp., *E. canis*, and *A. phagocytophilum*, 5% (4/73 samples) tested positive for co-infection with *Rickettsia* spp. only.

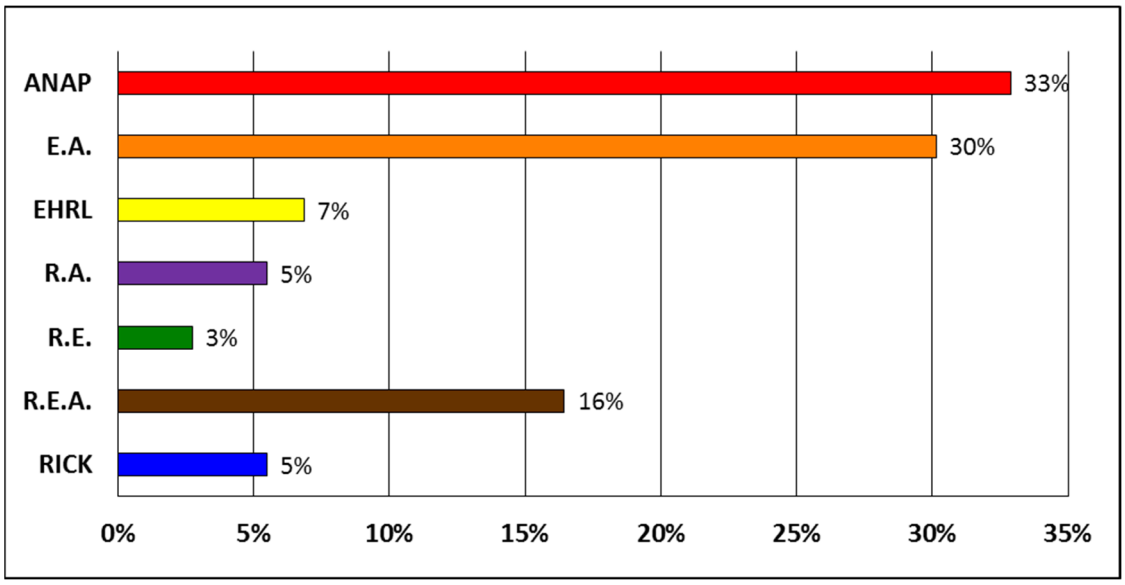

Figure 3. The percentage of positive samples with either single infections or co-infections of *Rickettsia* spp., *Ehrlichia canis*, and/or *Anaplasma phagocytophilum* (within the subset of the positive samples seen in Figure 2A). Abbreviations: RICK (*Rickettsia* spp. only), R.E.A. (*Rickettsia* spp., *E. canis*, and *A. phagocytophilum*), R.E. (*Rickettsia* spp., and *E. canis*), R.A. (*Rickettsia* spp., and *A. phagocytophilum*), EHRL (*E. canis* only), E.A. (*E. canis*, and *A. phagocytophilum*), ANAP (*A. phagocytophilum* only).

The different instant prevalence for singular infections as well as co-infections are illustrated in the bubble chart seen in Figure 4. The estimated prevalence for singular infections was calculated with respective 95% confidence intervals. For infections that only contained *Rickettsia* spp., the estimated prevalence was equal to 25% (16.6% - 35.6%). For infections that only contained the *E. canis* the estimated prevalence was equal to 52.3% (41.5% - 62.9%). For infections that only contained the *A. phagocytophilum* the estimated prevalence was equal to 70.5% (59.6% - 79.5 %). The estimated prevalence for co-infections was calculated with respective 95% confidence intervals. For co-infections that contained *Rickettsia* spp. and *E. canis* the estimated prevalence was equal to 17% (10.2% - 26.9%). For co-infections that contained *Rickettsia* spp. and *A. phagocytophilum* the estimated prevalence was equal to 18.2% (11.1% - 28.1%). For co-infections that contained *E. canis* and *A. phagocytophilum* the estimated prevalence was equal to 39.8% (29.7% - 50.8%). For co-infections that contained all three: *Rickettsia* spp., *E. canis*, and *A. phagocytophilum* the estimated prevalence was equal to 13.6% (7.5% - 23.0%).

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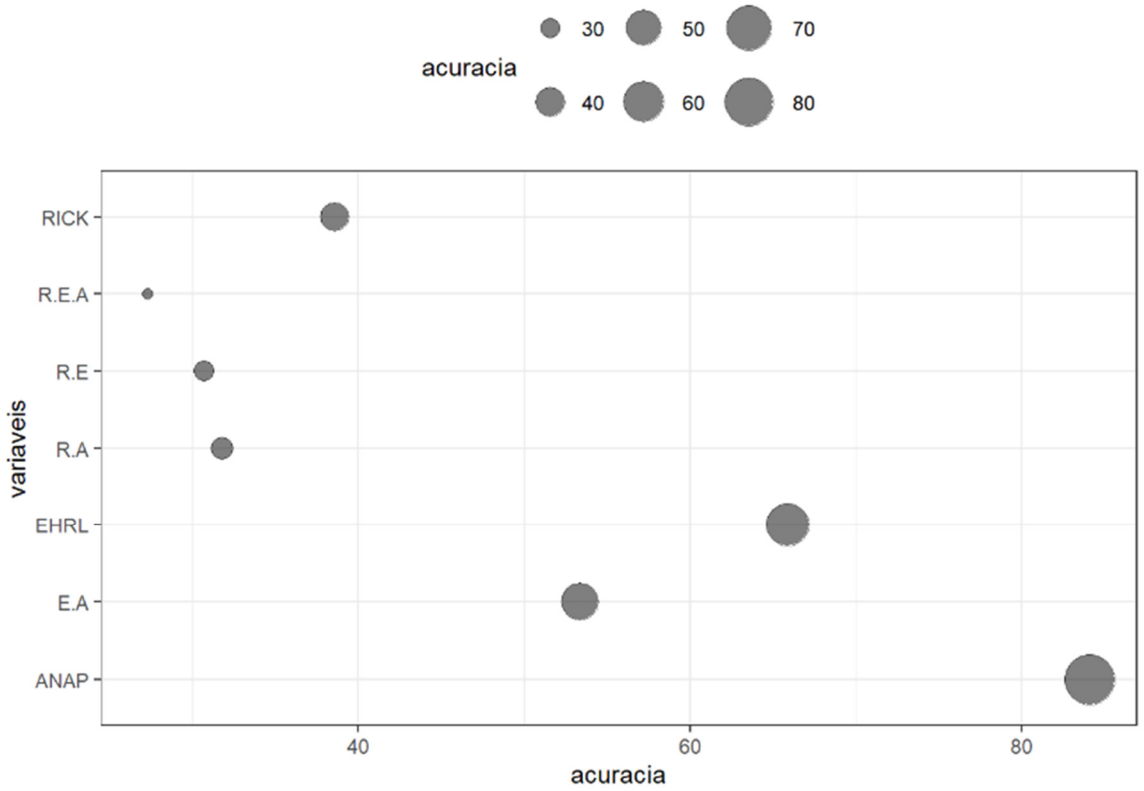

Figure 4. The prevalence of *Rickettsia* spp., *Ehrlichia canis*, and *Anaplasma phagocytophilum* infections and co-infections. Each bubble represents a respective infection or co-infection type, and the size indicates the prevalence of the respective infection or co-infection within the PNSC geographic area. Abbreviations: RICK (*Rickettsia* spp. only), R.E.A. (*Rickettsia* spp., *E. canis*, and *A. phagocytophilum*), R.E. (*Rickettsia* spp., and *E. canis*), R.A. (*Rickettsia* spp., and *A. phagocytophilum*), EHRL (*E. canis* only), E.A. (*E. canis*, and *A. phagocytophilum*), ANAP (*A. phagocytophilum* only).

A chi-square test of independence was performed to examine the relation between co-infections of *A. phagocytophilum* and *E. canis*, or between co-infections of *E. canis* and *Rickettsia* spp., or between co-infections of *A. phagocytophilum* and *Rickettsia* spp. (Figure 5). The chi-square statistic between *A. phagocytophilum* and *E. canis* co-infections is 5.7367. The p-value is 0.016614. The relation between *A. phagocytophilum* and *E. canis* co-infections was significant at $p < 0.10$. The chi-square statistic with Yates correction is 4.6697. The p-value is 0.030699. The relation between *A. phagocytophilum* and *E. canis* co-infections was significant at $p < 0.10$ with the Yates correction (Figure 5A). The chi-square statistic between *E. canis* and *Rickettsia* spp. co-infections is 3.425. The p-value is 0.064216. The relation between *E. canis* and *Rickettsia* spp. co-infections was significant at $p < 0.10$. The chi-square statistic with Yates correction is 2.5726. The p-value is 0.108731. The relation between *E. canis* and *Rickettsia* spp. co-infections was not significant at $p < 0.10$ with the Yates correction (Figure 5B). The chi-square statistic between *A. phagocytophilum* and *Rickettsia* spp. co-infections is 0.0728. The p-value is 0.787321. The relation between A. phagocytophilum and *Rickettsia* spp. co-infections was not significant at $p < 0.10$. The chi-square statistic with Yates correction is 0. The p-value is 1. Not significant at $p < 0.10$. The relation between *A. phagocytophilum* and *Rickettsia* spp. co-infections was not significant at $p < 0.10$ with the Yates correction (Figure 5C).

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A

	(+) EHRL	(-) EHRL	*Marginal Row Totals*
(+) ANAP	34 (28.89) [0.91]	28 (33.11) [0.79]	62
(-) ANAP	7 (12.11) [2.16]	19 (13.89) [1.88]	26
Marginal Column Totals	41	47	88 (Grand Total)

B

	(+) EHRL	(-) EHRL	*Marginal Row Totals*
(+) RICK	14 (10.25) [1.37]	8 (11.75) [1.2]	22
(-) RICK	27 (30.75) [0.46]	39 (35.25) [0.4]	66
Marginal Column Totals	41	47	88 (Grand Total)

C

	(+) RICK	(-) RICK	*Marginal Row Totals*
(+) ANAP	16 (15.5) [0.02]	46 (46.5) [0.01]	62
(-) ANAP	6 (6.5) [0.04]	20 (19.5) [0.01]	26
Marginal Column Totals	22	66	88 (Grand Total)

Figure 5. A chi-square test of independence was performed to examine the relation between co-infections of *Anaplasma phagocytophilum* and *Ehrlichia canis*, or between co-infections of *E. canis* and *Rickettsia* spp., or between co-infections of *A. phagocytophilum* and *Rickettsia* spp. 5A) The chi-square statistic between *A. phagocytophilum* and *E. canis* co-infections was significant at $p < 0.10$; and was significant at $p < 0.10$ with the Yates correction. 5B) The chi-square statistic between *E. canis* and *Rickettsia* spp. co-infections was significant at $p < 0.10$; but was not significant at $p < 0.10$ with the Yates correction. 5C) The chi-square statistic between *A. phagocytophilum* and *Rickettsia* spp. co-infections was not significant at $p < 0.10$; and was not significant at $p < 0.10$ with the Yates correction. Abbreviations: (+) RICK (samples that contain *Rickettsia* spp.), (-) RICK (samples that do not contain *Rickettsia* spp.), (+) EHRL (samples that contain E. canis), (-) EHRL (samples that do not contain E. canis), (+) ANAP (samples that contain *A. phagocytophilum*), (-) ANAP (samples that do not contain *A. phagocytophilum*).

4. Discussion

Between 2007 and 2023, the state of Minas Gerais, confirmed 451 cases of SF (the third highest number of confirmed cases in the country) [51], ranking second in Brazil in number of deaths from SF, with 145 deaths [52]. For many tick-borne organisms, dogs may be utilized as sentinel hosts in order to estimate the risk of human infection [35,47,53]. Previous studies have demonstrated a direct correlation between an increase of seropositivity in these animals and the resulting threat upon the public health of humans [54,55]. In this context, the serological survey in sentinels is essential, adequately reflecting the circulation of TBD in a given location, primarily indicating the presence of the bioagent in these animals.

For the purpose of screening for TBD, IFA serology, a methodology considered the gold standard in Brazil [16], is also frequently used in other countries, mainly because it is more practical and less costly. However, it is important to note that several studies have demonstrated cross-reactions between different members of the bioagents investigated here [25,56,57].

According to the BMH [16], titers that are greater than or equal to 1:64 for *Rickettsia* spp., 1:40 for *E. canis*, and 1:80 for *A. phagocytophilum* in a single sample confirm the diagnosis for the TBD studied. The results seen in Figure 2, demonstrate that positive titers (per the standards of the BMH) were detected in 83% of the collected samples (Figure 2A); and within those positive samples, 30% tested positive with *Rickettsia* spp. present, 56% tested positive with *E. canis* present, and 85% tested positive with *A. phagocytophilum* present (Figure 2B). These results demonstrate the circulation of these agents in the SCNP, and highlight the potential risk of infection, for both the human and animal population, within this location and the surrounding areas.

Previous research indicated that co-infection of multiple TBD pathogens in dogs appears more frequent in endemic areas [58,59]. The results in Figure 3 demonstrate the percentage of the samples

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where more than one infectious agent was present. Specifically, it was determined that 30% of the samples were co-infected with *E. canis*, and *A. phagocytophilum*; 16% of the samples were co-infected with *Rickettsia* spp., *E. canis*, and *A. phagocytophilum*; 5% of the samples were co-infected with *Rickettsia* spp., and *A. phagocytophilum*; and 3% of the samples were co-infected with *Rickettsia* spp., and *E. canis*.

Furthermore, a previous study found *E. canis* and *Anaplasma* spp. co-infecting the tick host *R. sanguineus*, thus explaining the common occurrence of these two infectious agents co-infecting dogs [60]. The chi-square test of independence seen in Figure 5 demonstrated a significant relationship between *A. phagocytophilum* and *E. canis* co-infections in the dogs sampled. The results in Figure 5A demonstrate that the chi-square statistic between *A. phagocytophilum* and *E. canis* co-infections was significant at $p < 0.10$; and was significant at $p < 0.10$ with the Yates correction. However, the co-infections between *E. canis* and *Rickettsia* spp. (Figure 5B) and between *A. phagocytophilum* and *Rickettsia* spp. (Figure 5C) were demonstrated to not be statistically related. Thus, our results not only support the previously findings [60], it also demonstrated that this co-infection also occurs in the new (sentinel) host.

Since surveillance in sentinel hosts has direct implications for the prevention, treatment, and control of zoonotic diseases [37], increasing the standard surveillance protocol to include even more endemic pathogens could be beneficial to public health. As such, the question was raised as to whether *A. phagocytophilum* should be included in FUNED's standard protocol for the surveillance of tick-borne diseases. The results in Figure 3 also demonstrated the percentage of positive samples with a single infection of either *Rickettsia* spp., *E. canis*, or *A. phagocytophilum*. Specifically, 33% of the samples were found to be infected with *A. phagocytophilum* only, 7% of the samples were infected with *E. canis* only, and 5% of the samples were infected with *Rickettsia* spp. only. The results in Figure 4 demonstrate that the most prevalent infection in this group of samples was for the single infection of *A. phagocytophilum*. Taken together these results confirmed a significant presence of *A. phagocytophilum* in the sentinel hosts monitored by FUNED, and establishes a potential threat upon the public health of humans. These results also imply that 33% of the sentinel hosts that were tested would have gone unnoticed and untreated, further permitting the potential spread of this disease.

5. Conclusions

The results of the present study: I) Confirmed the presence of *A. phagocytophilum* in the sentinel hosts, in addition to the pathogens that are currently included in the standard surveillance protocol performed by FUNED; II) Support the inference of the risk of TBD occurrence in humans and also in other vertebrate hosts in the SCNP; III) Could prove helpful in changing public policies to include testing for Anaplasmosis as a part of the standard surveillance protocol; and IV) Could prove helpful in monitoring, preventing, controlling, and treating another potential threat upon the public health of humans; as well as, providing education, health vigilance, and vector control measures in the respective areas.

Ideally, future studies should include: I) An increase in the serological surveillance methodologies to include research on ticks and etiological agents; II) Identifying and monitoring more land areas in order to determine the potential risk toward the public health of humans; and III) Monitoring the seropositive sentinel animals after treatment in order to quantify the success of the standard surveillance protocol upon the prevention, control and treatment for TBD.

Author Contributions: Conceptualization, L.D.; L.O and A.D.; Methodology, L.D; M.T. and A.D; Software, M.T..; Validation, L.D; M.T.; L.O.; A.D. and L.F.; Formal Analysis, L.D; M.T. and L.O.; Investigation, L.D.; A.D. and L.O.; Resources, L.D.; A.D.; G.F; V.R.; G.P.; D.R. and L.F.; Data Curation, L.D.; M.T.; L.O. and L.F.; Writing—original draft preparation, L.D.; L.O. and A.D.; writing—review and editing, L.D.; L.O. and A.D.; visualization, L.D.; L.O. and A.D.; supervision, L.D.; Project Administration, L.D.; A.D., L.O. and L.F.. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: This work was carried out by the Cipó Project, with support from the Chico Mendes Institute for Biodiversity Conservation, Ezequiel Dias Foundation, University of São Paulo, management of

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Parna Serra do Cipó, local community leaders, Log Nature and Fuller Laboratories, aiming to contribute to the conservation and collective health of the site, combining research and communication actions.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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