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Serological survey on *Ehrlichia* sp. among dogs in the central region of Rio Grande do Sul

Pesquisa sorológica de *Ehrlichia* sp. em cães da região central do Rio Grande do Sul

Felipe da Silva Krawczak[1]*; Marcelo Bahia Labruna[1]; Luís Antônio Sangioni[2]; Fernanda Silveira Flores Vogel[2]; João Fabio Soares[1]; Sônia Terezinha dos Anjos Lopes[3]

[1]Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo – USP, São Paulo, SP, Brasil

[2]Departamento de Medicina Veterinária Preventiva, Centro de Ciências Rurais – CCR, Universidade Federal de Santa Maria – UFSM, Santa Maria, RS, Brasil

[3]Departamento de Clínica de Pequenos Animais, Centro de Ciências Rurais – CCR, Universidade Federal de Santa Maria – UFSM, Santa Maria, RS, Brasil

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Abstract

A serological survey on *Ehrlichia canis* was conducted among dogs in the central area of the state of Rio Grande do Sul, where the tick *Rhipicephalus sanguineus* is a common parasite of dogs. Out of a total of 316 dogs attended at the veterinary teaching hospital in the municipality of Santa Maria, only 14 (4.43%) reacted positively to *E. canis* antigens in the indirect immunofluorescence assay, with the following endpoint titers: 80 (three dogs), 160 (five), 320 (four), 640 (one) and 1280 (one). Like in previous studies in other regions of the state of Rio Grande do Sul, only a very small portion of the dogs in Santa Maria presented antibodies reactive to *E. canis,* even though canine infestations due to *R. sanguineus* are very common in this study region. These results contrast with other regions of Brazil, where *E. canis* is endemic among canine populations, with seropositivity values generally higher than 30%. Genetic differences among the *R. sanguineus* populations in South America might be implicated in these contrasting results.

Keywords: *Ehrlichia canis*, Rio Grande do Sul, serology, dogs.

Resumo

Foi realizada uma pesquisa sorológica para *Ehrlichia canis*, em cães, na região central do estado do Rio Grande do Sul, onde o carrapato *Rhipicephalus sanguineus* é um parasita comum em cães. De um total de 316 cães atendidos no Hospital Veterinário Universitário no Município de Santa Maria, somente 14 (4,43%) reagiram positivamente para o antígeno de *E. canis* pela reação de imunofluorescência indireta, com os seguintes títulos finais: 80 (3 cães), 160 (5), 320 (4), 640 (1) e 1.280 (1). Semelhante aos estudos anteriores em outras regiões do estado do Rio Grande do Sul, apenas uma pequena parcela dos cães de Santa Maria apresentaram anticorpos reativos para *E. canis*, mesmo que as infestações caninas por *R. sanguineus* sejam muito comuns na região de estudo. Esses resultados contrastam com outras regiões do Brasil, nas quais *E. canis* é endêmica entre a população canina, com valores de soropositividade geralmente superiores a 30%. Diferenças genéticas entre as populações de *R. sanguineus*, na América do Sul, poderiam estar envolvidas nesses resultados contrastantes.

Palavras-chave: *Ehrlichia canis,* Rio Grande do Sul, sorologia, cães.

Ehrlichia canis is the etiological agent of canine monocytic ehrlichiosis (CME), a tick-borne disease that affects domestic dogs around the world (YU et al., 2007). The agent is primarily transmitted by the nymphs and adults of the brown dog tick, *Rhipicephalus sanguineus* (VIEIRA et al., 2011). Since there is no transovarial (vertical) transmission of *E. canis* in ticks (GROVES et al., 1975), and because dogs usually develop long-lasting infection, dogs are incriminated as the primary reservoirs of *E. canis* in nature (HARRUS et al., 1998; WANER et al., 2001). A recent review study showed that *E. canis* generally occurs endemically in Brazil (VIEIRA et al., 2011). However, it is noteworthy that seropositivity to *E. canis* among different canine populations was generally greater than 30% in all Brazilian regions, except for the southern region, where the lowest frequency of seropositivity to *E. canis* in Brazil has been reported (VIEIRA et al., 2011). For example, 1.7% and 4.8% were reported in previous studies in the state capital and the southern region, respectively, of the state

*Corresponding author: Felipe da Silva Krawczak
Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo – USP, Av. Prof. Dr. Orlando Marques de Paiva, 87, Cidade Universitária, CEP 05508-270, São Paulo, SP, Brasil
e-mail: fkrawczak@yahoo.com.br

of Rio Grande do Sul (LABARTHE et al., 2003; SAITO et al., 2008). Even though much lower winter temperatures generally occur in southern Brazil, this different climatic condition does not preclude establishment of *R. sanguineus*, which is a very common parasite of dogs in Rio Grande do Sul, especially in urban areas (RIBEIRO et al., 1997; EVANS et al., 2000). Here, we determined the seropositivity to *E. canis* among dogs in the central area of the state of Rio Grande do Sul, where *R. sanguineus* is a common parasite of dogs and no serological survey study had previously been done.

Between March and June 2010, blood serum samples were collected from 316 dogs attended at the veterinary teaching hospital of the Federal University of Santa Maria, located in the municipality of Santa Maria (29° 41' S and 53° 48' W). All the dogs were owned by clients of the veterinary practice and were brought to the hospital for routine clinical procedures not necessarily related to vector-borne diseases. Canine serum samples were tested by means of the immunofluorescence assay (IFA) against crude antigens of the *E. canis* strain São Paulo (AGUIAR et al. 2008), prepared in a cell culture as previously described (AGUIAR et al., 2007). Reactions were performed with FITC-labeled anti-dog IgG (Sigma-Aldrich) that had previously been titrated to the best working dilution (1:800), as described previously (RISTIC et al., 1972; AGUIAR et al., 2007). Serum was considered to contain antibodies reactive to *E. canis* if it displayed a reaction at the 1:80 dilution. On each slide, a serum sample that had previously been shown to be nonreactive (negative control) and one that was known to be reactive (positive control; endpoint titer of 1280) were tested at the 1:80 dilution. These control serum samples were derived from the study by Aguiar et al. (2007). Samples that reacted at the screening dilution (1:80) were then titrated using serial two-fold dilutions to determine endpoint titers.

A total of 14 dog serum samples (4.43%) reacted positively to *E. canis* antigens using IFA, with the following endpoint titers: 80 (three dogs), 160 (five), 320 (four), 640 (one) and 1280 (one). Like in previous studies (LABARTHE et al., 2003; SAITO et al., 2008), we found that only a very small portion of the canine population of Santa Maria presented antibodies reactive to *E. canis,* even though canine infestations by *R. sanguineus* are very common in this study region (unpublished data).

In a recent genetic study on ticks from different populations of *R. sanguineus* in Latin America, Moraes-Filho et al. (2011) demonstrated that the taxon *R. sanguineus* is likely to be represented by at least two distinct species in Latin America: one restricted to southern South America (Chile, Argentina, Uruguay and the state of Rio Grande do Sul), and another encompassing the remaining areas of Latin America, from Mexico to Brazil (excluding Rio Grande do Sul). It is possible that this supposedly distinct *Rhipicephalus* tick species that occurs in Rio Grande do Sul, Chile, Argentina, and Uruguay is not competent to transmit *E. canis,* although this condition has yet to be confirmed experimentally. Interestingly, one study in Uruguay failed to detect *Ehrlichia* DNA in 180 *R. sanguineus* ticks (VENZAL et al., 2007). In addition, there has not been any molecular study to prove the existence of *E. canis* in ticks or dogs in Rio Grande do Sul, or in Chile, Uruguay and Argentina, where *R. sanguineus* is also the major tick species infesting domestic dogs (GONZÁLES-ACUÑA; GUGLIELMONE, 2005; GUGLIELMONE; NAVA, 2005; VENZAL et al., 2007). A similar scenario is found in Rio Grande do Sul (RIBEIRO et al., 1997; VIEIRA et al., 2011). Finally, because serologically based studies are not species-specific (WANER et al., 2001), it is also possible that the few serologically positive dogs in the present study were infected by another *Ehrlichia* species, or even by an agent belonging to another genus of Anaplasmataceae that is yet to be identified in southern Brazil. In fact, the low endpoint titers presented by the dogs are compatible with cross-reactions.

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