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*Communication*

# Geographic distribution of *Ehrlichia canis* genotypes in Brazil

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**Abstract:** Tandem repeat proteins of 36 kDa (TRP36) are major immunoreactive proteins of *Ehrlichia canis*, which have been used in the serological diagnosis of different genotypes of the microorganism. The aim of this study was to evaluate the distribution of the American (USTRP36), Brazilian (BrTRP36) and Costa Rican (CRTRP36) genotypes of *E. canis* in Brazil, using ELISA assays. Serum samples of 815 dogs from 49 cities from all over Brazil were analyzed. Our results showed that 33.9% of the samples were reactive to the USTRP36 genotype and 32.6% to the BrTRP36 genotype. The two genotypes appeared to occur equally throughout Brazil, although the frequency of seropositivity was lower in the south than in the country's other regions. Co-positivity for the American and Brazilian genotypes was also observed in 16% of samples. A few dogs (n=5; 0.6%) reactive to *E. canis*-TRP36 genotype (CRTRP36) were also detected in the northeast and southern regions. We conclude that the American and Brazilian genotypes of *E. canis* are distributed evenly in Brazil, especially in the tropical region, while the temperate region in the south presented the lowest prevalence values. This study offers the first report of dogs seropositive for the Costa Rican genotype in Brazil.

**Keywords: dogs, ehrlichiosis, ELISA, tick-borne, TRP36, TRP19**

## 1. Introduction

*Ehrlichia canis* is the etiologic agent of canine monocytic ehrlichiosis (CME), a serious tick-borne disease of worldwide distribution [1]. A group of tandem repeat proteins of 19 and 36 kDa (TRP19 and TRP36) were characterized as important immunoreactive proteins of *E. canis* and were employed in the serological diagnosis of CME [2-3].

TRP19 is highly conserved among the known *E. canis* strains. It is a specific target for serological diagnostics [3-6] and a strong candidate for vaccine antigen for CME, due to its high degree of conservation and because it is a component of the morula membrane [7].

TRP36 provides more information about the genetic diversity of *E. canis* due to the divergence within amino acid tandem repeatsequences and/or in the number of tandem repeats [6,8-10]. TRP36 protein is secreted by *E. canis* at the time of intracellular colonization and is involved in the adaptive process between the microorganism and the host, and therefore has vaccine potential [3]. *Ehrlichia canis* TRP36 synthetic peptide antigens were used in enzyme linked immunosorbent assays (ELISA)[4], who found dogs seroreactive to two distinct genotypes in Brazil: American (USTRP36) and Brazilian (BrTRP36). Recently, dogs seropositive to the new zoonotic *E. canis* TRP36 genotype described in Costa Rica (CRTRP36) were identified in Colombia [9,11].

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In view of the epidemiological importance of CME in Brazil, and the antigenic relevance of these proteins, canine blood samples were obtained from various regions of Brazil to be evaluated in serological assays using crude *E. canis* antigens by immunofluorescence assay (IFA) and synthetic peptides from TRP19 and 36 (American, Brazilian and Costa Rican genotypes) by ELISA. Moreover, the use of TRP36 antigens revealed the widely distribution of different genotypes throughout Brazil and the first report of dogs seropositive for the Costa Rican genotype in Brazil.

## 2. Materials and Methods

The study included serum samples from 815 dogs suspected of CME in 49 cities (Tab 1 and 2) all over Brazil and treated at Veterinary Clinics and Hospitals and Zoonosis Control Centers. Canine serum samples from the city of Palotina, in the state of Paraná (n=30) were previously selected and analyzed, since one of them was known to be *E. canis* positive (SNAP 4Dx® Plus Test IDEXX Laboratories©, Westbrook, Maine). The presence of anti-*Ehrlichia* spp. serum antibodies was determined by IFA, using crude antigens of Cuiaba#1 strain of *E. canis* [12]. ELISA was performed with synthetic peptides corresponding to epitopes from *E. canis* TRP19 (HFTGPTSFEVNLSEEEKMELQEVS) [7], USTRP36 (TEDSVSAPATEDSVSAPA) [3], BrTRP36 (ASVVPEAEASVVPEAEASVVPEAE) [4] and CRTRP36 (EASVVPAAEAPQPAQQTEDEFFSDGIEA) [11]. All the ELISA assays were performed according to the protocol described [4-5]. Differences between results found per region and *E. canis* TRP36 peptides were evaluated by the chi-square test and $p \leq 0.05$ was considered significant. The statistical analysis was performed using Epi Info™ version 5.5.1 software. This study was approved by the Committee on Animal Research and Ethics of the Federal University of Mato Grosso (UFMT) under protocol no. 23108.122592/2015-10.

## 3. Results

The average number of samples evaluated per city was 14.5 in the Center West, 14 in the Northeast, 15.5 in the North, 17.5 in the Southeast and 28 in the South. Tables 1 and 2 list the number of dogs tested and seropositive in serological assays per city and region. A total of 349 (42.8%) dogs had antibodies to the TRP19 peptide, 277 (33.9%) had antibodies to the USTRP36 peptide, 266 (32.6%) had antibodies to the BrTRP36 peptide and 5 (0.61%) dogs had antibodies to the CRTRP36 peptide. Dual reactivity in dogs positive for both peptides (USTRP36 and BrTRP36) was detected in 131 samples (16.0%) from all the regions. Three hundred and seventy-five (46%) dogs were seroreactive to IFA. Overall, similar results were observed among dogs reactive to USTRP36 and BrTRP36 in all the Brazilian regions ($P>0.05$) (uppercase letters in Table 2). However, the values showed differences when evaluated as a function of antigen (lowercase letters in Table 2). The southern region, for example, differed from the others in all the tests ($P <0.05$). Exceptionally, samples from Barra do Quaraí and Curitiba were negative by IFA, and all the peptides and reactions against CR TRP36 peptides were observed only in Aracaju, SE and Londrina, PR. Figure 1 shows the location of each city and the ELISA results using TRP36 synthetic peptides.

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Table 1. Number of sampled and positive dogs in serological assays by city and region in Brazil

| Location | Map reference | No. of Dogs | ELISA (%) | | | | IFA (%) |
|---|---|---|---|---|---|---|---|
| | | | TRP19 | USTRP36 | BrTRP36 | CRTRP36 | |
| North | | 124 | 65 (52.4) | 38 (30.6) | 45 (36.2) | 0 | 58 (46.7) |
| Porto Velho, RO | 1 | 15 | 11 | 7 | 5 | 0 | 9 |
| Cacoal, RO | 2 | 15 | 8 | 2 | 6 | 0 | 8 |
| Brasiléia, AC | 3 | 15 | 8 | 4 | 4 | 0 | 6 |
| Rio Branco, AC | 4 | 16 | 9 | 3 | 3 | 0 | 10 |
| Boa Vista, RR | 5 | 18 | 14 | 13 | 15 | 0 | 12 |
| Belém, PA | 6 | 16 | 5 | 2 | 6 | 0 | 4 |
| Macapá, AP | 7 | 15 | 4 | 4 | 3 | 0 | 3 |
| Araguaína, TO | 8 | 14 | 6 | 3 | 3 | 0 | 6 |
| Northeast | | 235 | 114 (48.5) | 111 (47.2) | 94 (40) | 3 (1.27) | 124 (52.7) |
| São Luís, MA | 9 | 18 | 7 | 4 | 6 | 0 | 4 |
| Balsas, MA | 10 | 9 | 1 | 0 | 1 | 0 | 1 |
| Bacabal, MA | 11 | 10 | 4 | 2 | 4 | 0 | 4 |
| Raposa, MA | 12 | 12 | 5 | 3 | 6 | 0 | 7 |
| Guaribas, MA | 13 | 14 | 8 | 9 | 7 | 0 | 9 |
| Crato, CE | 14 | 15 | 9 | 8 | 5 | 0 | 10 |
| Mossoró, RN | 15 | 14 | 7 | 2 | 7 | 0 | 8 |
| João Pessoa, PB | 16 | 13 | 5 | 8 | 6 | 0 | 7 |
| Campina Grande, PB | 17 | 10 | 4 | 1 | 3 | 0 | 3 |
| Petrolina, PE | 18 | 15 | 11 | 13 | 8 | 0 | 15 |
| Serrita, PE | 19 | 15 | 6 | 11 | 10 | 0 | 11 |
| Lagoa Grande, PE | 20 | 15 | 8 | 10 | 4 | 0 | 8 |
| Salgueiro, PE | 21 | 20 | 3 | 11 | 2 | 0 | 2 |
| Maceió, AL | 22 | 15 | 13 | 6 | 10 | 0 | 10 |
| Cruz das Almas, BA | 23 | 10 | 6 | 9 | 8 | 0 | 9 |
| Salvador, BA | 24 | 15 | 9 | 5 | 6 | 0 | 8 |
| Aracaju, SE | 25 | 15 | 8 | 9 | 1 | 3 | 8 |
| Central West | | 131 | 75 (57.2) | 58 (44.2) | 51 (38.9) | 0 | 87 (66.4) |
| Cuiabá, MT | 26 | 15 | 15 | 10 | 5 | 0 | 15 |
| Barra do Garças, MT | 27 | 15 | 13 | 10 | 10 | 0 | 13 |
| Colniza, MT | 28 | 15 | 11 | 11 | 6 | 0 | 11 |
| Campo Grande, MS | 29 | 15 | 9 | 6 | 8 | 0 | 9 |
| Dourados, MS | 30 | 15 | 7 | 1 | 5 | 0 | 12 |
| Jataí, GO | 31 | 15 | 8 | 7 | 10 | 0 | 12 |
| Mineiros, GO | 32 | 15 | 4 | 6 | 2 | 0 | 7 |
| Goiânia GO | 33 | 11 | 5 | 2 | 3 | 0 | 5 |
| Brasília, DF | 34 | 15 | 3 | 5 | 2 | 0 | 3 |
| Southeast | | 123 | 62 (50.4) | 43 (34.9) | 54 (43.9) | 0 | 66 (53.6) |
| Uberlândia, MG | 35 | 17 | 11 | 13 | 8 | 0 | 13 |
| Itabirito, MG | 36 | 16 | 4 | 2 | 5 | 0 | 4 |

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|---|---|---|---|---|---|---|---|
| Niterói, RJ | 37 | 15 | 6 | 5 | 5 | 0 | 6 |
| Seropédica, RJ | 38 | 15 | 9 | 7 | 5 | 0 | 8 |
| Vitória, ES | 39 | 19 | 7 | 3 | 10 | 0 | 9 |
| Botucatu, SP | 40 | 15 | 12 | 3 | 8 | 0 | 15 |
| São Paulo, SP | 41 | 15 | 7 | 4 | 7 | 0 | 4 |
| Pres. Prudente, SP | 42 | 11 | 6 | 6 | 6 | 0 | 7 |
| South | | 202 | 33 (16.3) | 27 (13.3) | 22 (10.8) | 2 (0.9) | 40 (19.8) |
| Joinville, SC | 43 | 30 | 1 | 4 | 3 | 0 | 4 |
| Concórdia, SC | 44 | 40 | 2 | 0 | 2 | 0 | 1 |
| Londrina, PR | 45 | 14 | 5 | 3 | 2 | 2 | 6 |
| Palotina, PR | 46 | 30 | 25 | 19 | 14 | 0 | 27 |
| Curitiba, PR | 47 | 28 | 0 | 0 | 0 | 0 | 0 |
| Porto Alegre, RS | 48 | 30 | 0 | 1 | 1 | 0 | 2 |
| Barra do Quaraí, RS | 49 | 30 | 0 | 0 | 0 | 0 | 0 |
| Total | | 815 | 349 (42.8) | 277 (33.9) | 266 (32.6) | 5 (0.61) | 375 (46) |

Abbreviations of states: AC-Acre, AL-Alagoas, AP-Amapá, BA-Bahia, CE-Ceará, DF-Distrito Federal, ES-Espirito Santo, GO-Goiás, MA-Maranhão, MG-Minas Gerais, MS-Mato Grosso do Sul, MT-Mato Grosso, PA-Pará, PB-Paraíba, PE-Pernambuco, PR-Paraná, RJ-Rio de janeiro, RN-Rio Grande do Norte, RO-Rondônia, RR-Roraima, RS-Rio Grande do Sul, SC-Santa Catarina, SE-Sergipe, SP-São Paulo, TO-Tocantins

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Table 2. Number and frequency of seropositive dogs according serological assays, average and range of optical density by TRPs ELISA and titer range in IFA.

| Region | No. of Dogs | Number of positive dogs (frequency) (Optical Density average; range) | | | | IFA (frequency) (titer ranging) |
|---|---|---|---|---|---|---|
| | | TRP19 | USTRP36* | BrTRP36* | CRTRP36 | |
| North | 124 | 65 (52.4%) [a] (2.25; 0.42-3.77) | 38 (30.6%) [A b] (1.21; 0.33-3.58) | 45 (36.2%) [A a] (1.8; 0.33-3.8) | 0 | 58 (46.7%) [c b] (40 to 10,240) |
| Northeast | 235 | 114 (48.5%) [a] (2.05; 0.39-3.80) | 111 (47.2%) [A a] (1.48; 0.33-3.47) | 94 (40%) [A a] (2.06; 0.33-4.02) | 3 (1.27%) (1.6; 1.04-2.13) | 124 (52.7 %) [b] (40 to 10,240) |
| Center West | 131 | 75 (57.2%) [a] (2.22; 0.34-3.9) | 58 (44.2%) [A a] (1.63; 0.33-3.24) | 51 (38.9%) [A a] (1.82; 0.33-4.02) | 0 (0%) | 87 (66.4%) [a] (40 to 10,240) |
| Southeast | 123 | 62 (50.4%) [a] (2.01; 0.35-3.74) | 43 (34.9%) [A b] (1.45; 0.37-3.3) | 54 (43.9%) [A a] (1.72; 0.36-3.96) | 0 | 66 (53.6%) [b] (40 to 10,240) |
| South | 202 | 33 (16.3%) [b] (2.33; 0.37-3.95) | 27 (13.3%) [A c] (0.78; 0.33-2.51) | 22 (10.8%) [A b] (1.6; 0.33-3.65) | 2 (0.9%) (0.5; 0.35-0.66) | 40 (19.8%) [d] (40 to 10,240) |
| TOTAL | 815 | 349 (42.8) (2.17; 0.34-3.95) | 277 (33.9) [A] (1.51; 0.33-3.58) | 266 (32.6.3) [A] (1.8; 0.33-4.02) | 5 (0.61) (1.05; 0.35-2.13) | 375 (46) (40 to 10,240) |

* Same uppercase letter (A) on the same line indicates significantly similar values (P>0.05). Different lowercase letters (a, b, c, d) in a column indicates significantly different values (P<0.05).

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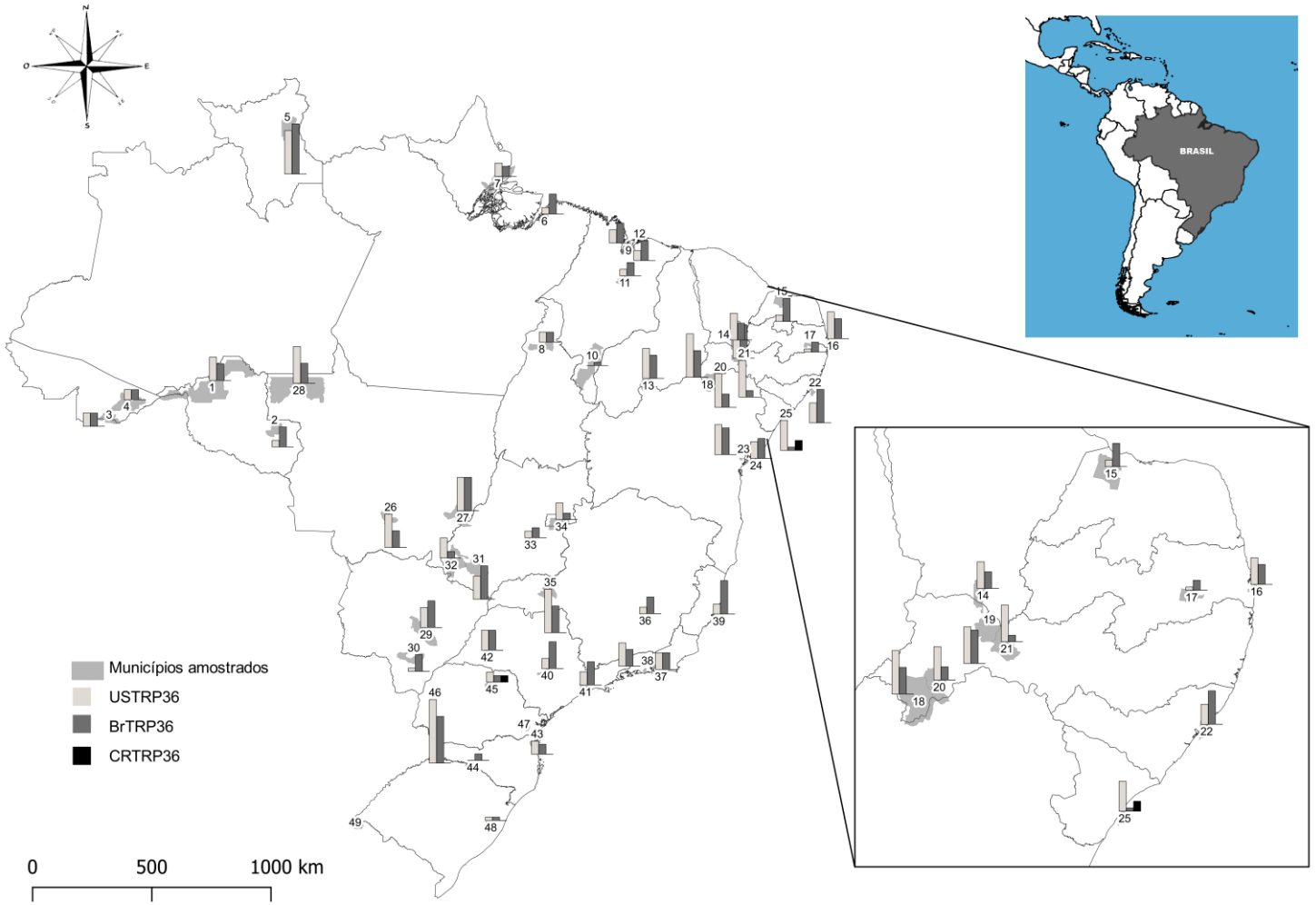


Figure 1- Distribution of *E. canis* genotypes in Brazil.

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## 4. Discussion

The present study involved a serological survey in dogs from all over Brazil, based on the detection of antibodies against antigens of *E. canis* TRPs proteins. In Brazil, with the exception to the southern states of Santa Catarina and Rio Grande do Sul [13], *Ehrlichia canis* infections in dogs are widespread around the country showing high prevalence rates and high IFA titers [14]. Our study is the first to assess the distribution of *E. canis* genotypes based on the detection of specific antibodies against *E. canis* TRP36 synthetic peptides in dogs suspected of CME from all over Brazil. Our findings indicated that the American and Brazilian genotypes are widely distributed throughout the country's various regions (Figure 1).

The presence of co-reaction between US and Br genotypes, suggestive of co-infection by the different TRP36 genotypes, was identified all the regions. Co-infection by different genotypes in the same host may lead to the development of genetic recombination of *E. canis*. This mechanism is commonly used by microorganisms as an immune evasion strategy and is also considered a major driver of genetic diversity in obligate intracellular microorganisms [15]. Examples of this phenomenon are reported the isolate Cuiabá # 16 of *E. canis* and several isolates of *E. ruminantium* [8,16]. The genetic recombination process can generate new strains of *E. canis* with different degrees of virulence, which may be fatal to dogs. Furthermore, new strains may be able to infect and adapt to new hosts, as probably occurred with *E. canis* and *E. minasensis* in bovines [17].

Unfortunately, our study was limited to testing dogs suspected of CME. Complete clinical data of the dogs in this study were not available for reasons of medical confidentiality. No clinical differences between US or Br genotypes were observed in a hospital population of dogs suffering from CME in an endemic area in Brazil [5], while clinical differences were observed in dogs infected by different genotypes in another study [18]. To date, the genetic diversity maybe related to the host-pathogen relationship. In this regard, a larger number of clinical cases of CME should be investigated in order to ensure more reliable conclusions about the clinical diseases and infections by different genotypes of *E. canis* in dogs.

Indeed, the fairly large number of co-infected dogs detected in Brazil supports the possibility of the emergence of new genotypes of *E. canis*. In our study, 24 (2.9%) dogs reactive only to TRP19 peptide suggests that these dogs may have been exposed to an as yet undescribed *E. canis* TRP36 genotype circulating in Brazil.

Despite the low detection of antibodies against the Costa Rica genotype in our study (n=5; 0.61%), this genotype may also have been a target of genetic recombination, since its amino acid sequence is similar to that of BrTRP36 [9,18]. In Brazil, this genotype was found pointwise in Aracajú – northeast region (three dogs) and Londrina – south region (two dogs). The genotype CRTRP36 was first detected in the blood of human donors in Costa Rica, exhibiting a potential zoonotic relationship [9]. However, data from South American serosurveys reveal evidence of infection by this genotype only in dogs from Colombia and Peru [11,19].

Our findings confirmed the low occurrence of seroreactive dogs in the south of Brazil. Similar data are reported but are based only on the IFA test [13]. In the present study, with the exception of samples from the city of Palotina PR, the other samples showed IFA results (~ 7.5%; 13/172 samples) similar to those reported [13]. However, 15% (2/13) of these samples presented seronegative results in all the ELISA assays using synthetic peptides of *E. canis*. This suggests that some of the reactions observed in dogs by IFA in the south are nonspecific, probably reflecting cross-reactions produced during antigenic stimulation after infections by closely related agents such as *A. platys* or unknown *Ehrlichia* species [20]. It should be noted that a relevant factor influencing the low prevalence of *E. canis* infection in the south of Brazil is the presence of the temperate strain of R. sanguineus ticks established in the region, which has low vector competence for the agent [21].

## 5. Conclusions

We conclude, from this study, that *E. canis* genotypes based on the TRP36 protein are distributed equally throughout Brazil. Comparisons between IFA and ELISA TRP19 reveal the occurrence of false positive reactions resulting from cross-reactions with other agents similar to *E. canis* in the indirect immunofluorescence reaction. Brazil's southern region has lower rates of seropositive dogs than other Brazilian regions. This paper reports for the first-time dogs seropositive for the *E. canis* Costa Rican genotype in Brazil.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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