Original article

# Seroprevalence and risk factors to *Ehrlichia* spp. and *Rickettsia* spp. in dogs from the Pantanal Region of Mato Grosso State, Brazil

Andréia L.T. Melo[a], Thiago F. Martins[b], Maurício C. Horta[c], Jonas Moraes-Filho[b], Richard C. Pacheco[a], Marcelo B. Labruna[b], Daniel M. Aguiar[a,*]

[a] *Faculdade de Agronomia, Medicina Veterinária e Zootecnia da Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil*
[b] *Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, São Paulo, SP, Brazil*
[c] *Curso de Medicina Veterinária da Universidade Federal do Vale do São Francisco, Petrolina, PE, Brazil*

## ARTICLE INFO

## ABSTRACT

Sera of 320 dogs from urban and rural areas of a Pantanal region of Brazil were evaluated for rickettsial (*Rickettsia rickettsii*, *R. parkeri*, *R. amblyommii*, *R. rhipicephali*, *R. felis*, and *R. bellii*) and ehrlichial (*Ehrlichia canis*) infection by the immunofluorescence assay (IFA). Risk factors for ehrlichiosis or rickettsiosis were also evaluated. Positive reaction against *Ehrlichia* spp. was detected in 227 (70.9%) dogs, 119 (74.3%) from an urban area and 108 (67.5%) from rural areas ($P > 0.05$). For *Rickettsia* spp., 152 (47.5%) dogs were positive, 31 (19.3%) from urban and 121 (75.6%) from rural areas ($P < 0.05$). Highest anti-*Rickettsia* spp. endpoint titers were observed for *R. amblyommii*, suggesting homologous reactions to this agent or a very closely related organism. While most of the urban dogs were found parasitized by the tick *Rhipicephalus sanguineus*, infestations on rural dogs were predominated by *Amblyomma cajennense*. Rickettsial infection was significantly higher ($P < 0.05$) in rural dogs, in dogs with hunting practice, or in *A. cajennense*-infested dogs.

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## Introduction

The Pantanal biome is the largest tropical wetland area of the world. Situated among 2 Brazilian states (Mato Grosso and Mato Grosso do Sul) and in portions of Bolivia and Paraguay, the Pantanal ecosystem is characterized by an immense diversity, where more than 80 species of mammals, among Canidae, Felidae, rodents, Tayassuidae, Cervidae, and others, share the same habitat (Alho et al., 1987) contributing to the maintenance of different tick species (Ito et al., 1998; Bechara et al., 2000; Martins et al., 2004; Labruna et al., 2005a; Cançado et al., 2009).

Infections by bacteria of the order Rickettsiales have been reported in other parts of Brazil (Labarthe et al., 2003; Aguiar et al., 2007; Labruna et al., 2007; Pinter et al., 2008; Saito et al., 2008; Souza et al., 2010). Regarding the Pantanal biome, to our knowledge there is only a single study that reported ehrlichial and rickettsial infections in free-living jaguars (*Panthera onca*) and their ticks in the southern part of the Pantanal, in the state of Mato Grosso do Sul (Widmer et al., 2011). Ticks infesting domestic dogs in Brazil are mostly *Rhipicephalus sanguineus* in the urban areas and a few

rural areas and different *Amblyomma* species in the rural areas (Labruna and Pereira, 2001; Labruna et al., 2005b). *R. sanguineus* is a recognized vector of *Ehrlichia canis*, the etiological agent of canine monocytic ehrlichiosis (CME) in many parts of Brazil (Vieira et al., 2011), whereas *Amblyomma* ticks (mostly *A. cajennense* and *A. aureolatum*) are recognized vectors of *Rickettsia rickettsii,* the etiological agent of Brazilian spotted fever (BSF) in southeastern Brazil (Labruna, 2009). In addition, *A. triste* and *A. ovale* are possible vectors of 2 different strains of *Rickettsia parkeri* in Brazil, where this rickettsial agent is an emerging human pathogen (Silveira et al., 2007; Spolidorio et al., 2010). Other *Rickettsia* species that have been reported infecting ticks in Brazil, namely *R. amblyommii*, *R. rhipicephali*, and *R. bellii*, are still regarded as potential pathogens or of unknown pathogenicity for humans (Labruna, 2009). Finally, the flea-associated *Rickettsia felis* agent is a recognized human pathogen in Brazil (Pérez-Osorio et al., 2008).

Considering that dogs are excellent sentinels to rickettsial infections that affect humans (Pinter et al., 2008; Sangioni et al., 2005; Demma et al., 2006; Horta et al., 2007; McQuiston et al., 2009), the present study aimed to determine for the first time in the Pantanal biome, the seroprevalence of *Ehrlichia* spp. and *Rickettsia* spp. in domestic dogs, and to determine possible epidemiological aspects of rickettsiosis. Furthermore, the present study also investigated the species of ectoparasites parasitizing domestic dogs in urban and rural areas of Poconé municipality, within the northern part of the Pantanal, in the state of Mato Grosso, Brazil.

* Corresponding author at: Faculdade de Agronomia, Medicina Veterinária e Zootecnia da Universidade Federal de Mato Grosso, Av. Fernando Corrêa, 2367, Boa Esperança, Cuiabá, MT 78060-900, Brazil. Tel.: +55 65 3615 8662.
*E-mail address:* danmoura@ufmt.br (D.M. Aguiar).

## Materials and methods

### Study area

The municipality of Poconé (56°37′S, 16°15′W) is located in the northeastern region of the Pantanal area, 100 km southwest of Cuiabá City, the Mato Grosso State capital, neighbored by the municipalities of Cáceres (west), Nossa Senhora do Livramento (north), Barão de Melgaço (east), and the state of Mato Grosso do Sul at the southern border (Fig. 1). Poconé has an estimated population of 32,162 inhabitants and 3111 dogs within a total area of 17,261 km², where 81% (~14,000 km²) are annually flooded during the rainy season (IBGE, 2010). This area is at an altitude of 142 m above sea level; the weather is hot and rainy in the spring–summer months, when mean temperatures are around 32 °C, and cool and dry in the autumn–winter period, when mean temperatures are around 21 °C. Mean annual rainfall ranges from 1000 to 1400 mm. The rainy season is between October and May. The Pantanal environment consists of 3 distinct regions: amazon, savannah, and chaco; the vegetation is not homogeneous, ranging according to altitude: In lower areas, grasses are predominant, in intermediate altitudes, the savannah is predominant, and in higher altitudes, the vegetation becomes arid and dry similarly to the Caatinga biome (Fernandes et al., 2010).

### Sampling procedures

Dogs were sampled during July to September 2009. This period of the year was selected because of better road conditions to access farms by car and also because it coincides with the period of highest activity of *A. cajennense* nymphs in Brazil (Labruna et al., 2002). A representative sample to be evaluated was determined to be 160 dogs, considering a 50% estimated prevalence, 10% absolute precision, and 99% confidence interval, using EpiInfo hardware version 6.0. For comparison purposes, 160 dogs were sampled in the urban area and 160 dogs in rural areas. In the urban area, dogs from all neighborhoods were sampled. In the rural areas, dogs from 25 farms and 3 rural communities were sampled. While 16 farms (F1 to F16) and all communities (C1 to C3) were accessed by federal or state roads (BR-070, MT-060, MT-360, MT-451), further 9 farms (F17 to F25) were accessed by boat through the Cuiabá River (in the borderline of Poconé and Barão de Melgaço municipalities). In all localities, geographic coordinates were completed by eTREX Vista HCx GPS (Garmin™, Kansas, USA). Farms F7, F8, F21, and community C2 were officially located outside Poconé Municipality, within neighboring municipalities, as shown in Fig. 1.

Blood samples were collected from dogs by jugular venipuncture to obtain serum. At the same time, dogs were evaluated by visual inspection and palpation for the presence of ectoparasites.
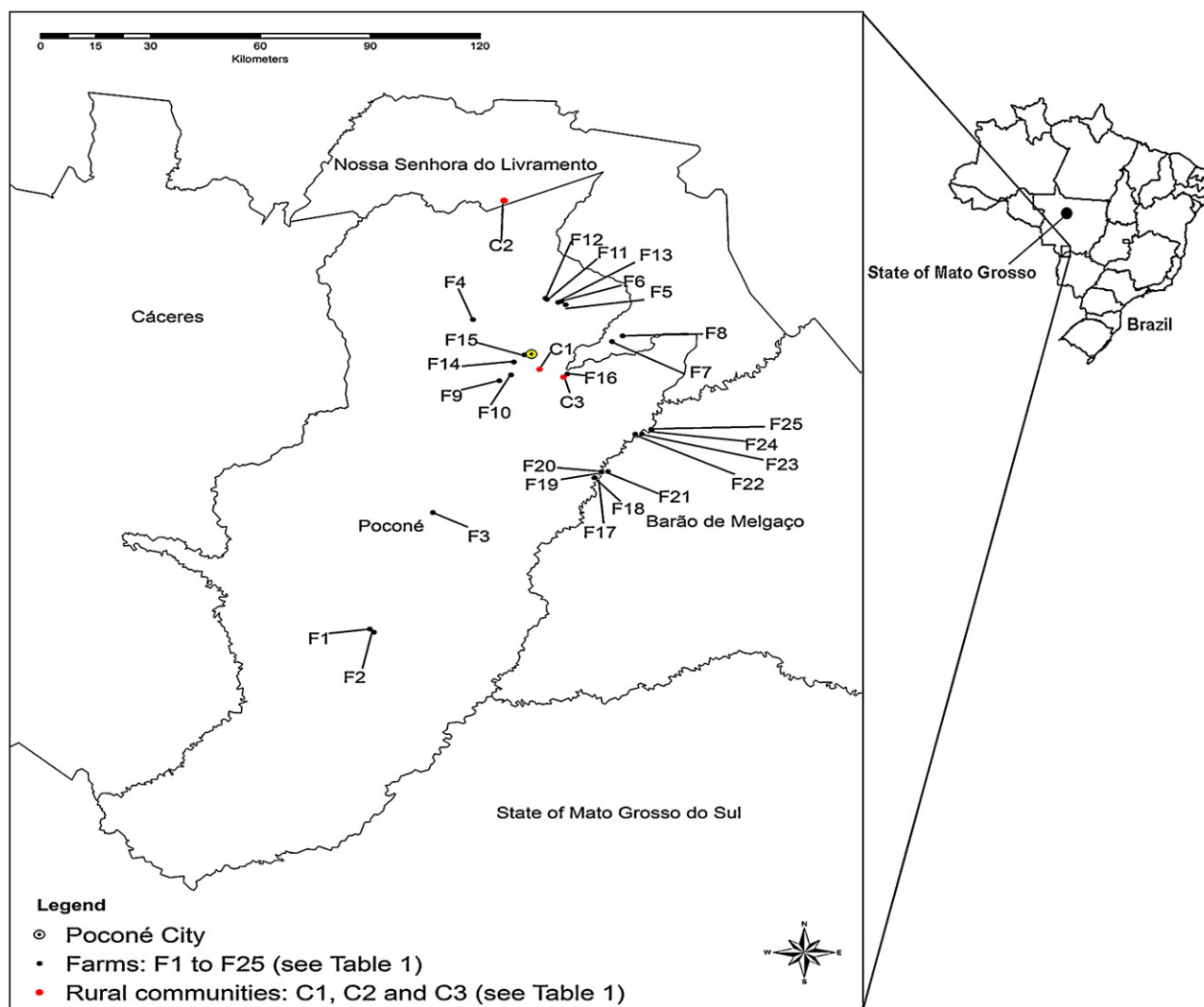


**Fig. 1.** The Poconé municipality and neighboring municipalities of Mato Grosso State, Brazil, indicating the urban area (Poconé city), 25 farms, and 3 rural communities where dogs were sampled in the present study.