Short Communication

Risk factors for leptospirosis and brucellosis in people living with human immunodeficiency virus who attended a referral hospital in southeastern Brazil

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Abstract

Introduction: Leptospirosis and brucellosis cause immunosuppression that worsens the clinical condition of people living with HIV/AIDS (PLWHA). We investigated the serological profile and risk factors of PLWHA.

Methods: Serum samples (n=238) were researched for Brucella spp. antibodies using Rose Bengal and tube agglutination tests and Leptospira spp. antibodies using the microscopic agglutination test.

Results: All samples were negative for Brucella spp. For leptospirosis, four samples (1.69%) were positive, and Andamana was the prevalent serovar.

Conclusions: Low or no detection of these zoonoses does not reduce their importance in PLWHA. Vigilant, educational, and preventive measures should be adopted.

Keywords: Anthropozoonosis. People living with HIV/AIDS. Public health.
Considering the importance of brucellosis and leptospirosis as anthropozoonoses, particularly in immunosuppressed patients, the purpose of this study was to determine *Brucella* spp. and *Leptospira* spp. antibodies and related risk factors in people living with HIV/AIDS (PLWHA) in a specialized infectious disease outpatient clinic in a referral hospital in southeastern Brazil.

This was a cross-sectional study and patients treated at the Domingos Alves Meira Specialized Infectious Diseases Outpatient Service (SAEI-DAM) of the Clinical Hospital (HC) of Botucatu Medical School, São Paulo State University (FMB-UNESP) were sampled. Botucatu is located in the mid-west region of São Paulo State (22°53’09” S; 48°26’42” W) with an estimated population of 146,497.

The SAEI-DAM registered patients were accompanied by a multidisciplinary team. The medical record system of the HC-FMB-UNESP was used to access patient data. Among them, 300 PLWA in several towns in the study area were identified, but only 238 patients met the study requirements: 129 (54.2%) men and 109 (45.8%) women, aged 18–76 years. No pregnant women were identified during the study period. Each patient was included in the study after obtaining an informed consent form.

Blood samples were collected using a vacutainer without anticoagulant by cephalic vein puncture to detect specific antibodies against each disease. Blood samples were centrifuged at 1,600 × g for 10 min, and the serum samples were stored at −4°C. In addition, an epidemiological questionnaire (“social and demographic characteristics”, “water, garbage, and sewer variables”, and “host-related characteristics”) was applied to the PLWA to determine the risk factors related to the studied disease.

The present study was approved by the Research Ethics Committee of the FMB-UNESP (protocol #821261).

*B. abortus* and *B. suis* antibodies were researched using the Rose Bengal test (RBT), a serum agglutination test in buffered acid-antigen stained with Rose Bengal, and the slow tube agglutination Rose Bengal test (RBT), a serum agglutination test in buffered acid.

*Leptospira* spp. antibodies were researched using the microscopic agglutination test (MAT). Cultures of *Leptospira* spp. standard serovars, maintained by weekly subcultures in Ellinghausen–McCullough–Johnson–Harris liquid medium, were used as antigens. Twenty-eight serovars were used: Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Canicola, Whitcombi, Cynopteri, Djasiman, Sentot, Grippotyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Javanica, Panama, Pomonae, Pyrogenes, Hardjo-Prajitno, Hardjo-Miniswajezak, Hardjo-C.T.G., Hardjo-Bovis, Wolffi, Shermanni, Tarassovi, Andamana, and Patoc. Serum samples were considered reagents for the presence of agglutination (≥ 50%) after infection, according to the World Health Organization (WHO). A cross-sectional study carried out in basic health units from Alagoas State, Brazil, reported 4.4% *Brucella* spp. antibodies in patients with brucellosis; however, no notification of the disease was identified in the Notifiable Diseases Information System.

Both humoral and cellular immune responses are required for brucellosis because the elimination of bacteria occurs in the intracellular environment. This fact increases the susceptibility of HIV/AIDS patients to *Brucella* infection. Brucellosis is rare in PLWA, although the eradication of intracellular bacteria is largely dependent on cellular immunity. In this way, it is hypothesized that HIV infection does not increase the incidence of brucellosis because most cases occur in asymptomatic patients with preserved immunity, and the epidemiology, clinical presentation, diagnosis, response to the therapy, and outcomes are similar to those observed in HIV-negative patients. A cross-sectional study carried out in basic health units from Alagoas State, Brazil, reported 4.4% *Brucella* spp. antibodies in patients with brucellosis; however, no notification of the disease was identified in the Notifiable Diseases Information System.

The close contact between humans and animals is evident and may indicate a related risk factor. The role of rodents in the transmission of many diseases, including leptospirosis, is widely
TABLE 1: Association (univariate analysis) between the Leptospira spp. antibody research and the social and demographic variables regarding the studied population

| Variable                                | N   | n  | % (95%CI)  | OR   | p-value |
|-----------------------------------------|-----|----|-----------|------|---------|
| **Sex**                                 |     |    |           |      |         |
| Male                                    | 129 | 3  | 2.3 (0.8-6.6) | 0.4 (0.0-3.8) | 0.63† |
| Female                                  | 109 | 1  | 0.9 (0.2-5.0) |      |         |
| **Age**                                 |     |    |           |      |         |
| 15 < x ≤ 30 years                       | 28  | 0  | 0.0 (0.0-11.9) |      |         |
| 30 < x ≤ 45 years                       | 94  | 3  | 3.2 (1.2-9.0)  |      |         |
| 45 < x ≤ 60 years                       | 89  | 1  | 1.1 (0.3-6.0)  | -    | 0.87† |
| 60 < x ≤ 76 years                       | 17  | 0  | 0.0 (0.0-18.5) |      |         |
| uninformed                              | 10  | 0  | 0.0 (0.0-28.5) |      |         |
| **Marital status**                      |     |    |           |      |         |
| Married                                 | 92  | 2  | 2.2 (0.7-7.6)  |      |         |
| Single                                  | 92  | 1  | 1.1 (0.3-5.9)  |      |         |
| Be living together                      | 10  | 1  | 10.0 (2.3-41.3) | -    | 0.42† |
| Divorced                                | 29  | 0  | 0.0 (0.0-11.6) |      |         |
| Widowed                                 | 14  | 0  | 0.0 (0.0-21.8) |      |         |
| **Educational level**                   |     |    |           |      |         |
| Undergraduate                           | 24  | 0  | 0.0 (0.0-13.7) |      |         |
| Incomplete under graduation             | 17  | 1  | 5.9 (1.4-27.3) |      |         |
| Completed the high school               | 67  | 2  | 3.0 (0.9-10.2) |      |         |
| Incomplete high school                  | 21  | 0  | 0.0 (0.0-15.4) | -    | 0.63† |
| Completed the primary/secondary school   | 39  | 0  | 0.0 (0.0-8.8)  |      |         |
| Incomplete primary/secondary school     | 61  | 1  | 1.6 (0.4-8.7)  |      |         |
| No educational level                    | 4   | 0  | 0.0 (0.0-52.2) |      |         |
| **Monthly wage**                        |     |    |           |      |         |
| up to 2 minimum wage                    | 165 | 3  | 1.8 (0.7-5.2)  |      |         |
| 3-5 minimum wage                        | 58  | 1  | 1.7 (0.4-9.1)  | -    | 1.00† |
| 6-10 minimum wage                       | 9   | 0  | 0.0 (0.0-30.8) |      |         |
| >10 minimum wage                        | 4   | 0  | 0.0 (0.0-52.2) |      |         |
| **Residence**                           |     |    |           |      |         |
| Urban area                              | 198 | 3  | 1.5 (0.6-4.3)  | 1.8 (0.2-17.4) | 0.51† |
| Rural area                              | 38  | 1  | 2.6 (0.6-13.5)|      |         |
| **Have you heard about leptospirosis or brucellosis?** |     |    |           |      |         |
| No                                      | 78  | 1  | 1.3 (0.3-6.8)  |      |         |
| Yes                                     | 154 | 2  | 1.3 (0.4-4.6)  | 1.0 (0.1-11.4) | 1.00† |

Legend: N: total number of sampled patients; n: number of positive patients for the microscopic agglutination test (MAT); %: percentage (95%CI, 95% confidence interval); OR: Odds Ratio; p-value for a = 5%; Fisher’s exact test.

known⁷. In urban areas, rodents are important reservoirs and sources of Leptospira infection with a higher probability of infection during rainy periods, mainly in tropical areas of developing and undeveloped countries⁸.

Although certain risk factors may be considered as indicators of the dissemination or, even, the severity of the disease in PLWHA, namely, tap water or artesian well water as “water source”, “if it floods when it rains” (Table 2), and even “if the animal stays at home or in the street” (Table 3), the low prevalence and sampled population limit the adequate characterization of the possible and eligible risk factors. The association between each variable and the serology results suggests a possible risk for PLWHA that experienced floods after rain. Despite this limitation, a higher seroprevalence was observed in males from urban areas, which could be related to the occupational risk. In Pernambuco State, Brazil, the authors also reported a higher occurrence of infection in male patients¹⁴. In non-PLWHA, leptospirosis has a high impact as an occupational disease. This fact was observed in São Paulo State, Brazil, among blood donors (1.3% reagents) from the Donor Center of the Clinical Hospital, FMB-UNESP⁹. This finding reinforces the relevance of continuous epidemiological surveillance and health education actions to control the disease in both animals and humans.

The observed range of the serological results seems reasonable, considering the different geographic regions and variations in
### TABLE 2: Association (univariate analysis) between the *Leptospira* spp. antibody research and the water, garbage, and sewer variables.

| Variable                                      | N   | n  | % (95%CI)  | OR  | p-value |
|-----------------------------------------------|-----|----|------------|-----|---------|
| Do you drink tap water?                       |     |    |            |     |         |
| No                                            | 85  | 1  | 1.2 (0.3-6.3) | 1.7 (0.2-16.5) | 1.00* |
| Yes                                           | 152 | 3  | 2.0 (0.7-5.6) |     |         |
| Water source                                  |     |    |            |     |         |
| Filtered water                                |     |    |            |     |         |
| Yes                                           | 91  | 0  | 0.0 (0.0-3.9) |     | 0.30*   |
| No                                            | 146 | 4  | 2.8 (1.1-6.8) |     |         |
| Tap water                                     |     |    |            |     |         |
| No                                            | 105 | 1  | 1.0 (0.2-5.1) | 2.4 (0.2-23.6) | 0.63*   |
| Yes                                           | 132 | 3  | 2.3 (0.8-6.4) |     |         |
| Spout’s water                                  |     |    |            |     |         |
| No                                            | 237 | 4  | 1.7 (0.7-4.2) | -   | 1.00*   |
| Yes                                           | 0   | 0  | 0.0 (0.0-0.0) |     |         |
| Artesian well water                           |     |    |            |     |         |
| No                                            | 223 | 3  | 1.4 (0.5-3.9) | 5.6 (0.5-58.1) | 0.22*   |
| Yes                                           | 14  | 1  | 7.1 (1.7-32.0) |     |         |
| Mineral water                                 |     |    |            |     |         |
| No                                            | 214 | 4  | 1.9 (0.8-4.7) | -   | 1.00*   |
| Yes                                           | 23  | 0  | 0.0 (0.0-14.2) |     |         |
| Does you have water tank?                     |     |    |            |     |         |
| No                                            | 46  | 2  | 4.4 (1.3-14.5) | 0.2 (0.0-1.7) | 0.18*   |
| Yes                                           | 187 | 2  | 1.1 (0.3-3.8) |     |         |
| How often is the water tank cleaned?          |     |    |            |     |         |
| Semiannual                                    | 23  | 0  | 0.0 (0.0-14.2) |     |         |
| Monthly                                       | 1   | 0  | 0.0 (0.0-84.2) |     |         |
| Annual                                        | 80  | 0  | 0.0 (0.0-4.4) | -   | 0.24*   |
| Biannual                                      | 26  | 1  | 3.8 (0.9-19.0) |     |         |
| Never                                         | 58  | 1  | 1.7 (0.4-9.1) |     |         |
| Sewer destination                             |     |    |            |     |         |
| Public sewer system                           | 207 | 3  | 1.4 (0.5-4.2) |     |         |
| Septic tank                                   | 25  | 1  | 4.0 (1.0-19.6) |     |         |
| Open sky                                      | 3   | 0  | 0.0 (0.0-60.2) | -   | 0.40*   |
| Rivers / streams                              | 0   | 0  | 0.0 (0.0-0.0) |     |         |
| When it rains, does it flood the street?      |     |    |            |     |         |
| No                                            | 203 | 0  | 0.0 (0.0-1.8) | -   | 0.00*   |
| Yes                                           | 33  | 4  | 12.1 (5.0-27.4) |     |         |
| What is the destination of your home garbage? |     |    |            |     |         |
| Public collect                                | 228 | 4  | 1.8 (0.7-4.4) |     |         |
| Wasteland                                      | 0   | 0  | 0.0 (0.0-0.0) | -   | 1.00*   |
| Backyard                                      | 0   | 0  | 0.0 (0.0-0.0) | -   |         |
| Burning trash                                  | 8   | 0  | 0.0 (0.0-33.6) |     |         |

Legend: N: total number of sampled patients; n: number of positive patients for the microscopic agglutination test (MAT); %: percentage (95%CI, 95% confidence interval); OR: Odds Ratio; p-value for a = 5%; Chi-square test; Fisher’s exact test.
TABLE 3: Association (univariate analysis) between the Leptospira spp. antibody research and the epidemiological variables related to the hosts.

| Variable                                      | N   | n   | % (95%CI)* | OR*  | p-value* |
|------------------------------------------------|-----|-----|------------|------|----------|
| Do you have animal at home?                   |     |     |            |      |          |
| No                                           | 51  | 1   | 2.0 (0.5-10.3) | 0.8 (0.1-8.1) | 1.00*   |
| Yes                                          | 185 | 3   | 1.6 (0.6-4.6)  |     |          |
| Which species?                                |     |     |            |      |          |
| Dog                                          |     |     |            |      |          |
| No                                           | 29  | 1   | 3.4 (0.8-17.2) | 0.4 (0.0-4.1) | 0.40*   |
| Yes                                          | 158 | 2   | 1.3 (0.4-4.5)  |     |          |
| Cat                                          |     |     |            |      |          |
| No                                           | 131 | 2   | 1.5 (0.5-5.4)  | 1.2 (0.1-13.2) | 1.00*   |
| Yes                                          | 56  | 1   | 1.8 (0.4-9.4)  |     |          |
| Bird                                         |     |     |            |      |          |
| No                                           | 146 | 2   | 1.4 (0.4-4.8)  | 1.8 (0.2-20.4) | 0.53*   |
| Yes                                          | 41  | 1   | 2.4 (0.6-12.6) |     |          |
| Pig                                          |     |     |            |      |          |
| No                                           | 178 | 3   | 1.7 (0.6-4.8)  | -   | 1.00*    |
| Yes                                          | 8   | 0   | 0.0 (0.0-33.6) |     |          |
| Wild animal                                   |     |     |            |      |          |
| No                                           | 180 | 3   | 1.7 (0.6-4.8)  | -   | 1.00*    |
| Yes                                          | 7   | 0   | 0.0 (0.0-36.9) |     |          |
| What is the food source to the animal(s)?    |     |     |            |      |          |
| Animal food (kibble)                         |     |     |            |      |          |
| No                                           | 11  | 1   | 9.1 (2.1-38.5) | 0.1 (0.0-1.4) | 0.17*   |
| Yes                                          | 169 | 2   | 1.2 (0.4-4.2)  |     |          |
| Homemade food                                 |     |     |            |      |          |
| No                                           | 128 | 2   | 1.6 (0.5-5.5)  | 1.3 (0.1-14.2) | 1.00*   |
| Yes                                          | 51  | 1   | 2.0 (0.5-10.3) |     |          |
| Leftovers                                     |     |     |            |      |          |
| No                                           | 179 | 3   | 1.7 (0.6-4.8)  | -   | 1.00*    |
| Yes                                          | 0   | 0   | 0.0 (0.0-0.0)  |     |          |
| Raw meat                                      |     |     |            |      |          |
| No                                           | 172 | 3   | 1.7 (0.6-5.0)  | -   | 1.00*    |
| Yes                                          | 7   | 0   | 0.0 (0.0-36.9) |     |          |
| Where does the animal stay?                  |     |     |            |      |          |
| Home (all day)                                | 123 | 1   | 0.8 (0.2-4.4)  | -   | 0.06*    |
| Street (all day)                              | 5   | 1   | 20.0 (4.3-64.1) | -   |          |
| Home + Street                                 | 47  | 1   | 2.1 (0.5-11.1) |     |          |
| If at home, where does the animal stay?       |     |     |            |      |          |
| Inside home                                   | 68  | 2   | 2.9 (0.9-10.1) | -   | 0.65*    |
| Backyard                                      | 97  | 1   | 1.0 (0.2-5.6)  | -   |          |
| Inside home + backyard                        | 11  | 0   | 0.0 (0.0-26.5) |     |          |
| Have you already found rats at home?          |     |     |            |      |          |
| No                                           | 96  | 0   | 0.0 (0.0-3.7)  | -   | 0.14*    |
| Yes                                          | 130 | 4   | 3.1 (1.2-7.6)  |     |          |

Legend: N: total number of sampled patients; n: number of positive patients for the microscopic agglutination test (MAT); %: percentage (95%CI, 95% confidence interval); OR: Odds Ratio; p-value for α = 5%; Fisher’s exact test.
environmental conditions, including rainfall, temperature and humidity, serovars, quality of the antigens, and interpretation of the results. Based on the serological results, the present study confirms that *Leptospira* spp. were circulating in the PLWHA population from São Paulo State, probably maintained by the animal population, even with low prevalence.

The low or no detection of the studied zoonoses does not reduce their importance in causing disease in PLWHA. Therefore, vigilant, educational, and preventive measures should be developed and maintained for the early identification of factors that predispose to the occurrence of these zoonoses.

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**AUTHORS’ CONTRIBUTION**

HL: conception and design of the study, data analysis, discussion and review; FBG: conception and design of the study, data analysis, discussion and review, final approval of the version to be submitted; ANB: conception and design of the study, data analysis, discussion and review; RCS: conception and design of the study, data analysis, statistical analysis, discussion and review, and in the final writing; KBM: drafting the article, participated in the review, discussion and analysis of the data and in the final writing; BDM: drafting the article, participated in the review, discussion and analysis of the data and in the final writing; CV: drafting the article, participated in the review, discussion and analysis of the data and in the final writing.

**CONFLICT OF INTERESTS**

The authors declare that there are no conflicts of interest.

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