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Canine infection by *Ehrlichia canis* and *Anaplasma platys* in the semiarid region of Brazil: frequency and molecular characterization

Infecção canina por Ehrlichia canis e Anaplasma platys na região semiárida do Brasil: frequência e caracterização molecular

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ABSTRACT Canine monocytic ehrlichiosis and canine thrombocytic anaplasmosis, caused by *Ehrlichia canis* and *Anaplasma platys*, are endemic diseases in Brazil. This study aimed to determine the occurrence of these pathogens in 224 dogs at a Veterinary Teaching Hospital in Piauí, northeastern Brazil. DNA samples were analyzed using PCR assays for *E. canis* and *A. platys*, followed by sequencing and phylogenetic analysis. Clinical data were collected from medical records. A total of 17.9% of the dogs (40/224) were positive for *E. canis*, 30.8% (69/224) for *A. platys*, and 16.5% (37/224) for both. Ticks were present in 50% of the dogs infected with *E. canis*, 59.4% with *A. platys*, and 62.2% with both bacteria. Common clinical and laboratory changes included lymphadenopathy, anemia, thrombocytopenia, hyperglobulinemia, and hypoalbuminemia. The partial 16S rDNA gene sequences of *E. canis* and *A. platys* from dogs in Piauí showed 99% to 100% and 99.74% identity to reference sequences from different regions of Brazil and other countries in GenBank, respectively. Phylogenetic analysis revealed that the sequences were grouped into their respective clades. This study represents the first molecular survey and phylogenetic characterization of these bacteria in dogs in the semiarid region of Piauí.

Keywords ehrlichiosis, anaplasmosis, canine tick-borne diseases, PCR, northeast

RESUMO *Erliquiose monocítica canina e anaplasmose trombocítica canina, causadas por Ehrlichia canis e Anaplasma platys, são doenças endêmicas no Brasil. Objetivou-se determinar a ocorrência desses patógenos em 224 cães atendidos em um Hospital-Escola Veterinário no estado do Piauí, no nordeste brasileiro. Amostras de DNA foram analisadas por meio de PCR para identificação de E. canis e A. platys, seguidos de sequenciamento e análise filogenética. Dados clínicos foram coletados a partir dos prontuários. Um total de 17,9% dos cães (40/224) foram positivos para E. canis, 30,8% (69/224) para A. platys, e 16,5% (37/224) para ambas as bactérias. Carrapatos estavam presentes em 50% dos cães infectados com E. canis, 59,4% com A. platys e 62,2% com ambas as bactérias. As alterações clínico-laboratoriais mais observadas foram linfadenopatia, anemia, trombocitopenia, hiperglobulinemia e hipoalbuminemia. As sequências parciais do gene 16S rDNA de E. canis e A. platys dos cães no Piauí mostraram identidade de 99% a 100% e 99,74%, respectivamente, com sequências de referência de diferentes regiões do Brasil e outros países no GenBank. A análise filogenética revelou que as sequências foram agrupadas em seus respectivos clados. Este estudo representa o primeiro levantamento molecular e caracterização filogenética dessas bactérias em cães na região semiárida do Piauí.*

Palavras-chave *erliquiose, anaplasmose, doenças caninas transmitidas por carrapatos, PCR, nordeste*

1. Introduction

In Brazil, canine monocytic ehrlichiosis (CME) and canine thrombocytic anaplasmosis (CTA) are common tick-borne diseases caused by Gram-negative bacteria of the species *Ehrlichia canis* and *Anaplasma platys*, respectively (Dumler et al., 2001). The transmission of these bacteria occurs primarily through the bite of ticks of the species *Rhipicephalus sanguineus* sensu lato (s.l.), which are highly prevalent in this country (Dantas-Torres, 2008; Vieira et al., 2018) and where the 'tropical species' of the *R. sanguineus* group occurs in most of its territory (Moraes-Filho et al., 2015). *R. sanguineus* from tropical America has high vector competence and can transmit *E. canis* and *A. platys* (Moraes-Filho et al., 2015; Snellgrove et al., 2020). It is noteworthy that, in a recent publication, Šlapeta et al. (2021) formalized the basis for using *R. linnaei* to refer to the *R. sanguineus* s.l. 'tropical lineage'.

CME is endemic in most Brazilian regions, with molecular and serological prevalence reaching around 80% (Soares et al., 2017; Vieira et al., 2018; Maggi & Krämer, 2019). In a previous study conducted in Teresina, Piauí, whole blood samples from 443 urban dogs were analyzed to detect *E. canis* DNA using the PCR technique, and the results revealed a positivity rate of 32% (Silva et al., 2017).

During *E. canis* infection, the bacterium displays tropism in relation to monocytes and macrophages and can disseminate in the organism through these cells (Nair et al., 2016). Clinical presentation of CME results in acute, chronic, or subclinical phases, with several clinical manifestations (Rodríguez-Alarcón et al., 2020). The clinical manifestations of acute-phase CME are fever, lethargy, anorexia, weight loss, lymphadenopathy, and bleeding disorders (Little, 2010; Vieira et al., 2011). In the subclinical phase, the animals usually do not show clinical signs (Rodríguez-Alarcón et al., 2020). It is not common that *E. canis* circulates in the blood, although it may be found in some target organs, such as the spleen, bone marrow, or liver (Gal et al., 2008; Faria et al., 2010). Nevertheless, clinical signs may be observed in the chronic phase of the disease, in which infected dogs show severe pancytopenia, haemorrhagic diathesis, and general debilitation (Harrus & Waner, 2011).

CTA is also considered an endemic disease in Brazilian regions, with molecular prevalence reaching around 50% (Maggi & Krämer, 2019). Although this disease does not cause severe clinical signs, infection of platelets by *A. platys* can promote thrombocytopenia. Moreover, coinfection with *E. canis* seems to worsen the clinical signs of CTA (De Caprariis et al., 2011; Gaunt et al., 2010).

Both CME and CTA are routinely diagnosed based on clinical signs, microscopic examination of peripheral blood smears, and serological and molecular tests, with varying sensitivity and specificity (Harrus & Waner, 2011; Lara et al., 2020). Molecular-based methods such as the polymerase chain reaction (PCR) and real-time PCR are frequently used in laboratories. These are more accurate and highly sensitive and specific for identifying bacterial infections in different clinical stages and with various bacterial loads (Ramos et al., 2009; Rufino et al., 2013). Furthermore, many studies have used PCR to amplify gene targets to investigate the molecular epidemiology and diversity among strains (Silveira et al., 2015).

It is known that coinfection by pathogens transmitted by vectors is a common condition among dogs in Brazil (Gonçalves et al., 2014; Soares et al., 2017; Maggi & Krämer, 2019). This highlights the need for differential diagnoses for dogs with nonspecific clinical signs, given that such conditions are frequently observed among dogs affected by these pathogens.

Although many studies on *E. canis* and *A. platys* infections have been carried out in different regions of Brazil, no reports from Teresina in northeast Brazil have been published. This city, located in the semiarid region of Brazil, presents climatic conditions that are favorable for the development of the tick vector. Thus, the aim of the present study was to investigate the occurrence of *E. canis* and *A. platys* infection using the PCR method, among dogs examined at a Veterinary Teaching

Hospital in Teresina. In addition, the molecular characteristics of the local bacterial strains were assessed, with comparison of the 16S rRNA gene between *E. canis* and *A. platys*, as characterized in other regions.

2. Material and methods

2.1. Study area

The municipality of Teresina is located at 05º05' south and 42º48' west in the north-central region of the state of Piauí (Figure 1), with 814,230 inhabitants spread over an area of 1,391 km². The biome consists of savanna (Cerrado), and the climate is tropical (IBGE, 2020), with an annual mean temperature and relative humidity of 28.2 °C and 69%, respectively, from 1980 to 2017 (Bastos & Andrade Júnior, 2019). The study was conducted at the Veterinary Teaching Hospital of the Federal University of Piauí, Teresina.

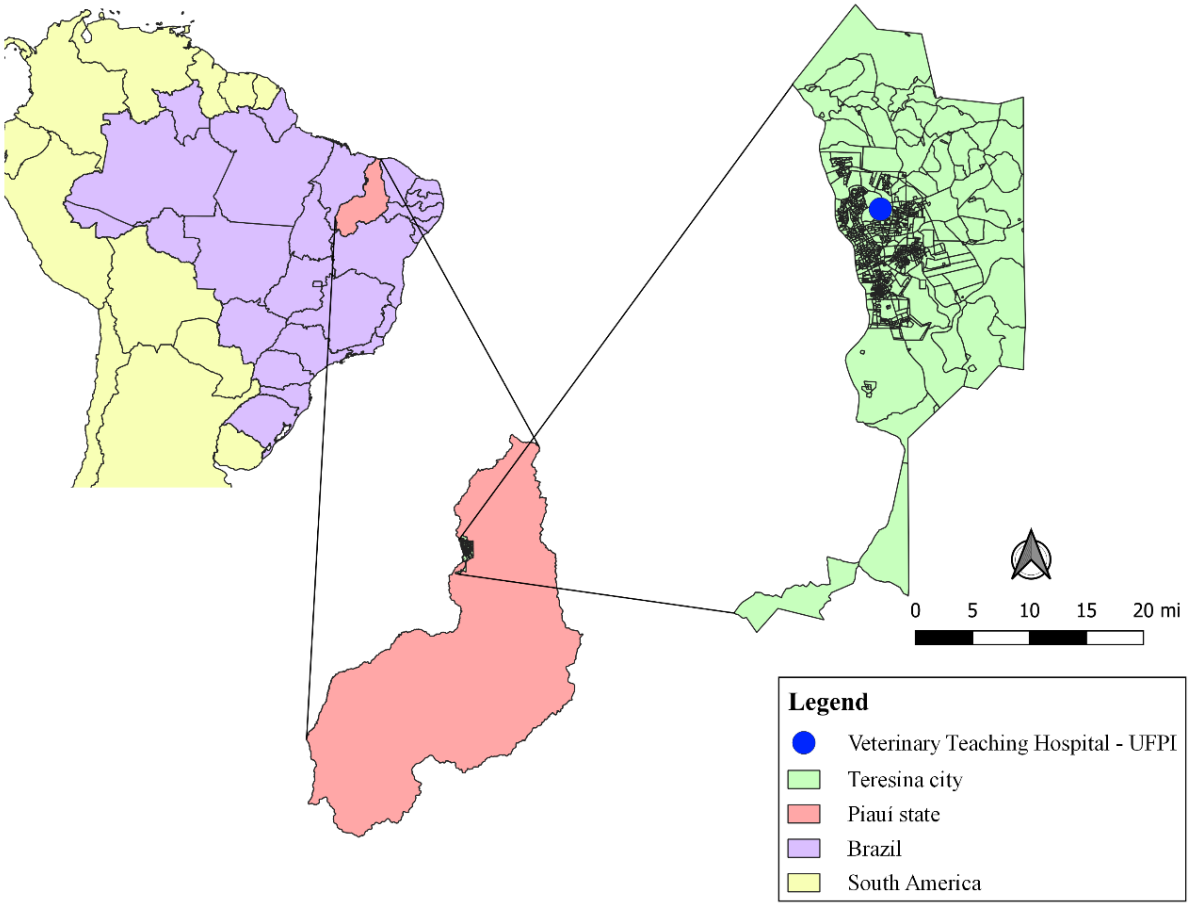


Figure 1 – Geographical location of the Veterinary Teaching Hospital in Teresina, located in the semiarid region of Brazil.

2.2. Sampling and ethics

This cross-sectional descriptive study included a convenience sample of 224 owned pet dogs from the Veterinary Teaching Hospital of the Federal University of Piauí. The sample size was calculated using the following criteria: estimated canine population as 11% of the human population

(Junqueira & Galera, 2019), an expected prevalence of CME of 25.6% (Guedes et al., 2015), with a 95% confidence interval, and a 5% margin of error.

The inclusion criteria included dogs under any clinical suspicion, regardless of age, breed, and sex, whose owners agreed to participate in the research and collection of biological samples by signing an informed consent form. All the procedures were conducted in accordance with the ethical principles for animal experimentation adopted by the National Council for Animal Experimentation Control (CONCEA), and approved by the ethics committee for animal experimentation of UFPI (protocol 070/12 – CEUA). Peripheral blood samples were collected from all dogs for molecular diagnosis of CME and CTA from the jugular vein. Subsequently, clinical and laboratory data were collected from the medical records of the positive dogs included in this study, comprising age, sex, breed, clinical signs, and hematological and biochemical alterations.

2.3. Molecular diagnosis of E. canis and A. platys infection

DNA was extracted from peripheral blood using the Wizard® Genomic DNA purification kit (Promega, USA), following the manufacturer's instructions. To evaluate PCR inhibitors in DNA samples, PCR for the GAPDH gene was performed using the primers 5'-CCAAAGTTGTCATGGATGACC -3'(F) and 5'- CCTTCATTGACCTCAACTACAT -3'(R) (Birkenheuer et al., 2003), which amplify a fragment of 450 bp.

Molecular detection of the 16S rRNA genes of *E. canis* and *A. platys* was performed using nested PCR (nPCR) (Wen et al., 1997). The primers for the first reaction for both bacteria were "EC1 sense" (5'-AGAACGAACGCTGGCGGCAAGCC-3') and "EC1 antisense" (5'-CGTATTACCGCGGCTGCTGGC-3'). For the second PCR for *E. canis* detection, the primers used were "EC2 sense" (5'-CAATTATTTATAGCCTCTGGCTATAGGAA-3') and "EC2 antisense" (5'-TATAGGTACCGTCATTATCTTCCCTAT-3'). For *A. platys* detection, the primers used in the second reaction were "A1 sense" (5'-TTTTTGTCGGAGCTTGCTATGATA-3') and "A2 antisense" (5'-TGTGGGTACCGTCATTATCTTCCCCA-3') (Inokuma et al., 2002). The PCR mixture contained 1x Taq buffer, 0.2 mM of each dNTP (Invitrogen, USA), 1.5 mM of $MgCl_2$, 0.4 µM of each primer, 0.62 U of Taq DNA polymerase (Invitrogen, USA), and 100 ng of DNA sample. The conditions of the second reaction were similar to those of the first one, except that 5 µL of previously amplified PCR product (from the first reaction) was used as the target. Amplification was performed in an automated thermocycler (Gene Bioer Pro®) as follows: 94 °C for 1 min; 40 cycles at 94 °C for 30 s; 60 °C for 1 min; 72 °C for 1 min; and a final extension at 72 °C for 5 min. The results from the second DNA amplification yielded products of approximately 390 and 386 base pairs (bp) for *E. canis* and *A. platys*, respectively. These PCR products were viewed using ethidium bromide staining (0.5 µg/mL) and electrophoresis on a 1.5% agarose gel, using a UV transilluminator (BioAgency).

2.4. Sequencing and molecular characterization

Randomly selected nPCR products from samples that had previously been amplified for *E. canis* (four) and *A. platys* (four) were purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, Massachusetts, USA), according to the manufacturer's recommendations. Purified amplified DNA fragments from positive samples were then subjected to sequence determination in an automated sequencer (ABI Prism 310 Genetic Analyzer; Applied Biosystems/Perkin Elmer, Foster City, California, USA) in both directions.

The sequences obtained were analyzed using the Phred-Phrap© software, version 23 (Ewing & Green, 1998), to evaluate the electropherograms' quality and obtain the consensus sequences from alignment of the sense and antisense sequences.

Identification and comparison of 16S rRNA sequences with those deposited in the GenBank database were conducted using the BLASTn program. The sequences, saved in "FASTA" format, were aligned with homologous sequences of each agent retrieved from GenBank using the MAFFT

software version 1.5.0 (Katoh & Standley, 2013) and further edited with Geneious Prime version 2024.0.7. The optimal evolution models were determined from the sequence alignment matrices using the jModelTest 2 software version 2.1.6 (Darriba et al., 2012). Phylogenetic inference was performed using the maximum likelihood (ML) method, with the evolutionary model chosen according to the BIC criterion in W-IQ-Tree software (available online: http://iqtree.cibiv.univie.ac.at/) (Trifinopoulos et al., 2016), which was also used for the phylogenetic analysis. Clade support indices were assessed through bootstrap analyses (Hoang et al., 2018) with 1,000 replicates. The phylogenetic tree was edited and rooted using an outgroup with the iTOL software version 6.9 (Letunic & Bork, 2024).

2.5. Statistical analysis

The frequencies of *E. canis* and *A. platys* DNA detection and the hematological and biochemical alterations in the dogs of this study were described. Associations between PCR positivity for *E. canis* and *A. platys* and the variables sex, breed, age, and presence of ticks at the time of sample collection were analyzed. Odds ratios were calculated, and the chi-square test ($\chi 2$) or Fisher test was applied, with a significance level of 0.05. The Shapiro-Wilk test revealed that the quantitative laboratory variables were normally distributed. Thus, comparative data analysis was performed using ANOVA, followed by Tukey's post hoc test. The statistical package GraphPad Prism 5 (GraphPad Software, California, USA) was used for these analyses.

3. Results and Discussion

Among the 224 dogs evaluated in this study, molecular analysis of blood-derived DNA samples revealed that 65.2% (146/224) tested positive by PCR for at least one of the two pathogens investigated (*E. canis* and/or *A. platys*). Of these dogs, 27.4% (40/146) were positive only for *E. canis*, and 47.3% (69/146) were positive only for *A. platys*. Coinfection with *E. canis* and *A. platys* was identified in 25.3% (37/146) of the animals. As expected, this study demonstrated that dogs attended at the Teaching Veterinary Hospital in Teresina exhibited a higher occurrence of infection by these pathogens. Since both pathogens are transmitted by ticks, primarily by *R. sanguineus* s.l. (Dantas-Torres, 2008), and this tick is abundant throughout Brazilian urban areas such as Teresina, where the climate is suitable for the proliferation of *R. sanguineus* s.l. — an environment conducive to the bacterial life cycle — it is expected that infections and coinfections may be frequent in dogs.

Indeed, a previous study conducted in Teresina, Piauí, analyzed whole blood samples from 443 urban dogs to detect *E. canis* DNA using the PCR technique, and the results revealed a positivity rate of 32% (Silva et al., 2017). These findings corroborate the endemic nature of *E. canis* in urban canine populations in the region, as also observed in our study, and reinforce the need for continued surveillance and vector control.

E. canis and *A. platys* are worldwide, predominantly found in tropical and subtropical areas, with a wide range of molecular prevalence (Sainz et al., 2015). In Brazil, disparate molecular prevalence levels have also been observed, ranging from 15% to 88% for *E. canis* (Vieira et al., 2011; Andre et al., 2015; Pereira et al., 2023) and from 1.6% to 55% for *A. platys* (Ramos et al., 2009; Lasta et al., 2013; Sousa et al., 2013). Many factors contribute to these differences, such as climate conditions, the abundance and density of vectors, socioeconomic factors, target DNA or PCR method, and sample type (Maggi & Krämer, 2019; Kabir et al., 2024). The high positivity (65.2%) for these bacteria observed in this study may be related to the fact that the dogs involved were owned by low-income individuals who lack access to proper health education, financial resources for periodic ectoparasite control, and live in environments with poor sanitary conditions that favor the endemicity of vectors and these infectious agents. Studies have shown that factors such as living outdoors, irregular sanitation, tick infestation levels, dog population density, age, and diet are associated with increased risk of *E. canis* and *A. platys* infections (Parham et al., 2015; Pérez-Macchi et al., 2019; Selim et al., 2021).

These bacteria have been identified through sequencing and phylogenetic analysis of the 16S rRNA gene in many states of Brazil (Macieira et al., 2005; Carvalho et al., 2024; Santos et al, 2009; Ramos et al., 2010; Duarte et al., 2013; Lasta et al., 2013). We have also identified the *E. canis* and *A. platys* species by gene sequencing and phylogenetic analysis. Analysis of four sequenced products based on the 16S rRNA region of *E. canis* from dogs in Teresina, Piauí, Brazil, yielded a fragment of approximately 392 bp. These sequences, which were deposited in the GenBank database (accession numbers JX437966, MT896772, MT896773, and MT896774), showed 99% to 100% sequence identity with *E. canis* (GenBank accession number KF972450). Similarly, analysis of four sequenced products based on the 16S rRNA region of *A. platys* from dogs in the same area produced a fragment of approximately 387 bp. These sequences, deposited in the GenBank database (accession numbers JX437967, MT897154, MT897155, and MT897156), showed 99.74% sequence identity with *A. platys* (GenBank accession number FJ755157).

The phylogenetic analysis by ML and K2P + G4 evolutionary model, based on the 16S rRNA gene of *Ehrlichia*, positioned the sequences detected in four dogs from Piauí state within the *Ehrlichia canis* clade. The clade has been previously detected in dogs, domestic cats, ticks (*R. sanguineus* s.l.), and marmosets (*Callithrix*) from Brazil, the United States, Mexico, Colombia, and India, with a branch support of 66% (Figure 2). Similarly, the analysis by ML and K2P + F4 evolutionary model, based on the 16S rRNA gene of *Anaplasma,* positioned the sequences detected in four dogs from Piaui state within the *Anaplasma platys* clade, which has been previously found in dogs and *R. sanguineus* s.l. ticks from Brazil and the United States, with a branch support of 74% (Figure 3).



Figure 2 - Phylogenetic tree based on alignment of 392 bp of *Ehrlichia canis* 16S rRNA gene, using maximum likelihood method and K2P + G4 as an evolutionary model. Numbers at nodes correspond to maximum likelihood bootstrap values. Sequences from the present study were highlighted in red (*E. canis*). *Neorickettsia risticii* (NR029162) was used as an outgroup.

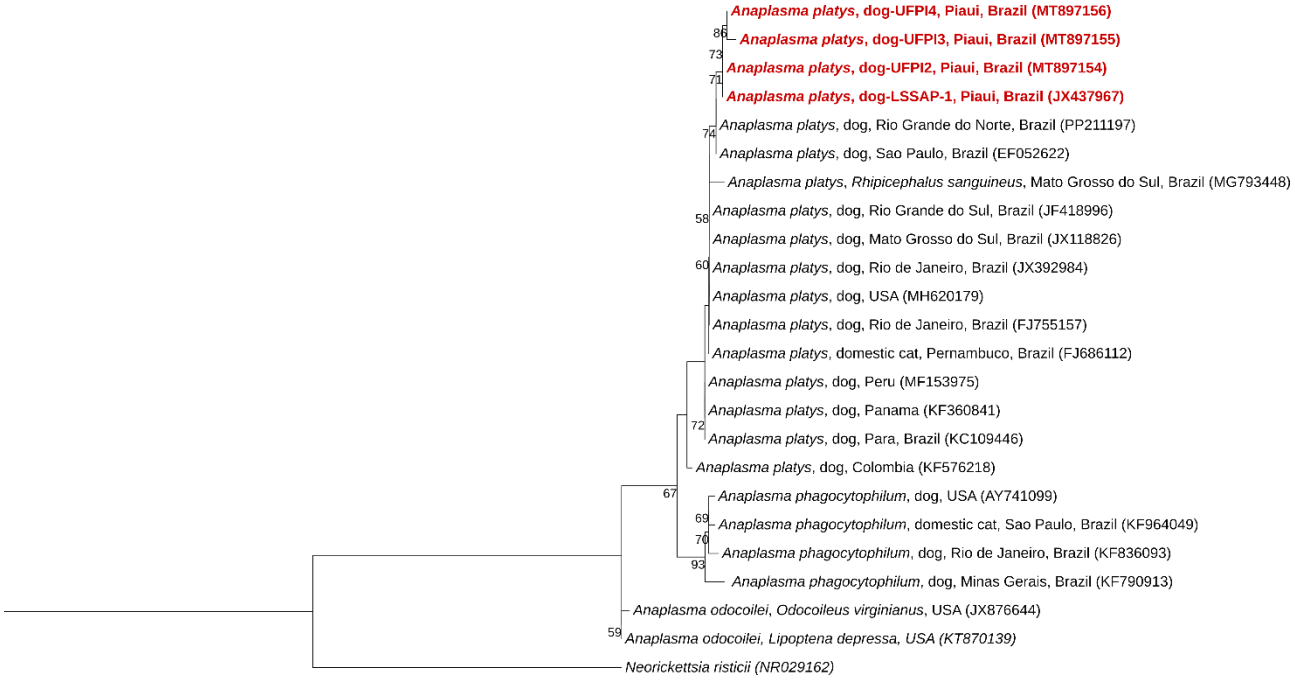


Figure 3 – Phylogenetic tree based on alignment of 387 bp of *Anaplasma platys* 16S rRNA gene, using maximum likelihood method and K2P + G4 as an evolutionary model. Numbers at nodes correspond to maximum likelihood bootstrap values over 50. Sequences from the present study were highlighted in red (*A. platys*). *Neorickettsia risticii* (NR029162) was used as an outgroup.

The sequences of *E. canis* and *A. platys* identified in this study exhibited 100% similarity to genotypes previously sequenced in various states of Brazil, including Bahia (Carvalho et al., 2024), Mato Grosso do Sul (Soares et al., 2017), Pernambuco (Ramos et al., 2010), Rio Grande do Sul (Lasta et al., 2013), Pará (Rufino et al., 2013), Rio de Janeiro (Ferreira et al., 2014), and São Paulo (Aguiar et al., 2008) (Figure 2), and in free-living primates of the genus *Callithrix* in Minas Gerais (Mafra et al., 2015). Due to this high degree of homology, we suggest that the *E. canis* and *A. platys* sequenced in the present study are likely the same species found in dogs across different geographical areas of Brazil.

Among the 146 dogs confirmed as infected, 54.8% (80/146) were male, 75 (51.4%) were mongrels, and 93 (85.4%) were aged between 1 and 7 years (Table 1). No statistically significant association was found in the analysis of these variables. There is no consensus regarding the association between phenotypic characteristics and susceptibility to infection and disease caused by *E. canis* and *A. platys* (Silva et al., 2012; Witter et al., 2013; Paschoal et al., 2020). A dog's genetic immunological susceptibility, environmental factors that favor tick proliferation, and the socioeconomic conditions of the human population can contribute to the increased prevalence of CME and CTA (Carlos et al., 2011; Paulino et al., 2018; Hazelrig et al., 2023; Ajith et al., 2024). Another aspect is that, regardless of sex, age, and breed, dogs are equally likely to become infected by *E. canis* and *A. platys*, especially when constantly exposed to ticks. Most dogs included in this study lived in domiciles with a history of tick infestations. On physical examination, ticks were found in 50% (20/40) of the dogs infected with *E. canis*, 59.4% (41/69) of those with *A. platys*, and 62.2% (23/37) of the coinfected dogs. Despite this finding, it was not possible to perform a statistical analysis between the presence of ticks and bacterial infection because we could not access data from the negative dogs. However, it is known that dogs exposed to ticks are more susceptible to infection.

The infected dogs presented nonspecific clinical signs and laboratory alterations that are frequently described in cases of CMA and CTA (Carlos et al., 2011; Harrus, 2015; Parashar et al., 2016; Guimarães et al., 2021; Mobarak et al., 2024). The clinical and laboratory findings were

observed in 60% of the dogs (24/40) with *E. canis*, 66.7% (46/69) with *A. platys*, and 59.5% (22/37) with both pathogens. The frequencies of all the clinical signs and laboratory changes recorded for dogs that were positive for *E. canis*, *A. platys*, or both (coinfection) are described in Table 2. Lymphadenomegaly was the most frequent clinical sign in all dogs infected with *E. canis* and/or *A. platys* and those coinfected. Other clinical signs observed included alopecia, apathy, anorexia, and pallor of mucous membranes, demonstrating the nonspecific nature of the clinical signs. This highlights how coinfection with two or more tick-borne pathogens, such as *E. canis* and *A. platys*, may complicate the association of specific clinical signs with a particular canine vector-borne disease. Differential diagnosis for other hemoparasites was not performed in the dogs evaluated in this study.

Table 1 – Risk factors associated with monoinfection or coinfection by *Ehrlichia canis* and *Anaplasma platys* in 224 dogs attended in the Veterinary Teaching Hospital at Teresina, Piauí.

Parameters	Total % (n)	*E. canis* % (n)	OR (IC 95%) *P value**	*A. platys* % (n)	OR (IC 95%) *P value**	Coinfection % (n)	OR (IC 95%) *P value**
Sex							
Female	46.9% (105/224)	37.5% (15/40)	1.515 (0.7394-3.105) *0.2545*	45% (31/69)	1.034 (0.5694-1.879) *0.9119*	54% (20/37)	0.7083 (0.3490-1.438) *0.3382*
Male	53.1% (119/224)	62.5% (25/40)		55% (38/69)		46% (17/37)	
Breed							
Mixed	47.8% (107/224)	52.5% (21/40)	1.433 (0.7110-2.890) *0.3128*	46.4% (32/69)	1.061 (0.5842-1.926) *0.8464*	59.5% (22/37)	1.760 (0.8594-3.604) *0.1192*
Pure	52.2% (117/224)	47.5% (19/40)		53.6% (37/69)		40.5% (15/37)	
Age							
< 1 year	17.9% (40/224)	12.5% (5/40)		24.6% (17/69)		16.2% (6/37)	
1 to 7 years	62.9% (141/224)	72.5% (29/40)	0.001286# *0.9714*	58% (40/69)	2.508# *0.1133*	64.8% (24/37)	0.002131# *0.9632*
> 7 years	16.1% (36/224)	15.0% (6/40)		17.4% (12/69)		19% (7/37)	

*Chi-square test; # Exact fisher test; Odds ratio (OR); Confidence interval (IC95%).

According to Gaunt et al. (2010), the clinical implications among dogs infected with *E. canis* or *A. platys*, or presenting coinfection, ranged from pronounced anemia to thrombocytopenia. This is in line with the observations of the present study, as the hematological analysis revealed that the most frequent changes were anemia and thrombocytopenia, regardless of whether the dogs were infected with *E. canis*, *A. platys,* or both (Tables 2 and 3). Infection with *E. canis* induces a systemic release of inflammatory mediators, which exert a suppressive effect on erythropoiesis in the bone marrow and promote the migration and consumption of circulating platelets at sites of tissue damage associated with the inflammatory response (Harrus, 2015). Thrombocytopenia is often recognized as a diagnostic criterion for canine monocytic ehrlichiosis (CME), and its pathogenesis involves the elevation of antiplatelet antibodies, which reduce the lifespan of circulating platelets, ultimately leading to their acute destruction (Parashar et al., 2016).

Table 2 – Clinical and laboratory findings observed in 92 dogs positive for PCR to *E. canis*, *A. platys*, and coinfected.

Parameters	*E. canis* % (n)	*A. platys* % (n)	Coinfected % (n)
Clinical signs			
Lymphadenomegaly	55% (22/40)	72.5% (50/69)	64.9% (24/37)
Alopecia	52.5% (21/40)	43.5% (30/69)	43.2% (16/37)
Pale mucous membrane	35% (14/40)	17.4% (12/69)	18.9% (7/37)
Weight loss	30% (12/40)	21.7% (15/69)	10.8% (4/37)
Anorexia	32.5% (13/40)	26.1% (18/69)	18.9% (7/37)
Apathy	30% (12/40)	24.6% (17/69)	18.9% (7/37)
Fever	22.5% (9/40)	15.9% (11/69)	8.1% (3/37)
Ocular changes	10% (4/40)	24.6% (17/69)	8.1% (3/37)
Vomit	10% (4/40)	10.1% (7/69)	8.1% (3/37)
Abdominal pain	5% (2/40)	1.4% (1/69)	0% (0/37
Splenomegaly	2.5% (1/40)	1.4% (1/69)	5.4% (2/37)
Icterus	5% (2/40)	0 % (0/69)	0% (0/37)
Lameness	2.5% (1/40)	5.8% (4/69)	5.4% (2/37)
Melena / Diarrhea	7.5% (3/40)	4.3% (3/69)	5.4% (2/37)
Dyspnea	2.5% (1/40)	0% (0/69)	2.7% (1/37)
Edema	0% (0/40)	4.3% (3/69)	5.4% (2/37)
Epistaxis/bleeding	0% (0/40)	4.3% (3/69)	5.4% (2/37)
Hind limb paresis	0% (0/40)	4.3% (3/69)	0% (0/37)
Nasal discharge/sneeze/cough	2.5% (1/40)	7.2% (5/69)	0% (0/37)
Laboratory results			
Anemia	60% (24/40)	39.1% (27/69)	59.5% (22/37)
Thrombocytopenia	55% (22/40)	39.1% (27/69)	59.5% (22/37)
Leukocytosis	22.5% (9/40)	32% (22/69)	29.7% (11/37)
Neuthrophilia	25% (10/40)	29% (20/69)	27% (10/37)
Lymphocytosis	15% (6/40)	14.5% (10/69)	16.2% (6/37)
Neuthropenia	5% (2/40)	2.8% (2/69)	2.7% (1/37)
Eosinophilia	7.5% (3/40)	16% (11/69)	13.5% (5/37)
Eosinopenia	17.5% (7/40)	7.2% (5/69)	10.8% (4/37)
Monocytosis	2.5% (1/40)	1.4% (1/69)	2.7% (1/37)
Leukopenia	15% (6/40)	7.2% (5/69)	8.1% (3/37)
Lymphopenia	0% (0/40)	0% (0/69)	2.7% (1/37)
Hyperproteinemia	92.5% (37/40)	87% (60/69)	78.4% (29/37)
Hyperglobulinemia	92.5% (37/40)	81.2% (56/69)	75.7% (28/37)
Hypoalbuminemia	50% (20/40)	33.3% (23/69)	59.5% (22/37)
Increased serum urea levels	35% (14/40)	37.7% (26/69)	32.4% (12/37)
Increased serum creatinine levels	10% (4/40)	31.9% (22/69)	24.3% (9/37)

Regarding biochemical alterations, hyperproteinemia due to hyperglobulinemia, in addition to hypoalbuminemia, was the most frequent laboratory finding in both infections (Tables 2 and 3), as described in other studies (Witter et al., 2013; Maggi & Krämer, 2019; Paschoal et al., 2020). Additionally, a significant decrease in albumin levels was observed in dogs with *E. canis* infection compared to dogs infected with *A. platys* (Table 3). Mobarak et al. (2024) also observed

hypoalbuminemia and hyperglobulinemia, but without an increase in total protein levels, which, according to the authors, may be due to compensatory synthesis and release of globulins acting as positive acute-phase reactants to trigger the inflammatory process.

Table 3 – Laboratory findings observed in 92 dogs PCR-positive for *E. canis*, *A. platys*, or coinfected with both.

Parameter	*E. canis*	*A. platys*	Coinfected	P value	RV
Hemathological					
Erythrocytes (x10^6/µL)	4.9 (±1.7)* [a]	5.4 (±1.6)[a]	4.9 (±1.5)[a]	0.1725	5.5-8.5
Hematocrit (%)	29.7 (±10.6)[a]	33.1 (±9.6)[a]	31.0 (±9.6)[a]	0.1441	37-55
Hemoglobin (g/dL)	10.7 (±3.7)[a]	12.0 (±3.5)[a]	11.2 (±3.6)[a]	0.1559	12-18
Total leukocytes (x10^3/mm^3)	13.4 (±8.4)[a]	14.5 (±6.6)[a]	13.4 (±7.2)[a]	0.6719	6-17
Lymphocytes (x10^3/mm^3)	2.9 (±1.9)[a]	3.5 (±1.5)[a]	3.2 (±1.8)[a]	0.2695	1-4.8
Neuthrophils (x10^3/mm^3)	9.5 (±6.2)[a]	9.5 (±4.7)[a]	8.9 (±5.0)[a]	0.8150	3-11.5
Monocytes (/mm^3)	430 (±357)[a]	537 (±968)[a]	477 (±338)[a]	0.7431	150-1350
Eosinophils (/mm^3)	533 (±570)[a]	776 (±666)[a]	748 (±898)[a]	0.2082	100-1250
Platelets (x10^3/mm^3)	205 (±165)[a]	249 (±142)[a]	198 (±125)[a]	0.1396	160-430
Biochemistry					
Total protein (g/dL)	8.9 (±1.5)[a]	9.1 (±1.7)[a]	9.0 (±2.0)[a]	0.7964	5.4-7.1
Albumin (g/dL)	2.5 (±0.9)[a,b]	3.1 (±1.1) [a,c]	2.7 (±1.0)[a]	0.0036	2.6-3.3
Globulin (g/dL)	6.4 (±1.8)[a]	6.0 (±1.9)[a]	6.4 (±2.3)[a]	0.5025	2.7-4.4
Creatinine (mg/dL)	1.0 (±0.7)[a]	1.5 (±1.0)[a]	1.3 (±1.6)[a]	0.1256	0.5-1.5
Urea (mg/dL)	59.8 (±48.6)[a]	59.2 (±47.4)[a]	61.8 (±50.2)[a]	0.9653	15-40

RV - Reference value for dogs to hemogram (Rizzi et al., 2010) and serum biochemistry (Kaneko et al., 2008); *mean (+ standard deviation); Different subscript letters show statistical significance ($p < 0.05$).

4. Conclusions

This was the first molecular survey and phylogenetic characterization of *E. canis* and *A. platys* in dogs in the semiarid region of Piauí. Furthermore, the results showed that *E. canis* and *A. platys* are highly prevalent in dogs in Teresina, in Piauí, northeastern Brazil. Thus, it is highly recommended that dog owners follow the veterinary recommendations for tick prevention. The analysis of the 16S rRNA gene was reliable for confirming infections caused by *E. canis* and *A. platys*. The amplification of this target can be a valuable tool in the routine molecular diagnosis of CME and CTA. Clinical, hematological, and biochemical findings may contribute to raising suspicion of occurrences of canine ehrlichiosis and anaplasmosis in areas endemic for these diseases. However, these tests are insufficient for confirmatory diagnosis, thus emphasizing the need for specific laboratory tests for diagnosing both diseases.

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Declaration of Conflict of Interest

The authors declared no conflicts of interest.

CRediT authorship contribution statement

Lucilene Santos Silva: Conceptualization, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing; **Flaviane Alves de Pinho:** Investigation, Methodology, Formal analysis, Visualization, Writing – original draft, Writing – review & editing; **Lidiany Viana Pires:** Investigation; **Juliana Fortes Vilarinho Braga:** Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing; **Thamires Carvalho da Luz:** Investigation; **Francisco de Assis Leite Souza:** Investigation, Formal analysis, Visualization, Writing – original draft; **Maria do Socorro Pires e Cruz:** Investigation; **João Pessoa Araújo Júnior:** Investigation; **Silvana Maria Medeiros de Sousa Silva:** Conceptualization, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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