

A survey for rickettsial agents on *Rhipicephalus sanguineus* (Ixodida, Ixodidae) ticks in Northeastern Brazil

Inquérito para agentes rickettsiais em carrapatos *Rhipicephalus sanguineus* (Ixodida, Ixodidae) no Nordeste do Brasil

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Abstract

In this study, rickettsial infection was searched in 108 canine blood samples and 22 *Rhipicephalus sanguineus* (Ixodida, Ixodidae) ticks collected on these dogs during 2011 and 2012 in Patos municipality, state of Paraíba, northeastern Brazil. Blood samples were tested through indirect immunofluorescence assay (IFA) by using antigens of six *Rickettsia* species isolates from Brazil. All 108 dogs tested seronegative for *R. rickettsii*, *R. parkeri*, *R. amblyommii*, *R. felis*, *R. rhipicephali*, and *R. bellii* antigens, suggesting a non-endemic status of the studied region for spotted fever rickettsiosis. Among 22 *R. sanguineus* ticks, *R. felis* was detected in one (4.5%) specimen by PCR targeting a portion of the rickettsial *gltA* gene. The possible implications of this unusual PCR finding are discussed.

Keywords: Rickettsiosis. Paraíba State. Brazil.

Resumo

No presente trabalho, foi pesquisada infecção riquetsial em amostras de sangue de 108 cães e 22 carrapatos *Rhipicephalus sanguineus* (Ixodida, Ixodidae) coletados destes animais durante 2011 e 2012, em Patos, Estado da Paraíba, Nordeste do Brasil. As amostras de sangue foram examinadas pela reação de imunofluorescência indireta (RIFI) utilizando-se antígenos de seis isolados de *Rickettsia* do Brasil. Todos os 108 cães foram soronegativos para os antígenos de *R. rickettsii*, *R. parkeri*, *R. amblyommii*, *R. felis*, *R. rhipicephali* e *R. bellii*, sugerindo que a região estudada não é endêmica para riquetsioses do grupo da febre maculosa. Dos 22 carrapatos *R. sanguineus*, *R. felis* foi detectada em um (4,5%) espécime por PCR do gene riquetsial *gltA*. São discutidas as possíveis implicações desse achado incomum na PCR.

Palavras-chave: Riquetsiose. Estado da Paraíba. Brasil.

In Brazil, four *Rickettsia* species have been associated with human diseases, namely, Brazilian spotted fever caused by *Rickettsia rickettsii*, murine typhus caused by *Rickettsia typhi*, Atlantic rainforest spotted fever caused by *Rickettsia parkeri*, and finally flea-borne spotted fever caused by *Rickettsia felis* (RAOULT et al., 2001; GALVÃO et al., 2003; SILVA; PAPAORDANOU, 2004; SPOLIDORIO et al., 2010). Reports of these agents have been restricted to southeastern Brazil and the southern portion of the state of Bahia, close to the southeastern region (LABRUNA et al., 2011). Herein, it was performed a search for rickettsial infection in dogs and their ticks

in an area of northeastern Brazil where no *Rickettsia* infection has ever been reported.

This study was performed in Patos municipality (7°1'S, 37°19'W), state of Paraíba. Patos is within a semiarid region, within the Caatinga biome, a xeric

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shrubland and thorn forest (ANDRADE-LIMA, 1981). The weather is characterized by a hot and semiarid climate, with temperatures averaging 27°C, and the mean annual rainfall is typically ≈500 mm. (BATISTA et al., 2007). During 2011 and 2012, a total of 108 blood samples and 22 ticks were collected from dogs from the county of Patos attended in the Veterinary Hospital of the Federal University of Campina Grande in Patos. Approximately 4 mL of blood was collected from each dog by venopunction of the cephalic or jugular veins. Sera was obtained and stored in sterile 1.5 mL tubes at -20°C for until serological tests. Tick specimens were removed and stored at -20°C after being taxonomically identified as adults of *R. sanguineus* on the basis of morphologic characters (SONENSHINE, 1991).

Serologic diagnosis was performed by indirect immunofluorescence assay (IFA) by using antigens of six *Rickettsia* isolates from Brazil: *R. rickettsii* strain *Taiacu* (PINTER; LABRUNA, 2006), *R. parkeri* strain *At24* (SILVEIRA et al., 2007), *R. amblyommii* strain *Ac37* (LABRUNA et al., 2004a), *R. felis* strain *Pedreira* (HORTA et al., 2006), *R. rhipicephali* strain *HJ#5* (LABRUNA et al., 2007), and *R. bellii* strain *Mogi* (PINTER; LABRUNA, 2006).

DNA extraction was performed using the guanidine thiocyanate protocol, as previously described (SANGIONI et al., 2005). Five microliters of extracted DNA of the each tick were used for amplification of a 401-bp fragment of the rickettsial *gltA* gene (citrate synthase gene) by a routine PCR with primers CS-78 (forward) and CS-323 (reverse) (LABRUNA et al., 2004b). PCRs (25 µL) were performed in an Applied Biosystems Thermocycler (Gene Amp PCR System 2700, Life Technologies do Brasil Ltda, São Paulo, SP) by adding 5 µL of the DNA template to 12.5 µL of the PCR iQSupermix (PCR SperMix, Life Technologies do Brasil Ltda, São Paulo, SP), 1.0 µL of each primer at 20 µM, and 5.5 µL of molecular-grade water. For each reaction, a negative control (water) and a positive control (*R. parkeri* DNA) were

included. Ten microliters of the PCR product were separated by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and examined by UV transillumination. PCR product was purified and sequenced (HORTA et al., 2007). Partial sequence was subjected to BLAST analysis to determine similarities to other *Rickettsia* species (ALTSCHUL et al., 1990). All DNA samples were further tested by a Taqman real-time PCR that has shown to successfully amplify a 150-bp fragment of *R. felis* and many other *Rickettsia* species (LABRUNA et al., 2004b).

All 108 dogs were seronegative for *R. rickettsii*, *R. parkeri*, *R. amblyommii*, *R. felis*, *R. rhipicephali*, and *R. bellii* antigens. In total, one *R. sanguineus* tick (4.5%) yielded expected band by PCR, which was demonstrated by DNA sequencing to be 100% (350/350 pb) identical to the corresponding sequence of the *gltA* gene of *R. felis* deposited in GenBank (CP000053). Only the sample previously positive by routine PCR was positive by real-time PCR.

In the present study, *R. sanguineus* was the only tick species found on the dogs. Interestingly, none of the known tick vectors of spotted fever agents in Brazil, namely *Amblyomma cajennense* (SOARES et al., 2012), *A. aureolatum* (PINTER; LABRUNA, 2006), and *A. ovale* (SZABÓ et al., 2013), have been reported in the study region (GUGLIELMONE et al., 2003; ESTRADA-PENÁ; GUGLIELMONE; MANGOLD, 2004). In addition, *R. sanguineus* was the only tick species previously reported to infest wild Canidae in the semi-arid region of Paraíba (LABRUNA et al., 2011). The fact that all dogs were seronegative to rickettsial antigens in the present study, plus the scarcity or absence of *Amblyomma* ticks in the study region, suggested that Patos municipality is currently non-endemic for tick-borne rickettsioses. Environmental factors, such as the semiarid climate and the Caatinga typical xeric forest could be associated with the absence of tick vectors of the genus *Amblyomma* in the study region. Therefore, other areas with similar characteristics to Patos in

northeastern Brazil are likely to have the same status for tick-borne rickettsioses.

Flea-borne rickettsiosis, caused by *Rickettsia felis*, is an emerging disease that has been reported worldwide, including in southeastern Brazil (RAOULT et al., 2001). It was first detected by Adams, Schmidtman and Azad (1990) in the midgut cells of a cat flea (*Ctenocephalides felis*), and later described as a new *Rickettsia* species (BOUYER et al., 2001). Cases of human infection have been reported worldwide with dengue fever-like symptoms including fever, headache, myalgia, and macular rash (PEREZ-OSORIO et al., 2008).

It was detected DNA of *R. felis* in one of the ticks feeding on dogs. This rickettsial agent is primarily associated with fleas of the genus *Ctenocephalides*, which are among the most common ectoparasites of dogs in Brazil, including the northeastern region (DANTAS-TORRES et al., 2009). Since *Ctenocephalides* fleas have been reported to be infected by *R. felis* worldwide (PEREZ-OSORIO et al., 2008), it is likely that *R. felis*-infected fleas were the source of infection of *R. felis* for *R. sanguineus* while feeding on dogs, although the mechanisms for such horizontal transmission remain unknown. Interestingly, dogs appear to be refractory to *R. felis* infection because several studies failed to detect *R. felis*-reactive antibodies in dogs living under natural infestations by *R. felis*-infected fleas (CARDOSO et al., 2006; HORTA et al., 2007; PINTER et al., 2008).

There have been two previous reports of *R. felis* detection in *R. sanguineus* ticks in Brazil, both in

regions endemic for Brazilian spotted fever in southeastern Brazil (CARDOSO et al., 2006; OLIVEIRA et al., 2008). The present study reports the first detection of a *Rickettsia* species in northeastern Brazil (excluding southern Bahia, which is in the southern limit of northeastern Brazil). In the previous studies (CARDOSO et al., 2006; OLIVEIRA et al., 2008), *R. felis* was detected in ticks only by nested-PCR targeting a small fragment of the rickettsial *htrA* gene. Herein, it was detected *R. felis* by a routine PCR and a Taqman real-time PCR, both targeting the rickettsial *gltA* gene. While Cardoso et al. (2006) and Oliveira et al. (2008) failed to amplify other gene fragments of *R. felis* from their tick samples, we also failed to amplify other rickettsial genes (i.e., *ompA*, *htrA*) by routine PCR that was run on the only *gltA*-PCR positive tick (data not shown). These inconsistencies should be related to low rickettsial load in *R. felis*-infected *R. sanguineus*, resulting in PCR negative results while using other PCR protocols that amplify larger fragments; therefore, less sensitive (BUSTIN 2000). At the same time, this result suggests that *R. felis* is not able to cause a disseminate infection in ticks, a condition yet to be further evaluated. Until the real capacity of *R. felis* to infect ticks is not proven by other methods, fleas remain as the only suspected vector of *R. felis* in Brazil.

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