

Original article

Rickettsial infection in ticks (Acari: Ixodidae) of wild animals in midwestern Brazil



Rute Witter^a, Thiago F. Martins^b, Artur K. Campos^c, Andréia L.T. Melo^a, Sandra H.R. Corrêa^a, Thaís O. Morgado^a, Rafael W. Wolf^a, Joares A. May-Júnior^d, Afonso L. Sinkoc^a, Christine Strüssmann^e, Daniel M. Aguiar^a, Rogério V. Rossi^f, Thiago B.F. Semedo^g, Zilca Campos^h, Arnaud L.J. Desbiezⁱ, Marcelo B. Labruna^b, Richard C. Pacheco^{a,e,*}

^a Programa de Pós Graduação em Ciências Veterinárias, Faculdade de Agronomia, Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil

^b Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, SP, Brazil

^c Departamento de Veterinária, Universidade Federal de Viçosa, Viçosa, MG, Brazil

^d Universidade do Sul de Santa Catarina, Tubarão, SC, Brazil

^e Departamento de Ciências Básicas e Produção Animal, Faculdade de Agronomia, Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil

^f Departamento de Biologia e Zoologia, Instituto de Biociências, Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil

^g Programa de Pós Graduação em Zoologia, Instituto de Biociências, Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil

^h Embrapa Pantanal, CP 109, CEP 79320-900 Corumbá, MS, Brazil

ⁱ Royal Zoological Society of Scotland, Murrayfield, Edinburgh EH12 6TS, Scotland, United Kingdom

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ABSTRACT

Ticks collected in the last two decades from free-living and captive wild animals from 28 municipalities of the Brazilian state of Mato Grosso were identified and tested using molecular methods for the presence of rickettsial agents. A total of 4467 ticks (229 larvae, 1676 nymphs, 1565 males, 997 females) representing 27 ixodid species were collected from 235 species of amphibians, reptiles, birds, and mammals from three different ecoregions (Pantanal, Cerrado, and Amazonia). The species *Amblyomma parkeri*, *Amblyomma romitii*, *Amblyomma varium* and *Ixodes luciae* are reported for the first time in the state of Mato Grosso. Amongst 538 ticks tested by molecular methods for rickettsial infection, we detected 'Candidatus Rickettsia amblyommii' infecting *Amblyomma cajennense sensu stricto* and *Amblyomma coelebs*, *Rickettsia* sp. strain Atlantic rainforest infecting *Amblyomma ovale*, *Rickettsia* sp. strain NOD infecting *Amblyomma nodosum*, and 'Candidatus Rickettsia andeanae' infecting *Amblyomma sculptum*. Our results represent an impressive expansion of knowledge on tick fauna and rickettsiae and are essential for understanding the ecology of ticks and tick-borne diseases in the Neotropical region, particularly in midwestern Brazil.

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1. Introduction

Ticks are classified in the subclass Acari and are highly specialized, bloodsucking, nonpermanent ectoparasitic arthropods that feed on mammals, birds, reptiles, and amphibians worldwide (Anderson and Magnarelli, 2008). During recent years, there has been a growing number of reports of tick parasitism on wild animals in different ecoregions of Brazil (Horta et al., 2011; Saraiva et al.,

2012; Spolidorio et al., 2012; Martins et al., 2014; Soares et al., 2015; for ecoregions definition and delimitation see Olson et al., 2001). However, many aspects on the taxonomy, biology, ecology, geographical distribution, natural hosts, and capacity of transmitting biological agents are still poorly known for tick species parasitizing wildlife (Barros-Battesti et al., 2006).

Ticks are the most important vectors of infectious diseases of animals and are second only to mosquitoes as vectors of human diseases, transmitting viruses, bacteria, protozoa and helminths (Nava et al., 2009). The most important zoonotic disease transmitted by ticks in Brazil is Brazilian Spotted Fever (BSF), caused by *Rickettsia rickettsii* (Labruna, 2009). Bacteria of the genus *Rickettsia* (Rickettsiales: Rickettsiaceae) are obligate intracellular, Gram-negative

* Corresponding author at: Departamento de Ciências Básicas e Produção Animal, Av. Fernando Corrêa da Costa, 2367, Boa Esperança, 78060-900 Cuiabá, MT, Brazil. E-mail address: richard@ufmt.br (R.C. Pacheco).

organisms that infect invertebrate hosts worldwide, measuring 0.8–2.0 μm in length and 0.3–0.5 μm in width (Dumler et al., 2001). Currently valid species have been classified into four phylogenetic groups, namely the spotted fever group (SFG), the typhus group (TG), the *Rickettsia bellii* group, and the *Rickettsia canadensis* group (Parola et al., 2013).

In Brazil, SFG rickettsiae, which are chiefly associated with ticks, may cause mild to severe human infectious disease (Szabó et al., 2013a). Until 2000 only one SFG rickettsia, *R. rickettsii*, was known to be endemic in the country, but during the last 12 years this number has risen to six with the inclusion of *Rickettsia parkeri*, *Rickettsia rhipicephali*, *Rickettsia felis*, ‘*Candidatus Rickettsia amblyommii*’, and ‘*Candidatus Rickettsia andeanae*’ (Labruna et al., 2011; Nieri-Bastos et al., 2014).

With nearly one thousand square kilometers, the state of Mato Grosso is located in midwestern Brazil, encompassing biotic elements from Amazonia, Cerrado, and Pantanal ecoregions. Forests from southern Amazonia correspond to nearly 53% of the state and are located in its northern portion. The Cerrado – a huge tropical savannah that extends across the Brazilian Central Plateau – occupies nearly 40% of the state, in its central portion. The Pantanal – the largest tropical wetland in the world – occupies another 7% of the state area, in its southwestern portion (SEMA, 2014).

Knowledge of tick parasitism of wildlife is very useful when analyzing tick ecology and distribution, host relationships, and aspects related to tick-borne diseases (Barros-Battesti et al., 2006), such as vector species and potential foci of infection. In this report, we provide information on tick species infesting free-living and captive wild animals (anuran amphibians, reptiles, birds, and mammals) in 28 municipalities in the state of Mato Grosso (Fig. 1). We also provide results of a molecular screening for rickettsial agents in a subsample of the collected ticks.

2. Materials and methods

Ticks were collected between 1995 and 2015 from free-range and captive wild animals from 28 municipalities within the Amazonia, Cerrado, and Pantanal ecoregions in the state of Mato Grosso (Fig. 1). Samples were collected during clinical care and examination of wild animals at the Veterinary Hospital and at the Zoo of the Federal University of Mato Grosso, as well as from road-killed animals. Collected ticks were preserved in absolute isopropanol and sent to the laboratory for taxonomic identification following Barros-Battesti et al. (2006), Martins et al. (2010), and Nava et al. (2014). Some engorged ticks (nymphs) were kept alive in the laboratory in dry plastic tubes in an incubator at 25 °C and RH 90%, according to Labruna et al. (2002), in order to molt to the adult stage. Nymphs that died in the laboratory before reaching the adult stage were preserved in absolute isopropyl alcohol. Some of the larvae-positive for rickettsial infection after a polymerase chain reaction (PCR) – were identified to species level through molecular analysis (see below). Voucher tick specimens have been deposited at the following Brazilian tick collections: “Coleção Nacional de Carrapatos” (University of São Paulo, São Paulo, Brazil) and “Coleção de Carrapatos da Universidade Federal de Mato Grosso” (Cuiabá, Mato Grosso, Brazil).

After taxonomic identification, random samples of different species were subjected to DNA extraction by the guanidinium thiocyanate protocol, as described by Sangioni et al. (2005). Adult ticks were processed individually, while immature ticks were tested in pools of five nymphs or 10 larvae. Extracted DNA samples were tested by PCR, firstly using the CS-78 and CS-323 primers, targeting a 399 to 401-bp fragment of the citrate synthase gene (*gltA*) of presumably all *Rickettsia* species (Labruna et al., 2004a). Samples positive in this PCR protocol were further tested by another

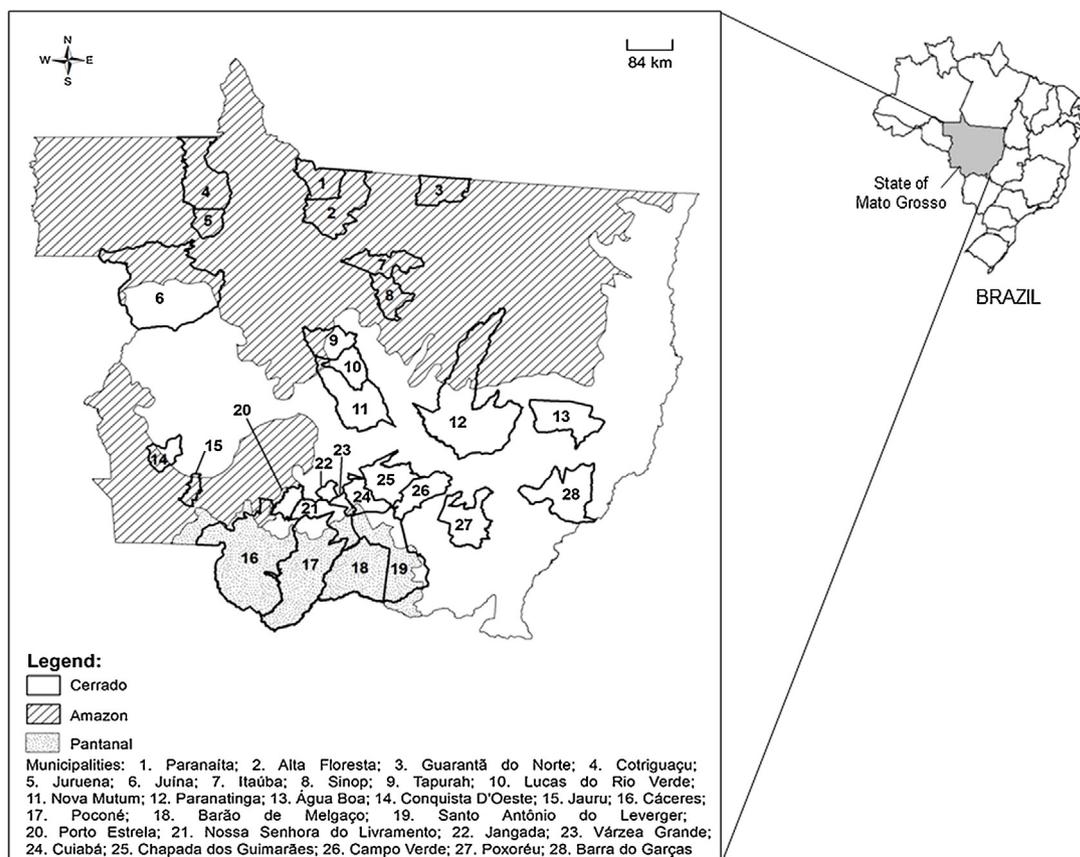


Fig. 1. The Brazilian state of Mato Grosso and its municipalities (1–28) where ticks of the present study were collected.

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