

First molecular evidence of *Rickettsia* spp. in human blood samples, with clinical and epidemiological profile of tick-borne disease in the Mato Grosso do Sul state

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Resumo

Patient 44 years, male, farm worker, non-drinker and non-smoker, born and residing in MS, previously healthy, was admitted for investigation of fever (38.5 to 40°) recurrent for 1 year, associated with confusion, diplopia, confused speech (“dragged”), axial and appendicular ataxia, imbalance and difficulty while walking with “dance of the tendons”; generalized tonic-clonic seizures, dysphagia transmission (including liquid), asymmetric migratory arthritis of large joints (mainly left shoulder and right knee) and later small joints (proximal interphalangeal and wrists). By verification of positive epidemiology for tick borne disease, associated with the patient’s report of contact and constant tick-bite, was suggested diagnosis of Lyme disease-like. The patient was treated, in home care, with ceftriaxone 1g 12/12 h for 30 days and then maintained with doxycycline 100 mg 12/12h, with clinical remission. As the patient had complete remission with this treatment, he stopped using the drug, and after stopped developed recurrent disease with neurological and articulate signs. It was por-

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trayed with the same scheme, with full recovery and remains asymptomatic and with doxycycline use. Later it was verified that the Lyme-like serology was negative for antibodies against *Borrelia* spp. Having new clinical recurrence, after discontinuation of doxycycline, we send samples for PCR of *Borrelia* spp., *Babesia* spp. and *Rickettsia* spp. In laboratory, the blood sample was subjected to DNA extraction, protocol using a combination of guanidine isothiocyanate and phenol. The extraction buffer was prepared the day before the procedure, adding one volume of phenol in an amount of guanidine isothiocyanate (6 M) and incubated at 4°C overnight. The DNA was incubated overnight for rehydration. The samples were then quantified GeneQuant™ spectrophotometer (Pharmacia) and the concentration of total DNA from each sample were adjusted to 200 ng μL^{-1} . The PCR was utilized for detection of *Rickettsia* spp., using the primers CS 78F (GCA-AGTATCGGTGAGGATGTAAT) and CS 323R (GCTTCCTTAAAATTCA-ATAAATCAGGAT) which target a partial sequence synthase citrate gene (*gltA*), delimiting a 401-bp fragment. The sequence of this gene is relatively well conserved in all *Rickettsia* spp. Positive sample was subjected to second PCR with primers Rr 190.70p (ATGGCGAATATTCTCCAAAA) and Rr 190.602n (AGTGCAGCATTGCTCCCCCT) that amplified a fragment of 732 bp of the *ompA* gene of *Rickettsia* spp.

Keywords: Tick borne diseases, *Borrelia* spp., *Rickettsia* spp., rickettsiosis.

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