

Short Communication

Seropositivity for *Rickettsia* spp. and *Ehrlichia* spp. in the human population of Mato Grosso, Central-Western Brazil

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Abstract

Introduction: The epidemiology of *Rickettsia* and *Ehrlichia* species infection is underestimated in Mato Grosso State. **Methods:** Serum samples obtained during a Dengue outbreak in 2011-2012 were tested via indirect immunofluorescence and/or ELISA. **Results:** Samples from 19/506 (3.8%) patients presented antibodies for at least one of three *Rickettsia* species; 2/506 (0.4%) samples reacted against *Ehrlichia canis*. Most afflicted patients are residents of cities from the south-central region of the state, where these diseases have been reported in animals. **Conclusions:** These results show serological evidence of human exposure to *Rickettsia* and *Ehrlichia* species in Mato Grosso State.

Keywords: Rickettsiosis. Ehrlichiosis. Immunofluorescence assay.

Rickettsiosis and ehrlichiosis are tick-borne emerging zoonosis with worldwide distribution. These diseases are transmitted to animals and humans by infected arthropods, mainly ticks of the family Ixodidae. Patients with these infections may present with clinical symptoms that can lead to high hospitalization and lethality rates depending on the pathogen^{1,2}.

Both rickettsiosis and ehrlichiosis are caused by aerobic α -proteobacteria, obligate intracellular pleomorphic Gram-negative coccobacilli that multiply through binary fission. Species of the genus *Ehrlichia* are members of the family Anaplasmataceae, order Rickettsiales. The same order includes the family Rickettsiaceae, to which the genus *Rickettsia* belongs. *Rickettsia* (*R.*) species have been allocated into the following phylogenetic groups: spotted fever group (SFG), typhus group, and the *Rickettsia bellii* and *Rickettsia canadensis* groups^{1,2}.

Ehrlichia (*E.*) *chaffeensis* and *Ehrlichia canis* species have been implicated in the etiology of human monocytic ehrlichiosis (HME). In Brazil and other South American countries, cases of ehrlichiosis in humans have already been serologically diagnosed; however, the species associated with these diseases have not been identified even though *E. canis* was amplified

from serum samples of patients in Venezuela who exhibited clinical signs of HME^{3,4}.

Currently, several species belonging to the SFG are endemic in Brazil, including *Rickettsia rickettsii*, the etiological agent of Brazilian spotted fever (BSF). *R. rickettsii* was the only species of *Rickettsia* transmitted by ticks that was thought to infect humans in South America until 2000. Since then, *Rickettsia parkeri*, *Rickettsia rhipicephali*, *Rickettsia felis*, '*Candidatus Rickettsia amblyommii*' and '*Candidatus Rickettsia andeanae*' have been reported as well⁵. Furthermore, *Rickettsia* sp. strain Atlantic rainforest (*Rickettsia parkeri*-like), was found to be a new etiological agent for spotted fever in the country⁶.

Rickettsiosis and ehrlichiosis acute clinical phase is characterized by non-specific symptoms. These diseases are more frequently reported as fever associated with myalgia and headache. The misdiagnosis of these diseases increases the risk of severity and lethality owing to the lack of adequate treatment. This situation is exasperated when the symptoms are initially suspected of being other endemic diseases, such as dengue or leptospirosis^{1,2}.

Studies conducted in Mato Grosso (MT) revealed evidence of rickettsial and ehrlichial infections in animals and ticks^{7,8}. However, there have been no investigations of these bacteria in humans. For this reason, the present study aimed to investigate the presence of anti-*Rickettsia* spp. and anti-*Ehrlichia* spp. antibodies among the residents of MT.

To achieve this, serum samples collected from patients presenting with febrile illnesses for less than five days between

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October 2011 and July 2012 in 19 cities in MT, and who were clinically suspected of having dengue or yellow fever (n=506), were analyzed. These samples had previously been tested for arboviruses^{9,10}. Among all patients, 398 (78.6%) were residents in Cuiabá, the State's capital; furthermore, 490 (96.8%) were from urban areas and nine (1.8%) were from rural zones, while seven (1.4%) did not report their area of residence. Additionally, 249 (49.21%) patients were women and 257 (50.8%) were men. Sampled patients ranged in age from seven months to 80 years; the majority (233/506; 46%) were 20-39 years old.

Samples were tested using an indirect immunofluorescence assay (IFA) to search for antibodies against *Rickettsia* spp.¹¹ and *E. canis*¹². The endpoint antibody titers present in each serum sample was determined individually for antigens against *R. rickettsii* (strain Taiaçu), *R. parkeri* (strain At24), *R. amblyommii* (strain Ac37), all tested from 64 to 16384, and for *E. canis* (strain Cuiabá 16; tested from 40 to 10240). Sera showing antibody titers at least four-fold higher than that observed for any other *Rickettsia* species were considered to be homologous to the target (or closely related) species, deemed a *possible antigen involved in a homologous reaction*¹³. Enzyme-linked immunosorbent assay (ELISA) was performed with anti-*E. canis* and anti-*E. chaffeensis* antibodies (peptides p19 and TRP32) in samples that were positive for *E. canis* on IFA¹⁴.

Overall, 19/506 (3.7%) patients were seroreactive (i.e., titer ≥ 64) against at least one *Rickettsia* species; titers ranged from 64 to 1,024. Additionally, three samples positive for *R. parkeri*, as well as one for *R. rickettsia* and one for *R. amblyommii*, showed endpoint titers that were at least four-fold higher than those for the remaining rickettsial antigens tested, suggesting homologous reactions to these antigens or their closely related variants (**Table 1**).

Antibodies against *E. canis* were identified in 2/506 (0.4%) patients, with antibody titers of 80 and 1280. However, the ELISA (which was performed to identify the possible etiological agent responsible for the primary infection) were negative.

Among 19 patients who were positive for anti-*Rickettsia* spp., five were positive for dengue virus 4; among the two patients with anti-*E. canis*, one was positive for dengue virus 4 (**Table 1**). There were no statistical differences between the epidemiological variables we examined among patients that presented antibodies titers against *Rickettsia* or *Ehrlichia* spp. (**Table 2**).

The frequency and distribution of ehrlichiosis and rickettsiosis had never been previously studied in the population of MT. The presence of antibodies against *E. canis* and *Rickettsia* spp. as detected in this study provides evidence of human exposure to these agents in this region.

TABLE 1

Distribution of antibody titers against antigens of *Rickettsia* spp. and *Ehrlichia canis* in the sera of patients collected between 2011-2012 in the State of Mato Grosso, Brazil, as determined by indirect immunofluorescence assay.

Sample	<i>R. rickettsii</i>	<i>R. amblyommii</i>	<i>R. parkeri</i>	<i>E. canis</i>	PAIHR*	RT-PCR
1	NR**	64	NR	NR	-	Negative
2	64	128	NR	-	-	Negative
3	64	NR	NR	-	-	DENV-4
4	128	128	128	-	-	DENV-4
5	64	64	NR	-	-	Negative
6	64	NR	256	-	<i>R. parkeri</i>	Negative
7	256	128	128	-	-	Negative
8	64	128	NR	-	-	Negative
9	128	NR	NR	-	<i>R. rickettsii</i>	Negative
10	256	256	128	-	-	DENV-4
11	64	64	NR	-	-	Negative
12	256	256	512	-	-	Negative
13	512	512	1,024	-	-	Negative
14	128	128	256	-	-	DENV-4
15	64	256	NR	-	<i>R. amblyommii</i>	Negative
16	512	256	256	-	-	Negative
17	256	128	1,024	-	<i>R. parkeri</i>	Negative
18	64	64	128	-	-	Negative
19	NR	NR	256	-	<i>R. parkeri</i>	DENV-4
20	NR	NR	NR	1,280	-	Negative
21	NR	NR	NR	80	-	DENV-4

R.: *Rickettsia*; **E.:** *Ehrlichia*; **PAIHR:** possible antigen involved in a homologous reaction; **RT-PCR:** reverse transcription-polymerase chain reaction; **NR:** non-reaction; **-:** not determined; **DENV-4:** dengue virus 4. * A homologous reaction was considered when an endpoint titer to a *Rickettsia* species was at least 4-fold higher than those observed for the other *Rickettsia* species. The *Rickettsia* species associated with the highest endpoint titer was considered the PAIHR. ** NR of serum within titers of ≥ 64 and ≥ 40 for *Rickettsia* spp. and *Ehrlichia canis*, respectively.

TABLE 2

Epidemiological data of patients seropositive for *Rickettsia* spp. and *Ehrlichia canis* from Mato Grosso, Brazil, between 2011-2012.

Variable	<i>Rickettsia</i> spp.	<i>p</i> -value	<i>Ehrlichia canis</i>	<i>p</i> -value
	N (%)		N (%)	
Sex				
female	11 (57.9)	0.489	1 (50.0)	1,000
male	8 (42.1)		1 (50.0)	
Age (years)				
<5	1 (5.3)	0.395	-	-
5-9	2 (10.5)	0.341	1 (50.0)	-
20-39	10 (52.6)	0.641	1 (50.0)	1,000
40-59	5 (26.3)	0.349	-	0.317
≥60	1 (5.3)	0.541	-	-
Ethnicity			2 (100.0)	
brown	10 (52.6)	1.000	-	0.499
white	4 (21.0)	0.757	-	-
black	2 (10.5)	0.297	-	-
yellow	1 (5.3)	0.142	-	-
not reported	2 (10.5)	0.268	-	-
Area of residency			2 (100.0)	
urban	18 (94.7)	0.463	-	1,000
not reported	1 (5.3)	0.236	-	-
Visit to rural/sylvatic areas				
yes	2 (10.5)	0.646	2 (100.0)	-
no	15 (78.9)	0.151	-	0.528
not reported	2 (10.5)	0.075	-	-
Residency city			2 (100.0)	
Cuiabá	16 (84.2)	1.000	-	1,000
Várzea Grande	1 (5.3)	0.709	-	-
Poconé	1 (5.3)	0.174	-	-
Pontes e Lacerda	1 (5.3)	0.418	-	-

%; Percentage of positive patients for each variable.

A high number of cases of undifferentiated acute febrile disease are registered annually in MT, and are mainly attributed to arboviruses such as dengue virus, which is endemic in the State. However, other diseases with similar clinical presentations are poorly investigated or documented in MT. No cases of Brazilian spotted fever cases have been confirmed in the state since the implementation of compulsory notification in 2001 (according to data published on the Ministry of Health's website as of 2015).

Moreover, most patients who were seropositive in this study were negative for arboviruses (which were the reason that the serum bank was first established^{9,10} on reverse transcription-polymerase chain reaction). This demonstrates that other agents might have been responsible for the acute febrile symptoms exhibited by the patients at the time of sample collection. However, rickettsiosis and ehrlichiosis are often confused with the febrile illnesses caused by arboviruses; therefore, it is important to emphasize the need for differential diagnosis for these diseases.

In the present study, 0.4% of the tested samples were positive for antibodies against *E. canis* antigens. The negative result

obtained on ELISA may indicate that another *Ehrlichia* sp. is responsible for producing antibodies in these patients, especially as the test was performed using peptides of proteins specifically expressed by *E. chaffeensis* (TRP32) and *E. canis* (p19)¹⁴. Molecular studies might be necessary to identify possible *Ehrlichia* species circulating within the State's population. To date, *E. canis* has been reported in dogs in the City of Poconé¹⁵ and in domestic cats in the cities of Cuiabá and Várzea Grande in MT¹⁶. Another species that is similar to *E. canis* was reported in bovines in North Pantanal⁷.

Studies involving human ehrlichiosis are scarce in Brazil. One such study described an *Ehrlichia* spp. seroprevalence of 5% in 100 individuals enrolled in a rural area considered endemic in Paraná State in the south of Brazil⁴.

In this study, random sampling may have influenced the relatively low prevalence of antibodies against *E. canis*. These findings demonstrate the importance of pinpointing the endemic areas in the state so that they can undergo epidemiological surveillance.

Antibodies against *Rickettsia* spp. were found in 19 (3.7%) patients investigated in the study; these patients carried antibodies against at least one tested species. Sera from three

patients showed a homologous reaction for *R. parkeri* or a closely related species, one patient each showed the same for *R. amblyommii* and *R. rickettsii*. Taken together, these data indicate that more than one species of *Rickettsia* may circulate in MT. Moreover, all the patients with positive serology for *Rickettsia* and *Ehrlichia* identified in the study were residents in cities from south-central MT and near North Pantanal, where species of these bacteria are frequently reported in vectors and animals^{8,17}.

IFA constitutes the gold standard test for the detection of antibodies in patients suspected of ehrlichiosis and rickettsiosis. This test may involve cross-reactions among species classified in the same genus¹. For purposes of diagnosis, studies have demonstrated that antibody titers that are at least four times higher against antigens of a specific species compared to others tested from the same genus are evidence of homology, i.e., the antibodies were likely produced by the species itself or another that is closely related¹³.

The detection of antibodies against Rickettsiae in individuals from MT supports the findings of previous studies that detected *Rickettsia* species in animals and several species of ticks in the region. 'Candidatus *Rickettsia amblyommii*' was demonstrated in *Amblyomma cajennense* complex and *Acanthodica coelebs*¹⁷. *R. parkeri* was found in *Amblyomma triste*⁸, while another species related to *R. parkeri* was found in *Amblyomma nodosum* and *Amblyomma ovale*¹⁵. Furthermore, *R. rhipicephali* was reported in *Haemaphysalis juxtakochi*, and 'Candidatus *Rickettsia andeanae*' and *R. bellii* in *Amblyomma cajennense* complex ticks¹⁷.

Identification of these bacteria via serological and molecular tests is evidence of their circulation in vectors and animals in MT; however, this is the first study to show *Rickettsiae* and *Ehrlichiae* evidence of infection in humans in this State.

The fact that most of the positive samples in this study were derived from urban residents may indicate that these individuals may have had contact with infected vectors sporadically during visits to sylvatic or rural areas where these ticks are present, or even to endemic areas of other regions of the country. MT contains three biomes, a wide range of wildlife, and tracts of preserved areas that are in proximity to populated urban areas. Ecotourism is frequent in the state; bathing in rivers and waterfalls, as well as sports fishing, are popular outdoors activities that can expose visitors to infected ticks.

Recognizing the impact of these diseases on public health is key to raising awareness among health professionals regarding the identification of these diseases and establishing a compulsory system of notification, including differential diagnose for these diseases. Moreover, it is necessary to institute awareness strategies in the population to promote health education that aims at preventing tick-borne diseases in areas where these parasites are common. Understanding the transmission cycle and the epidemiological aspects of rickettsiosis and ehrlichiosis, particularly the seasonal aspects and specificities of each vector species in the territory of MT, remain to be clarified.

Ethical considerations

As it involved human clinical samples stored in serum banks, the study was approved by the Ethics Committee on Research, Hospital Universitário Júlio Müller, Universidade Federal de

Mato Grosso (approval number 100/2011). Additionally, the Committee authorized the use of the bank for the present study (approval number 952.476/2014).

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Conflict of interest

The authors declare that have no conflict of interest.

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