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Serological trail of *Brucella* infection in an urban slum population in Brazil

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Abstract

Introduction—Brucellosis is a re-emerging zoonosis with new cases reported each year in many Latin American countries, but it is mostly under-recognized. This study presents a serological investigation of infection with *Brucella* abortus and *Brucella* canis in a poor urban community in the city of Salvador, Brazil.

Methodology—Human sera (n = 180) were randomly selected from 3,171 samples taken from healthy individuals during 2003-2004 and tested with C-ELISA for *B. abortus* and I-ELISA for *B. canis*.

Results—Thirteen percent (24/180) of the individuals were positive for *B. abortus* and 4.6 % (8/174) were positive for *B. canis*. Among the variables studied only age (older than 45 years) appeared to be a risk factor for the detection of *Brucella* antibodies.

Conclusion—These results indicate the presence of *Brucella* infection in this settlement and highlight the need to understand the epidemiology of infection under these circumstances to establish the necessary measures for surveillance and control.

Keywords

serology; *Brucella abortus*; *Brucella canis*; zoonosis; urban slum

Introduction

Brucellosis is a zoonotic disease with widespread global distribution, and is associated with a high degree of morbidity and minimal mortality [1]. The etiological agent is a coccobacillus of the genus *Brucella*, which presents four different species of interest for

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human public health: *Brucella abortus, Brucella canis, Brucella suis*, and *Brucella melitensis*. The transmission of brucellosis to humans occurs by ingestion of contaminated raw milk or dairy products or by direct contact with infected animals and/or with aborted fetuses or fomites [2]. Clinical features in humans are polymorphous; brucellosis usually presents as an acute febrile syndrome that may evolve to chronic disease with reproductive, osteoarticular, or nervous system complications [1,3]. Traditionally this disease in humans has been linked with occupational hazards [3,4] and to economically deprived social groups [5] resulting in a large number of missed diagnoses [3,5]. A few studies have been conducted on human brucellosis in urban areas [6,7], but in Brazil no such study has yet been reported.

With these considerations, we aimed to determine the presence of antibodies against smooth (*B. abortus, B. melitensis* and *B. suis*) and rough (*B. canis*) *Brucella* species in human sera as an indicator of the presence of these bacteria in a poor urban community in Salvador, Bahia, Brazil.

Methodology

A convenience sampling of 180 human serum specimens were randomly selected using an R program from a serum bank consisting of 3,171 samples held in Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil. These sera were collected during the period of 2003 and 2004 for an epidemiological study of leptospirosis in healthy human subjects living in the Pau da Lima community, a densely populated slum settlement situated in the periphery of Salvador, a city with 2,443,107 inhabitants, in Northeast Brazil. Of the 180 samples, 174 had sufficient serum volume for an Indirect ELISA (I-ELISA).

Competitive ELISA (C-ELISA) was used to detect antibodies against *Brucella* S-LPS (*B. abortus* S 1119-3). Briefly this test uses a monoclonal antibody (M84) specific for an epitope of the polysaccharide O chain of *Brucella* S-LPS and goat anti-mouse IgG antibody conjugated with horseradish peroxidase. Control sera (strongly positive, weakly positive, and negative bovine serum) were standardized and supplied by the Brucellosis Center of Expertise and OIE Reference Laboratory, Animal Diseases Research Institute (ADRI), Canada. Results are expressed as percentage inhibition (PI) of the monoclonal antibody (mAb) activity. For interpretation, sera with PI values of 28% or more were considered positive. This test has been demonstrated to be accurate for the detection of antibodies to *B. abortus, B. melitensis* and *B. suis* [8].

For the detection of *B. canis*, an I-ELISA was used. This technique is recommended by the Manual of Procedures for Diagnosis of Human Brucellosis [9] and briefly consists of the following steps: antigen obtained from a less mucoid (M-) variant of *B. canis* is coated onto ELISA plates and the control or problem serum is added; antibodies adhering to the antigen are revealed with the help of horseradish peroxidase conjugated A/G protein (ImmunoPure, Pierce Biotechnology, Rockford, IL, USA) and the corresponding chromogen. A cut value OD414 > 0.281 is considered positive.

Among the variables studied in a previous *Leptospira* project it was possible to analyze and relate the following factors to our serological results (positive or negative): demographics (gender, age); socioeconomic aspects (number of inhabitants in the house, per capita income per month, race, educational level, occupation); housing (open sewage, standing water and mud); presence of animals in the house; and positivity to *Leptospira* antibodies by the standard serologic micro-agglutination test (MAT) conducted previously. Bivariate analysis was used to examine a possible association between anti-*Brucella* status and the above-mentioned variables. Data were analyzed using the Epi-Info for Windows software (Centers

for Disease Control and Prevention, Atlanta, GA, USA). Individual subjects were linked by location of residence to spatially coded information for households and environmental attributes within the study site. Chi-square and Wilcoxon rank sum tests were used to compare categorical and continuous data, respectively. A p value ≤ 0.05 in two-sided testing was used as the criterion for statistical significance.

Results and discussion

From 180 serum samples, 24 (13%) were positive for smooth *Brucella* spp. and 8 serum samples out of 174 (4.6%) were positive for *B. canis*. Two individuals were positive for both types of *Brucella* (1.1%). After analysis of potential risk factors, only age (older than 45 years) appeared to be a factor for the detection of *Brucella* antibodies (Table 1); this result is contrary to the findings of a previously reported study in Italy (2005) where patients suffering from brucellosis showed a fairly uniform age distribution [10]. This difference in results between the Italian study and our observations may indicate that the source of contact with the bacteria is no longer present in this geographic location or that older people may have been infected earlier in life in different locations. Unfortunately the available data with respect to the occupations of the study participants was incomplete, so it was not possible to determine occupational association with *B. abortus, B. melitensis* or *B. suis*. Contact with dogs correlates with positivity to *B. canis* [4]; however, the presence of *B. canis* antibodies was not related to the presence of a dog in the household ($x2 = 0.62$, $p < 0.43$); this pattern was also found for smooth *Brucella* species ($x2 = 2.03$, $p < 0.15$).

According to the World Health Organization (WHO), human brucellosis is present in Latin America, with Mexico presenting the largest infected population: 2,599 new cases in 2010 [11]. In the same year, Peru, Argentina and Uruguay reported 375, 279 and 15 cases, respectively; no cases were reported in Colombia [11]. Bovine brucellosis caused by *B. abortus* is the most prevalent *Brucella* infection in Brazil and the economic impact is estimated at 32 million US dollars annually [12], followed by *B. suis* in pigs, *B. ovis* in sheep, and *B. canis* in dogs. *B. melitensis* and *B. neotomae*, as well as newly recognized species, including those from marine hosts [13], have never been isolated in Brazil.

In 2010, 26 new human cases were reported in Brazil, up from 22 cases in 2009; no records of submission to the WHO were available for 2007 or 2008 (WAID Interface, 2009). However, in 2008, Ramos and colleagues reported 4.4% seropositivity in an occupational group exposed to risk factors in Tocantins in the north of the country [14] and, in the same year, a report targeting individuals in rural populations in Pernambuc in northeast Brazil with occupational risk factors such as working with cattle or in slaughterhouses revealed an incidence of 1.8% [15]. The seropositivity found in the present study (13%) is also higher than that reported in Costa Rica (0.87%) [16] and Tanzania (5.5%) [17] for individuals with occupational hazards, but is lower than that reported in Colombia for individuals working in *Brucella* vaccination programs (34%) [18].

According to Mantur *et al.*, [3], the true worldwide incidence of human brucellosis could be 25 times higher than officially reported, due to misdiagnosis and to the fact that it is not a disease requiring mandatory reporting in some countries. Part of the change in the geographic distribution of brucellosis (*B. melitensis, B. abortus* and *B. suis*) is caused by the increased exposure to pathogens resulting from socioeconomic and political events such as open borders, people transit (travel and migration), population displacements, and the inveterate consumption of unpasteurized dairy products [5,12]. Infection and disease caused by *B. canis* have not been included in this map, but the increase in the dog population in urban areas and the lack of specific health policies concerning canine brucellosis have led to

an increasing number of cases of infections and disease in humans in close contact with infected dogs [14,19-21].

With respect to *B. canis*, there are serological studies in dogs in Brazil [22-25] but not in humans. Moreover, very few studies have been conducted in humans worldwide: in the United States, in 1973 researchers found 0.4% of military recruits were positive [25]. Two additional studies that were also conducted in 1975 involving people in close contact with dogs [26] indicated low positivity, while in the State of Florida 3 positives out of 303 individuals in the general population [27] were detected. Recently global reports about *B. canis* infections in humans have become more frequent. Human cases associated with the ownership of infected pets have been reported with isolation of the bacterium from humans with or without disease [4,19,21]; and in Argentina, an outbreak was reported that involved a family of six persons (three children and three adults) and a bitch and its puppies living under conditions of close daily contact with the family [20].

The Ministry of Health of Brazil recognizes brucellosis as an occupational disease, but it is still not considered a disease of mandatory notification [28]. In 2001 the program for control and eradication of animal brucellosis and tuberculosis was implemented in Brazil, but the huge national bovine herd presents a challenge for the control of the disease [14]. However, in Brazil, as well as in many other countries, there is no legislation for *B. canis*.

To our knowledge this is the first *Brucella* survey performed with samples from individuals living in an urban community with characteristics of strong poverty in Brazil.

Despite the limitations of our study, using only serological techniques to detect seropositive brucellosis and only in one specific human population, we believe that these results may reflect a widespread situation in Brazil, where 37% of the urban population lives in similar conditions [29]. This study is an effort to uncover the infection to alert the academic and health authorities of the country.

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Table 1

Risk factors for anti-*Brucella* antibodies* among subjects at the slum community site

Variables	*Brucella* antibodies		
	Yes (n = 30) No. (%)	No (n = 150) or median (IQR)†	*P* ‡
Demographics			
Male gender	9 (30.0)	68 (43.8)	-
Age, years			
5-14	8 (26.7)	48 (30.9)	NA
15-24	9 (30.0)	38 (24.5)	-
25-34	3 (10.0)	34 (21.9)	-
35-44	1 (3.3)	19 (12.2)	-
≥45	9 (30.0)	16 (10.3)	<0.05
Socioeconomic indicators			
No. of inhabitants	4 (3-7)	4 (3-6)	-
Per capita income, R$/month	7.5 (0-75.5)	15.0 (0-240.0)	-
Black race	29 (96.6)	145 (93.5)	-
Did not complete primary school	5 (16.6)	44 (28.4)	-
Work	9 (30.0)	61 (39.6)	
House characteristics			
Open sewage < 10m	8 (26.6)	54 (34.8)	-
Standing water < 10m	7 (23.3)	51 (32.9)	-
Mud < 10m	14 (46.6)	59 (38.1)	-

*ELISA was used for *Brucella* identification.

†Median and inter-quartile range (IQR) values are shown for continuous variables.

‡Values are not shown for non-significant associations.