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Research Paper

Zoonoses in humans from small rural properties in Jataizinho, Parana, Brazil

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Abstract

The aim of this study was to conduct a serological survey for Lyme diseases, brucellosis, leptospirosis and toxoplasmosis and identify the risk variables related to these zoonoses in humans living in the rural area of Jataizinho, state of Parana, Brazil. A total of 63 rural properties were surveyed. Additionally, 207 serum samples collected from these rural area inhabitants were tested for indirect immunofluorescence (IFI) and western blots (WB) were performed to detect *Borrelia burgdorferi* (sensu lato); a tamponated acidified antigen test (AAT) and 2-mercaptoethanol (2-ME) were used to detect antibodies of *Brucella abortus*; the microscopic agglutination test (MAT) was carried out to detect antibodies anti-*Leptospira* spp. and IFI was used to find antibodies of *Toxoplasma gondii*. Two of the samples (0.96%) were reactive for Lyme borreliosis, three (1.4%) for brucellosis, 25 (12.1%) for leptospirosis and 143 (69.1%) for toxoplasmosis. Although the town of Jataizinho has a human development index (IDH) that was considered to be average (0.733) in the state of Parana, the low social, economic and cultural conditions of the population from small rural properties have resulted in lack of basic information on animal health and direct or indirect contact with the various species of domestic animals, wildlife and ticks have probably contributed to the prevalence levels found. These results show the need for additional regional studies in order to determine the epidemiological characteristics of these diseases as well as their respective vectors and reservoirs so that effective prophylaxis can be administered in the human population.

Key words: Lyme disease, leptospirosis, brucellosis, toxoplasmosis, human, antibody.

Introduction

Leptospirosis, brucellosis and toxoplasmosis are widely distributed zoonoses. Domestic and wild animals act as reservoirs for the etiological agents for these diseases. For Lyme disease, tick acts as a vector for the etiological agent and human beings and as accidental hosts in the epidemiological cycle (Gill and Johnson, 1992; Macedo, 1997; Corradi *et al.*, 2005; Langoni *et al.*, 2008).

Seroepidemiological studies have described these zoonoses in human populations from different rural areas in Brazil. They have shown the importance of adopting preventive measures for residents exposed to infections (Garcia and Navarro, 2001; Gonçalves *et al.*, 2006; Naka *et al.*, 2008). In rural workers, infections become more frequent due to either absence or inappropriate use of protective equipment (Schwabe, 1984; Gonçalves *et al.*, 2006).

The town of Jataizinho, located in the northern region of Paraná, has a total area of 201.847 km^2, with a humid

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subtropical climate and is bordered, by the Tibagi River. According to the 2007 report from the basic health unit (BHU), its population was estimated at 11,604 inhabitants, with 1,850 residents in rural areas. This city has a human development index (HDI) of 0.733 according to PNUD (2010) that is considered as average in the state of Parana. However, the population in small rural properties faces lower social, economic and cultural conditions.

According to data from the BHU that was collected in 2009 in Jataizinho, there has been no diagnosis of brucellosis, leptospirosis or toxoplasmosis in the human population since 2003 (Gonçalves *et al.*, 2006). In contrast, the incidence of Lyme borreliosis has never been investigated. The aim of this study was to perform a seroepidemiological survey in residents from rural areas of Jataizinho and identify variables associated with these four zoonoses.

Materials and Methods

Samples, collection sites and conduction of tests

This research was approved by the Committee of Ethics in Research involving Humans from the Universidade Estadual de Londrina (UEL) (No. 319/06). According to data from the town under study, which was provided by the National Institute of Colonization and Agrarian Reform (INCRA), 95 out of 127 properties in the town of Jataizinho, Paraná state, were characterized as small, but only owners from 63 agreed to participate in this project. From February to November 2007, blood samples were collected voluntarily, from all 207 persons who were between 15 and 72 years old living in the 63 small rural properties. Serological tests were performed in laboratories at the Department of Preventive Veterinary Medicine (DMVP) at UEL and the Leptospirosis and Lyme Borreliosis laboratory at the Institute of Hygiene and Tropical Medicine (IHMT), New University of Lisbon (UNL).

Laboratory tests

Sera were screened for antibodies against *Borrelia burgdorferi* from the complex sensu lato (s.l.) by indirect immunofluorescence (IFI), glass slides coated with immobilized spirochetes (antigen) from the reference strain (B31) from *B. burgdorferi* sensu stricto (s.s.) were used (Collares-Pereira *et al.*, 2000). The results were subsequently confirmed by western blots (WB) using the following commercial kits "*recom* Line Borrelia IgM" and "*recom* Line Borrelia IgG" (Mikrogen®, Germany). Highly purified recombinant *B. burgdorferi* s.s., *B. afzelii*, *B. garinii* and *B. spielmanii* antigens were used in the western blotting procedure (Camargo *et al.*, 1995; Garcia *et al.*, 1999b). In IFI, sera presenting *Borrelia* with titers ≥ 256 were considered positive, and the results of antigenic fractions (p100, VlsE, p58, p41, p39, OspA, OspC and P18) were performed according to the manufacturer's instructions.

To detect antibodies against *Brucella* spp., sera were subjected to screening using the tamponated acidified antigen test (AAT), and positive results were confirmed with a 2-mercaptoethanol (2-ME) test (Technology Institute of Paraná-Tecpar) (Alton *et al.*, 1976). In AAT, positive samples were defined as samples presenting macroscopic agglutination. In 2-ME, positive samples formed a precipitate with a film at the bottom of the tube and a clear supernatant. Samples that had signs of *Brucella* spp. in both serological tests were considered to be positive.

For the detection of anti-*Leptospira* spp. antibodies, sera were subjected to the microscopic agglutination test (MAT) with 22 reference serovars (Myers, 1985). Serum samples that presented at least 50% of leptospires agglutinated in a 1:100 dilution were considered to be positive. These samples were further diluted to determine maximum positive dilution. In the analysis of the results, the more probable serovars had the highest agglutination. Those presenting co-agglutination in the highest dilution were considered to be positive for *Leptospira* spp (Myers, 1985).

To detect anti-*Toxoplasma gondii* antibodies, sera were subjected to an indirect immunofluorescence assay (IFI) (Camargo, 1973), using a commercial conjugate (an anti-human immunoglobulin G fluorescein-labeled-Sigma®, USA). Sera were tested in serial dilutions until a dilution of 1:4,096 was reached, and the samples presenting fluorescent tachyzoites with a titer ≥ 16 were considered to be positive.

Epidemiological research

To obtain epidemiological information, each human person individually answered a questionnaire that included information on variables related to the zoonoses studied (education level; presence of dog and/or cat in the property; presence of other species of domestic or wild animals in the property, presence and control of rodents in the property; whether the person aids and/or performs bovine castrations, assists in delivering animals on artificial insemination or culling of animals; daily work with animals; ingestion of raw milk or raw meat; eating vegetables without washing; habit of walking barefoot; use of personal protective equipment; ticks attached to the body; ticks in the house; flu outbreak up to 15 days before collection; and, reports of joint pain and night sweats).

Statistical analysis

The results were statistically analyzed with a chi-square (χ^2) correction using Yates or Fisher's exact test. Tabulation of epidemiological data and analyses was performed using the EpiInfo statistical program version 6.04 (CDC) with a 5% significance level. As an association measure, odds ratio (OR) calculation was used with confidence interval of 95% (Dean *et al.*, 1994).

Registration of rural properties for research of epidemiological occurrences was done through a global posi-

tioning system (GPS). A global positioning system was used in this study, and the geo-processing package ArcGIS 9.3 -ESRI was later used to map the resident properties that were studied as to the different zoonoses considered.

Results

For Lyme disease, a total of 18 (8.7%) serum samples were positive in IFI, but only two (0.96%) presented IgM reactivity for *Borrelia garinii* and *B. burgdorferi* s.s. in a western blot (Figure 1). For brucellosis, three samples (1.4%) were considered positive in 2-ME test with titers of 1:50 each.

Analysis of variables associated with anti-*Borrelia burgdorferi* s.l. and anti-*Brucella abortus* antibodies is shown in Table 1.

For leptospirosis, a total of 25 (12.1%) samples were considered to be reactive in MAT. From these samples, 11 (44%) had antibodies for one serotype, three (27.3%) samples were positive for Hardjo serovar, three (27.3%) for Castellonis, two (18.2%) for Grippotyphosa and for Australis, Pomona and Shermani one sample (9.1%) each. There were titers ranging between 100 and 1,600 for all samples. In 14 (56%) samples, antibodies against two serotypes were simultaneously detected with titers between 1:100 and 1:200, which made it impossible to characterize the most likely serovar. The analysis of the variables associated with the presence anti-*Leptospira* antibodies is shown in Table 2.

For toxoplasmosis, 143 (69%) samples were considered to be reactive, with titers between 16 and 4,096. There were no statistically significant differences between these variables and infection by *T. gondii*.

Table 1 - Variables associated with the presence of anti-*Borrelia burgdorferi* s.l. and anti-*Brucella abortus* antibodies in 207 human sera samples from people of the rural area in the state of Parana, Brazil, 2007.

Disease	Positive samples	p	OR (CI 95%)
Variables	Total (%)		
Lyme disease			
Ticks attached to body			
Yes	02/16 (12.5)	0.0056 *	
No	00/191 (0.0)		
Ticks in the house			
Yes	02/27 (7.4)	0.0164 *	
No	00/180 (0.0)		
Brucellosis			
Performing artificial insemination			
Yes	02/13 (15.0)	0.0105 *	
No	01/194 (51.0)		
Joint pain and night sweats			
Yes	03/07 (42.8)	0.0001 *	
No	00/200 (0.0)		

p = probability; * Fisher's exact test; OR = Odds ratio; CI = confidence interval.

In 59 (93.7%) properties, positive results for the zoonoses were found. Also, in 37 (62.7%) properties, a single infection was observed; there was a single infection (2.7%) for leptospirosis and 36 (97.3%) for toxoplasmosis. Mixed infections were also observed at 22 properties: one property

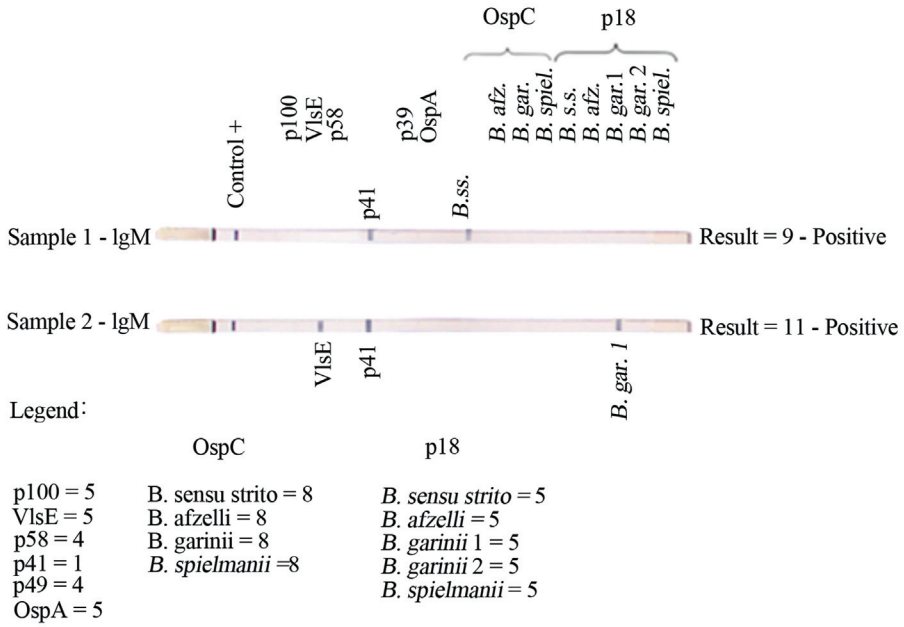

Figure 1 - Western blot positive for IgM in serum samples from two asymptomatic humans for *Borrelia burgdorferi* s.l. The subjects were from the rural area of Jataizinho in the state of Parana, Brazil.

Table 2 - Variables associated with the presence of anti-*Leptospira* antibodies in 207 humans sera samples from the rural area of Jataizinho in the state of Parana, Brazil.

Variables	Positive samples Total (%)	p	OR (CI 95%)
Education level°			
Literate	11/170 (6.47)	0.0001*	0.11 (0.04-0.31)
Illiterate	14/37 (37.00)		
Control of rodents on the property**°			
Yes	02/78 (2.56)	0.0138	0.11 (0.02 to 0.52)
No	23/121 (19.0)		
Assistance in animal deliveries			
Yes	21/74 (28.3)	0.0001	12.78 (3.89-46.38)
No	04/133 (3.0)		
Practice of castrating animals***			
Yes	18/49 (36.7)	0.0001	18.73 (5.43-70.97)
No	04/133 (3.0)		
Slaughtering of animals			
Yes	23/110 (20.9)	0.0001	12.56 (2.75-79.47)
No	02/97 (2.1)		
Daily work with animals			
Yes	21/110 (19.0)	0.0020	5.49 (1.69-19.71)
No	04/97 (4.1)		
Flu outbreak up to 15 days prior to collection			
Yes	20/21 (95.0)	0.0001*	724.0 (73.65-17781.32)
No	05/186 (2.7)		

p = probability; OR = Odds Ratio CI = Confidence interval.
* = Fisher's exact test.
** = Question not answered by eight rural residents.
*** = Question not answered by 25 rural residents.
° = Protective factors.

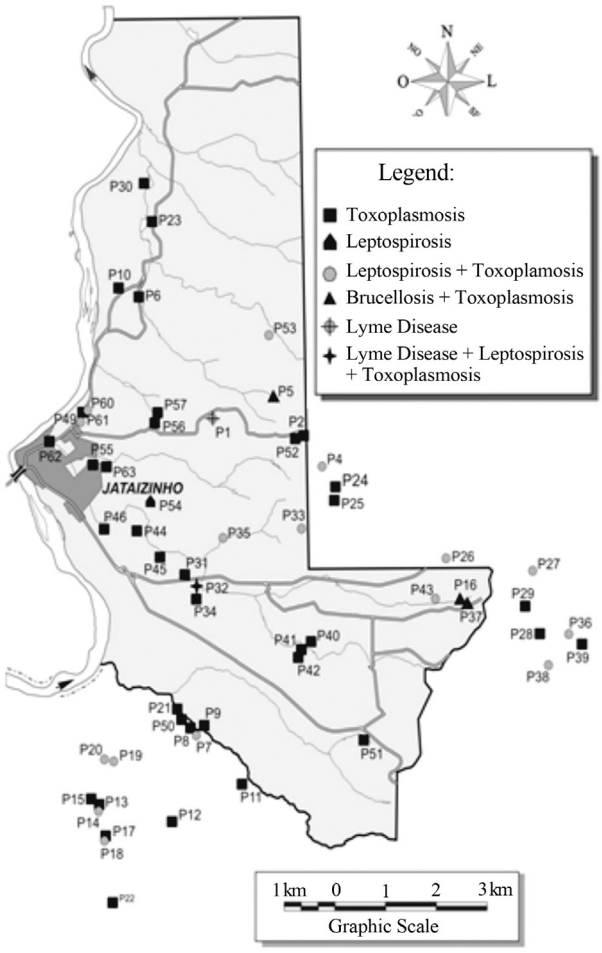

Figure 2 - Map of the rural area in Jataizinho (PR) indicating the properties with reactive humans for the various zoonoses surveyed, Parana, 2007.

each (4.5%) for Lyme disease and toxoplasmosis; one property each (4.5%) for Lyme disease, leptospirosis and toxoplasmosis; three properties (13.6%) for brucellosis and toxoplasmosis; and 17 properties (77.2%) for leptospirosis and toxoplasmosis (Figure 2).

Discussion

Different researchers have demonstrated the presence of antibodies and variations in clinical manifestations for the zoonoses in different regions of Brazil to understand the occurrence of Lyme disease(Yshikawa, 1996; Costa *et al.*, 2001; Yoshinari *et al.*, 2007; Naka *et al.*, 2008). In the state of Parana, this was the first detection of antibodies against *B. burgdorferi* s.l. in rural area residents who were directly or indirectly exposed to domestic or wild animals and ticks, the latter being considered vectors for *Borrelia* spp (Bennett, 1995).

In this study, 18 positive serum samples were found in IFI, and after using a screening test for the disease, only two (0.96%) samples were confirmed by a western blot. This test was performed according to the criteria establish by Center for Disease Control and Prevention (CDC) (Yoshinari *et al.*, 2003) and by the criteria for European *Borrelia* species (Hauser *et al.*, 1999) for having identified specific proteins for *B. burgdorferi* s.s. and *B. garinii*, respectively. In Brazil, the presence of anti-*B. burgdorferi* s.s. antibodies in symptomatic patients with history of contact with ticks has been demonstrated by western blot (Yoshinari *et al.*, 2003). In another study, using ELISA, anti-*B. garinii* antibodies were detected in patients with facial paralysis (Pirana *et al.*, 2000). In the present study, the main risk variables found were the ticks attached to the body (p = 0.0056) and their presence indoors (p = 0.0164), which was consistent with other studies that have reported cases of Lyme disease in humans Yoshinari *et al.*, 2007.

In Brazil, despite the high geographical distribution of both invertebrate and vertebrate hosts for *Borrelia* spp., there are few descriptions of these spirochetes. As such, further serological and molecular studies are necessary whether in humans or in different species of domestic and

wild animals and particularly in ixodid ticks, in order to better understand the disease.

Bovine brucellosis is a disease endemic to many regions of the world, and the cases diagnosed in humans are almost always associated with direct contact with infected animals or their contaminated products (Gómez *et al.*, 2008). In this study, a prevalence of 1.4% for brucellosis in rural residents was lower than the results found in the states of Pernambuco and Tocantins, where prevalence values were 21.1% and 8.1%, respectively (Moura *et al.*, 2000; Ramos *et al.*, 2008). This low prevalence of human brucellosis among rural residents in Jataizinho was probably related to the low prevalence (3%) of bovine brucellosis in the state of Parana (Dias, 2004).

The main risk of infection by *B. abortus* in humans was related to environmental contamination from abortion products (Wray, 1975). In this research, it was found that owners of small rural properties did not have basic information on animal health, especially bovine brucellosis, and those owners failed to evaluate the consequences of these risks to human health when purchasing an animal infected with *B. abortus*. Serological positive results for brucellosis in three residents showed a relationship to their occupation. The positive results were likely due to the individuals direct contact with infected vaginal secretions, performance of artificial insemination and lack or inadequate use of protective equipment. This finding suggests the need for developing an educational program designed to raise awareness about the health of animal herds. Additionally, there should be periodic serological monitoring of these animals as well as education on sanitary measures and individual protection for populations that are at risk of infection.

In humans, brucellosis appears as a chronic disease with symptoms of fever, night sweats, joint pain and myalgia (Ferreira *et al.*, 2002; Ramos *et al.*, 2008). These symptoms are compatible with the present results, as night sweats and joint pain ($p = 0.0001$) were found in three humans that had positive results in the 2-ME test.

In the human population studied from the rural area of Jataizinho, the prevalence of leptospirosis (12%) was similar to the prevalence found in the state of Rondônia (10%) and lower than the prevalence found in the state of São Paulo (23.5%) (Myers, 1985; Aguiar *et al.*, 2007). These results may have been influenced by differences in the prevalence of animal leptospirosis from corresponding Brazilian states and differences in the study periods. Therefore, there may have been higher or lower odds of infection in humans who came into contact with urine, organs, viscera and secretions from infected animals.

The risk variables found by this study included aiding with animal deliveries ($p = 0.0001$), castrating animals ($p = 0.0001$), slaughtering animals ($p = 0.0001$), and daily contact with animals ($p = 0.0020$). These results were consistent with those obtained in the states of Piauí (Macedo, 1997) and Parana (Garcia *et al.*, 1999a), which have also reported that occupational exposure is linked to work activities in rural areas. The association with occupational activities identified in this study can be attributed not only to close and continuous contact with different species of infected animals, such as cattle, horses, dogs and wild animals, but also work activities associated with wetlands as well as agricultural and livestock structures contaminated with urine from infected rodents or other animal species.

In humans, leptospirosis may cause serious illnesses or only sub-clinical infections, which is similar to the difference between the flu and colds (Gonçalves *et al.*, 2006); similar to the variable detected in this study, flu outbreak up to 15 days before blood collection ($p = 0.0001$) in surveyed residents.

The prevalence of 69% for toxoplasmosis was similar to other studies, which were also conducted in the state of Parana with prevalences of 66% and 83%, respectively (Garcia *et al.*, 1999a; 1999b).

Despite the high prevalence found in the humans studied, there was no association between the variables that were analyzed and the presence of anti-*T. gondii* antibodies. The high prevalence in rural areas may be due to greater exposure of this population to an environment where there is more contamination by oocysts or domestic cats. This result demonstrates that the inhabitants of this town were exposed to common sources of infection regardless of gender, age, activities or eating habits. Another study in the state of Parana found a prevalence of 71%, and the authors were not able to identify variables associated with this zoonotic disease in rural area residents (Garcia and Navarro, 1995). Other authors considered consumption of food and/or water contaminated with sporulated oocysts as well as, ingestion of raw or undercooked meat and sausages containing tissue cysts of *T. gondii* as the main sources of disease transmission to humans (Avelino *et al.*, 2004; Dubey *et al.*, 2005).

Even though the town of Jataizinho has a human development index (HDI) that is considered to be average (0.733) in the state of Parana (PNUD, 2010), the low social, economic and cultural conditions of the population in small rural properties could be associated with both the lack of basic information on animal health and the direct or indirect contact with the various species of domestic and wild animals as well as ticks. It is likely that these factors contributed to the prevalence levels that were found. These results show the need for regional studies to determine the epidemiological characteristics of these four diseases as well as their respective vectors and reservoirs. The ultimate goal of these studies would be to develop effective prophylaxis for the human population.

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