

ANTI-LEPTOSPIRA SPP AND ANTI-BRUCELLA SPP ANTIBODIES IN HUMANS FROM RURAL AREA OF MONTE NEGRO MUNICIPALITY, STATE OF RONDÔNIA, BRAZILIAN WESTERN AMAZON

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ABSTRACT

Sera from 276 humans living in 71 farms located in Monte Negro Municipality, RO., Western Amazon, Brazil were examined for anti-*Leptospira* spp antibodies by Microscopic Agglutination Test and for anti-*Brucella* spp antibodies by Tube Agglutination Test. *Leptospira* spp antibodies were detected in 28 (10.2%) of them with at least one positive case in 23 farms (32.4%). The most frequent leptospira serovars were Patoc (46.7%), Autumnalis (10.0%) and Shermani (10.0%). The proportion of positive males (14.5%) were higher than females (5.0%; $P < 0.05$) and the contact with river water presented association with *Leptospira* spp infection (OR: 27; $P = 0.01$). A total of 04 (1.4%) humans reacted against *Brucella* antigens; three farms (4.2%) presented at least one positive case of brucellosis.

Key-words: Human, Leptospirosis, Brucellosis, Amazon, Brazil

INTRODUCTION

Leptospirosis and brucellosis are important zoonoses affecting humans and domestic animals all over the world. Leptospirosis occurs in urban and rural areas in both tropical and temperate regions and is an occupational hazard for those who work outdoor or with animals and as a recreational hazard for those who swim or wade in contaminated waters and inundations (12). In Brazil 4,128 cases were recorded in 2000, according to the National Foundation of Health (17). Prevalence of the brucellosis diverge widely from country to country, in Brazil, it was not regularly verified but the consumption of contaminated food and occupational contact remain as the major sources of infection (4).

The Brazilian Amazon region is a new frontier where agricultural development and livestock activities have been attempted. The state of Rondônia is a developing area in the

western Amazon of Brazil, where it is not available enough information about the occurrence of some zoonoses. This region is a typical frontier of human occupation, where the human inhabitants, domestic and wild animals share the same habitat, and also pathogenic microorganisms (10). The present study examined the seroprevalence and risk factors associated with *Leptospira* spp and *Brucella* spp infections in humans of the rural area of Monte Negro Municipality, Rondônia state, Western Amazon, Brazil.

MATERIAL AND METHODS

Monte Negro Municipality, located in Rondônia state, Western Amazon, Brazil (10° 15' 35"S, 63° 18' 06"W), has population of approximately 13,000 inhabitants, most of them rural dwellers in small, family-employed farms. The region has a high rainfall index that averages 2,000 mm per year; there is a

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moderate drought period during April-October. Temperature ranges from 25 to 29°C and the relative humidity is 70% - 80% throughout the year. From 722 cattle farms registered in the area surveyed, 86 were randomly selected using an estimated prevalence of 50%, absolute precision of 10% and confidence interval of 95%. The people living in those farms were invited to collaborate in this study. In 71 farms it was obtained the permission for blood sample collection.

Serum samples were collected from 276 humans from May to October 2002 and stored at -20°C until serologic analysis. During the visit to each farm, a questionnaire was applied to identify possible independent variables associated with seropositivity. The variables questioned were based on the regional characteristics regarding the water source, contact with domestic animals (dog, pig, and horse) and production herd-type of cattle (dairy, beef or mixed).

Serum samples were examined for leptospirosis by Microscopic Agglutination Test (MAT) (3,5) with the cut off 1:100 dilution against 24 leptospira serovars cultivated in modified EMJH medium (2). Positive sera were retested against each reactant serovar in two fold serial dilutions. The most probable serovar was the one that presented the highest titer. For brucellosis, serum samples were examined by Tube Agglutination Test (SAT) (1). The antigen used was an inactive suspension of *B. abortus* 1119-3 produced by the Institute of Technology of Paraná, Brazil. Reactions were considered positive when a titer of at least 80 was detected.

The variables identified in the questionnaire were subjected to univariate analysis and those with statistical association ($P < 0.20$ in Qui-square test (χ^2) or Fischer's exact test) were tested in the multivariate model by the stepwise forward method (8). Variables were included in the multivariate model if they displayed statistical significance of $P < 0.05$. Analyses were performed using SPSS for Windows (1999). For evaluation of the differences between age groups and sex occurrence, the χ^2 was used with EpiInfo version 6.0.

RESULTS

Leptospira spp antibodies were detected in 28 (10.2%; 95% CI: 7 – 14%) of the 276 sera against one or more serovar with MAT titers of 100 in ten, 200 in 12, 400 in four and 800 in two. From the 71 farms examined, 23 (32.4%; 95% CI: 22 – 44%) had at least 1 seropositive person. In relation to age, no difference were observed ($\chi^2 = 3.2$; $P = 0.07$) (Table 1), however there was significant difference in the seroprevalence of male 14.5% (21/144) and female 5.0% (07/132; $\chi^2 = 6.2$; $P = 0.01$). The serovars Patoc, Autumnalis and Shermani were the most prevalent (Table 2). Four (1.4%; 95% CI: 0.4 – 3.4%) humans reacted against *Brucella* antigens with SAT beings two titers of 80 (two males; with 30 and 72 years old) and two titers of 160 (one male with 20 years old and one female with 24 years old). In only three farms

Table 1. Antibody titers to *Leptospira* spp by age groups in sera of 276 humans of the rural area from Monte Negro Municipality, Rondônia State, Brazilian Western Amazon, by the Microscopic Agglutination Test (MAT).

Age (years)	No. tested	No. positive (%)	MAT titers			
			100	200	400	800
0-6	21	0	0	0	0	0
7-12	30	03 (10.7)	0	03	0	0
13-19	49	02 (7.3)	0	02	0	0
20-40	76	11 (39.2)	03	05	02	01
>40	100	12 (42.8)	07	02	02	01
Total	276	28	10	12	04	02

Table 2. Seroprevalence according to different *Leptospira* serovars among humans of the rural area from Monte Negro Municipality, Rondônia State, Brazilian Western Amazon.

Serovar	Positive sample	
	N	%
Patoc	14	46.7
Autumnalis	03	10.0
Shermani	03	10.0
Pomona	02	6.7
Hardjo	01	3.33
Bratislava	01	3.33
Pyrogenes	01	3.33
Canicola	01	3.33
Copenhageni	01	3.33
Icterohaemorrhagiae	01	3.33
Others serovars*	0	0
Not characterized serovars	2	6.7
Total	30	100

* Hebdomadis, Wolffi, Grippothyphosa, Panama, Butembo, Whitcombi, Australis, Castellonis, Andamana, Cinoptery, Sentot, Tarassovi, Bataviae, Javanica.

(4.2%; 95% CI: 1 – 11%) there were at least one seropositive, and for that reason the statistical analysis was not accomplished. The results of univariate and multivariate analysis for *Leptospira* spp are presented in Table 3.

DISCUSSION

This is the first serological survey of the infection by *Leptospira* spp and *Brucella* spp in humans from the rural areas

Table 3. Statistical analyses of association between studied variables and presence of antibody anti-*Leptospira* spp in humans from rural area from Western Amazon, Brazil.

Variables	Farms			Analysis				
	Sampled	Positive	%	Univariate		Multivariate		
				χ^2	P	OR	P	95% CI
Water for work								
Well	38	10	26.0					
River	07	05	71.4	5.4	0.02	27.0	0.01	2.8–351.0
Hunting consumption								
No	29	06	20.0					
Yes	39	16	41.0	3.1	0.07	1.7	0.20	—————
Dairy farms								
No	28	07	25.0					
Yes	43	16	37.2	1.15	0.28			
Beef farms								
No	63	20	31.7					
Yes	08	03	37.5	0.23	0.74			
Mixed farms								
No	51	18	35.2					
Yes	20	05	25.0	0.70	0.40			
Dog presence								
No	07	03	42.8					
Yes	64	20	31.2	0.53	0.67			
Pig Presence								
No	29	08	27.5					
Yes	42	15	35.7	0.51	0.47			
Horse presence								
No	06	01	16.6					
Yes	65	22	34.0	0.55	0.65			

of the Western Amazon, Brazil. Our study indicated that the prevalence of anti-*Leptospira* spp antibodies in humans beings was 10.2% and 32.4% of the farms presented at least one seropositive sample. The farm seroprevalence in the present study was similar as found by Homem et al. (6) in the Eastern Brazilian Amazon. Most tropical countries are also developing countries, and there are great opportunities for exposure of human population to infected animals, whether livestock, domestic pets, or wild or feral animals. Additionally, the incidence of human leptospirosis is significantly higher in warm-climates countries than in temperate regions. According to WHO, it probably ranges from 0.1 to 1 per 100 per year in temperate climates to 10 or more per 100 per year in the humid tropics (17).

The occurrence of leptospira antibodies in males (14.5%) was higher than in females (5.0% - $P < 0.05$). Maybe because,

males have more probability of acquiring the infection at work. The contact with river water was the variable that presented the highest association with *Leptospira* spp infection. In this survey, *Leptospira* spp prevalence had no association with presence of domestic animals, however, water-borne transmission have been documented, and agree with Levett (12) who pointed water contamination as responsible for several leptospirosis outbreaks.

The serovar Patoc was the most prevalent. This serovar belongs to the *Leptospira biflexa* group and is frequently used in serological investigations as genus specific antigen, because it presents crossed-reactions with pathogenic serovars and could detected antibodies earlier in the infection course by pathogenic serovar (13). The second more prevalent serovar were Autumnalis and Shermani. Few studies in Brazil described

the occurrence of Autumnalis in humans from north region as well the serovar Shermani that is maintained by wild rodents and has frequently been found in serological studies in cattle and humans from the northern area of Brazil (14, 6). Other serovars worldwide reported in rural areas like Pomona, Hardjo, Bratislava, and Pyrogenes were detected in low levels.

Antibodies against *Brucella* spp was found in 04 (1.4%) people from 03 (4.2%) farms. In the Eastern Brazilian Amazon, Homem *et al.* (7) found a higher prevalence of up to 29% among the studied farms. The true incidence of human brucellosis is unknown. Reported incidence in endemic disease areas varies widely, from <0.01 to > 200 per 100,000 population (4). In Brazil human brucellosis is caused mainly by *B. abortus* and *B. suis* (15,16). Most cases of human brucellosis were essentially an occupational hazard, occurring primarily in abattoir workers and meat processors (9). Additionally, the consumption of unpasteurized fresh cheese is very common and could be another way of transmission for humans (11,15).

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RESUMO

Anticorpos anti-*Leptospira* spp e anti-*Brucella* spp em humanos da área rural do município de Monte Negro, estado de Rondônia, Amazônia Ocidental Brasileira

Foram avaliados soros de 276 humanos procedentes de 71 fazendas localizadas no município de Monte Negro, RO, pela Soroaglutinação Microscópica para verificar a presença de anticorpos anti-*Leptospira* spp e pela Soroaglutinação Lenta em Tubos para verificar a presença de anticorpos anti-*Brucella* spp. Anticorpos anti-*Leptospira* spp foram detectados em 28 (10,2%) humanos procedentes de 23 fazendas (32,4%). Os sorovares mais freqüentes foram Patoc (46,7%), Autumnalis (10,0%) e Shermani (10,0%). A positividade foi maior no sexo masculino (14,5%) que no feminino (5,0%; $P < 0,05$) e o contato com a água de rio apresentou associação com a infecção por *Leptospira* spp (OR: 27; $P < 0,05$). Quatro humanos (1,4%) reagiram contra antígenos de *Brucella* spp, com três (4,2%) fazendas apresentando pelo menos uma reação positiva para *Brucella* spp.

Palavras-chaves: Humanos, Leptospirose, Brucelose, Amazônia, Brasil

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