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Factors Associated With Human Visceral Leishmaniasis: Confirmed Cases And Co- Inhabitants in Minas Gerais State, Brazil

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ABSTRACT

Human Visceral Leishmaniasis (HVL) is increasing gradually in all regions of Brazil which is the third largest country focus of infection. Individuals with positive serology and are asymptomatic, are at high risk of becoming symptomatic if they are living with a family member showing HVL symptoms. This study aimed to evaluate demographic and serologic characteristics of confirmed cases (CC) and their co-inhabitants (Co-In) living in Governador Valadares (2010 to 2012). This study included 166 participants, 23 were CC and 143 Co-In. The results showed that males were more likely to be infected and 73.7% of the CC was unaware of forms of prevention. There was an association trend between infection and the habit of staying in the backyard.

Key words: Human Visceral Leishmaniasis, Risk Factors and Co-Inhabitants.
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INTRODUCTION

Human Visceral Leishmaniasis (HVL) is a neglected tropical disease and in Brazil is caused by *Leishmania* (*Leishmania*) *chagasi* (Badaro and Duarte, 2002). The disease can manifest in different clinical forms and can progress to death if untreated (Pastorino et al., 2002; Gama et al., 2004). Thus, early diagnosis and treatment are of utmost importance to the reduction of mortality rates and the morbidity of the disease (WHO, 2011; Chappuis et al., 2007). The main parasite reservoir is the dog responsible for the spread of the disease in large Brazilian cities (Maia et al., 2008). Brazil is considered the third-largest country focus of infection (Miles et al., 1999; Desjeux, 2004; WHO, 2010; Belo et al., 2013). Statistics provided by government agencies recorded the appearance of new cases of HVL and its increase gradually in all regions of Brazil, setting broad endemicity of the disease. According to data from the Notifiable Diseases Information System (SINAN) of the Ministry of Health, throughout the month of December 2011 the disease was found in 21 of the 27 states, with an average

annual incidence of 2 cases / 100,000 inhabitants (Brasil, 2011). More recently, Ortiz and Anversa (2015) showed that the control measures carried out in Bauru-SP so far, had not been able to eliminate the transmission of the parasite.

In Minas Gerais State, the first case of HVL came from the Municipality of Itanhomi in 1953 (Monteiro et al., 1994). Between 1981 and 1991, 614 cases of HVL were reported by FUNASA (National Health Foundation) and the State Health Secretariat of Minas Gerais (Borges, 2006). In the period 2000 to 2005, there were 2,018 confirmed cases of HVL, resulting in 188 deaths. From 2004 to 2008, there were a total of 2,374 cases of HVL in the state, accounting for 68% of cases registered in the Southeast and 14% of the country. Minas Gerais was considered the second state with the highest number of notifications of the disease and the mortality rate in this period was 8.9% (Brasil, 2009). The Vale do Rio Doce and Mucuri regions, in eastern Minas Gerais State, were considered endemic for HVL and ACL (American cutaneous leishmaniasis) by Mayrink et al. (1979) presenting similar epidemiological characteristics as those observed in northeastern Brazil. According to Coelho and Falcão (1966) prior to the application of control measures, 130 cases of human HVL were diagnosed in the Vale do Rio Doce most of them in Governador Valadares and surrounding towns. After the implementation of the Canine visceral leishmaniasis (CVL) control program, only cases of ACL and a small number of allochtonous HVL cases were diagnosed in the region. However, in the early 90s, the CVL control program was interrupted and the surveillance did not occur regularly in the region. According to Malaquias et al. (2007), 18 cases of HVL were diagnosed in the period of 2002 to 2007 in this region and there was an equal distribution of the disease in both genders (male and female) and most of the confirmed cases were represented by individuals up to 15 years old.

A canine serological survey conducted in the municipality of Governador Valadares in 2007, confirmed the re-emergence of CVL in urban and rural areas of the municipality (Malaquias et al., 2007). The animals showed seropositivity with an incidence of 13.7% in urban areas and 12.4% in rural areas. It is important to consider that the absence of intervention with appropriate control measures possibly contributed to the re-emergence of HVL in the region (Malaquias et al., 2007). In 2008, the Municipal Hospital of Governador Valadares attended 14 autochthonous human cases, including three deaths. Patients were predominantly male, aged between 24 and 45 years old. In 2009, 30 cases were diagnosed with a total of eight deaths. In 2010, seventeen cases occurred and no deaths. Therefore, in the period between 2008 and 2010, ninety-three cases of HVL had been diagnosed of which 61 were considered positive for HVL and 32 suspect. The lethality rate in this period was significant reaching the value of 18.03%, while the average rate in the country was 6.5% from 2001 to 2011.

In Minas Gerais State, it reached 3.20% in 2000 and 5.60% in 2008 (Brasil, 2013, 2010). The insect vector, *L. longipalpis* was identified in 2006 and has dispersed throughout the Municipality of Governador Valadares, being found in several neighborhoods (Barata et al., 2013). Studies on risk factors associated with asymptomatic infection reported that the presence of an asymptomatic case of HVL is almost three times more likely in a family with a symptomatic case than in those without cases (Custodio et al., 2012). According Bern et al. (2005) the most important determinant of the presence of asymptomatic infection is related to the proximity to a previous HVL case. The closer a case of HVL, the greater the likelihood of an asymptomatic infection becoming symptomatic. Individuals residing up to 50 meters away from a home that has a case of leishmaniasis have a three times higher chance of acquiring the disease. While those individuals who possessed cases of HVL in the family and resided in the same household (co-inhabitants) had a twenty-six times higher chance of developing the disease. Lima et al. (2012) stress that family members and neighbors of patients with classic HVL history include groups of people more susceptible to acquiring the disease, since there are epidemiological, demographic and environmental characteristics that are common among these individuals and similar to those in the reported case.

In this sense, these individuals have a high likelihood of exposure to the parasite. The HVL control strategies in Brazil are based on the occurrence of symptomatic cases, and most of these strategies only identify risk factors associated with progressive disease. The investigation of asymptomatic infection is very important for diagnosis in the early phase. However, this is extremely difficult due to the low level of antibodies and small parasitic load (Moreno et al., 2006). More recently, Marques et al. (2012) showed that asymptomatic children had a low and fluctuating quantity of Leishmania DNA and a significant decrease in parasitemia one year later. Thus, our study aimed to evaluate demographic and serologic characteristics of confirmed cases of HVL and their co-inhabitants living in Governador Valadares in the period from 2010 to 2012, in order to identify and monitor high-risk groups and implement more effective control measures.

MATERIALS AND METHODS

Study Design and Subjects

The study was conducted in Governador Valadares, an economic hub located in the Vale do Rio Doce, which is in the east of Minas Gerais. Individuals considered

eligible were those with confirmed diagnosis of visceral leishmaniasis, from July 2010 to March 2012, residents in this Municipality. Initially, the confirmed cases of HVL were identified by the Epidemiological Surveillance Service of the Municipal Health Department of Governador Valadares (SMS/GV). A list containing the home addresses of all 38 confirmed cases was provided by SMS/GV. All households were visited, though some families had changed their address and could not be found. Residents of those selected households that were willing to participate voluntarily entered the study. A total of 23 households was contemplated, that is, 19 households of confirmed cases that developed to cure and 4 households of cases that evolved to death. The residents of these 23 households that did not show any symptoms of HVL at the time of the visit were called co-inhabitants and also included in this study.

Collection of Demographic and Socioeconomic Data

For data collection a structured, pre-coded questionnaire was elaborated containing the variables: (a) demographic (gender, age, skin color, educational level, etc.); (b) socioeconomic (family income, occupation, number of rooms in the home, number of individuals per household); (c) household characteristics (presence of animals, household waste destination, urbanized garbage collection and presence of yard; (d) knowledge of the disease (prevention, symptoms) and (e) specific variables contemplating confirmed cases (symptoms, diagnosis and treatment). Interviewers were previously trained to standardize the procedures and interviews. From the identification of the confirmed cases, a spreadsheet for each residence was developed. With the support of Community Health Agents linked to the Family Health Strategy home visits were conducted to apply the questionnaire. It is noteworthy that children and the elderly who were contemplated had the questionnaires answered by adults living in the same household. After the questionnaires, the responses were coded in double entry, using the Epi Data 3.1. Following validation and correction of inconsistencies, the analysis of the results were obtained.

Immunoassay-Serological Test

Blood collection of participants was carried out by properly trained professionals using vacuum tubes of 10 ml without anticoagulant. Blood samples were centrifuged and the serum was separated and stored at -20° until the completion of the test that followed the manufacturer's instructions (Bio LINE Leishman SD-CAA). Briefly, the diluent was added, 3 drops (about 100 microliters) to a test tube provided by a "kit". Then, 20 microliters of serum from each participant were pipetted and added to the test tube. The mixture was gently mixed. The test strip was removed from the package provided by the manufacturer and was placed vertically in the test tube containing the mixture of serum and diluent. There was a 10 to 15 min interval before reading the result. The test was considered positive when there were two designated colored lines, test line and control line. The result was considered negative when only the control line was displayed. The test was considered invalid if no color development occurred in row C (control). It is worth mentioning that this test uses recombinant protein K-39 (rK39) according to Chappuis et al. (2007).

Statistical Analysis

The characterization of the participants in this study was obtained using the frequency distribution of all qualitative variables and analysis of central tendency for quantitative variables. A comparison of the infected individuals (CC-confirmed cases) and co-inhabitants (Co-In), who did not have any symptoms for (HVL) was performed using univariate analysis and odds ratio. To calculate the lethality coefficient, the numerator used the total number of individuals who died from the disease and the denominator the total of infected individuals. The results were analyzed using Stata / IC 11 (Stata Corp LP, 1985 to 2009).

Ethical Aspects

The study was approved by the Research Ethics Committee of the University Vale do Rio Doce / MG, with the protocol No.024/ 09-09, and observed the recommendations of Resolution 196 of 10/10/1996 - National Health Council for Research on Humans. The Term of Informed Consent (IC) was applied to study participants. In the case of minors, the inclusion was made only after signing the consent by a parent or guardian.

RESULTS

Clinical and Demographic Characteristics of Study Group

A total of 166 individuals, including 23 CC and 143 co-inhabitants Co-In participated in the study. Interviews and questionnaires were conducted and blood sampling for the immunoassay. The CC group had 18 males and 5 females, with ages ranging 1 to 76 years. Of the 4 cases who died, all were male, a child of two years, an elderly 76 years and 2 adults. The lethality rate for HVL in this study group was 17.4% in the period 2010 to 2012. The immunoassay was performed in 19 subjects (CC), 11 positive (57.9%) and 08 negative (42.1%). Regarding the 143 Co-In, 83 answered the questionnaire. Sixty

Table 1. Demographic characteristics of CC and Co-in of Human Visceral Leishmaniasis, municipality of Governador Valadares, Minas Gerais, 2010 to 2012.

Variables	Confirmed cases (n=23)	Co-inhabitants (n=83)
	N (%)	N (%)
Gender[a]		
Male	17 (73.9)	25 (30.1)
Female	6 (26.1)	58 (69.9)
Age		
Mean ±SD	29.1±21.8	35.5±15.6
Min-Max	1-76	1-78
Median (1Q/3Q)	M=30 (5/54)	M=34 (23/44)
Skin color[a]		
White	8 (42.1)	21 (25.6)
Brown	4 (21.0)	31(37.8)
Black	6 (31.6)	30 (36.6)
Education [a,b]		
Never Studied/ incomplete elementary	12 (75.0)	41 (49.4)
Elementary school	-	17 (20.5)
High school /graduated	4 (25.0)	25 (30.1)
Employment status[a]		
Rural worker	7(43.7)	27 (33.3)
Unemployed	4 (25.0)	19 (23.5)
Technician	1 (6.3)	16 (19.7)
Student	4 (25.0)	4 (4.9)
Housewife	-	10 (12.4)
Retired	-	5 (6.2)

[a]Excluded Individuals without information [b]excluding under 6 years old.

individuals including children and elderly did not answer the questionnaire, since only adults living in the same house were chosen to do so. All 143 Co-In were subjected to the immunoassay, with only 2 subjects (1.4%) positive and 141 (98.6%) negative results to the test.

Demographic Characteristics of CC And Co-In

The Table 1 shows data on demographic characteristics of CC and Co-In. In the CC group, male individuals predominated (73.9%), while in the Co-In group predominated female subjects (69.9%). The median ages were 30 and 34 years for CC and Co-In groups, respectively. With regard to skin color, there was no predominance of a particular type of skin color. Educational level was considered low in both groups, as 75.0% of the CC never studied or had only incomplete primary education. In the Co-In group, 49.4% of participants also never studied or had not completed primary school. Only 25.0 and 30.1% of subjects on the CC and Co-In groups, respectively, reported having completed elementary or higher school. Regarding the main occupation in the CC group, 43.7% were manual workers (mason, gardener, for example). The same characteristic was observed in the group Co-In (33.3%). There was a considerable percentage of unemployed individuals in both groups (25.0%-CC; 23.5%-Co-In), which could be contributing to the low socioeconomic status of individuals at risk of becoming infected.

Socioeconomic and Domestic Characteristics

The socioeconomic characteristics of the participants and their respective households were assessed. The average number of people living in each household was (4.60 ± 1.55), the houses had an average of 5.4 rooms, the number of people who shared the same room averaged (2.2 ± 0.9). Family income varied from less than 1 minimum wage to more than 2 wages / month. Fifty (50.0%) of families had an income of 1 to 2 minimum wages / month. When it was inquired about the fate of domestic waste in 91.3% of households the individuals said that the garbage is collected in plastic bags, in 13.1% of households the garbage is discarded on the streets or in vacant lots. In 95.7% of households, the garbage collection is performed by the urban cleaning service. The households that did not have this service accounted for a total of 4.3%. There were backyards in most households visited (91.3%). Some yards had beaten floor (66.7%) with shading (42.9%) and wetland (23.8%). In 52.6% of households the

Table 2. Knowledge and habits about Human Visceral Leishmaniasis of CC and Co-in, municipality of Governador Valadares, Minas Gerais, 2010 to 2012.

Variables	Confirmed cases n=19 (%)	Co-inhabitants n=83 (%)
Could a person have visceral leishmaniasis and not present symptoms?[a]		
Yes	5 (27.8)	31 (37.8)
No	11 (61.1)	49 (59.8)
Is there a way to prevent the disease?[a]		
Yes	5 (26.3)	43 (52.4)
No	14 (73.7)	39 (47.6)
If so, what can be done? [a,b]		
Do not leave trash in the streets, lots, yards	3 (60.0)	32 (74.4)
Perform cleaning around the environment	3 (60.0)	33 (76.7)
Spraying insecticide indoors	2 (40.0)	7 (16.3)
Use insecticide collars on dogs	-	5 (11.6)
Interventions to prevent the disease[a,b]		
Use insecticide collars on dogs	13 (86.7)	70 (88.7)
Spray insecticide indoors	15 (100.0)	75 (94.9)
Spray insecticide outside around the home	14 (93.9)	75 (94.9)
Perform cleaning around the house	15 (100.0)	78 (98.7)
Whereabouts between 6 pm and 10 pm		
Indoors	12 (63.2)	65 (83.3)
Outdoors	7 (36.8)	13 (16.7)
Is visceral leishmaniasis treatable?		
Yes	14 (77.8)	43 (52.4)
Do not know	4 (22.2)	39 (47.6)

[a]Excluded Individuals without information [b]Some individuals indicate more than one intervention.

individuals answered that the yard was cleaned more than 3 times a week. While 47.4% said that cleaning the yard was held once or twice a week. In 73.9 % of the households interviewed insecticides were not used. It was observed that 43.5% of households kept some kind of animal. Of these, 40.0% had chickens, 30.0% dogs, 20.0% cats and those households that had other animals totaled 20.0%. Households who reported having owned dogs diagnosed with leishmaniasis accounted for 75.0% (n=9). And, 88.9 % (n=8) of those households had dogs that had been collected by the Animal Control Center (CCZ) of the city of Governador Valadares. It was also found that in 4 households, the animal had been collected by the CCZ, one year or more before the date of the interview. In 37.5% of households, the dogs were collected under one year and 12.5% of households the individuals could not answer how long the dog had been seized. Finally, it was observed that in 6 households the animal waste was discarded into the environment, in 4 households in the backyard or even within their own homes.

Features Relating To the Confirmed Cases

Regarding the symptoms identified by the participants, the most common were fever (94.7%), hepatomegaly (68.4%), weight loss (63.2%), weakness (36.8%), pain (36.8%) and pallor (21.1%). Other symptoms reported by 2 individuals were headache, hair loss, arthralgia, and other unspecific symptoms. Of the 19 individuals in the CC group 79.0% (n = 15) said they had initially sought the primary care unit. As for treatment, 77.8% (n = 14) reported knowing that the disease is treatable. In 84.2% (n = 16) of cases the diagnosis was made at the Municipal Hospital of Governador Valadares, followed by 10.5% in private clinics and 5.3% in Basic Health Units (UBS). As for the time interval between the demand for health care and disease diagnosis, 73.6% (n=14) of the subjects said that the delay was on average 10 days to one (1) month. Noteworthy is the significant number of individuals (21.1%) whose diagnosis was confirmed 6 months to 1 year after the first consultation. The beginning of treatment occurred 10 days to one month after diagnosis in 73.6% (n = 14) of cases.

Acknowledgment and Attitudes of Participants In Relation To HVL

Regarding the symptoms of HVL most individuals in both groups (CC = 61.1%, Co-In = 59.8%) stated that a person cannot have leishmaniasis without presenting any symptoms (Table 2). On prevention, 73.7% of the CC

Table 3. Risk factors associated with clinical CC of Human Visceral Leishmaniasis, municipality of Governad or Valadares, Minas Gerais, 2010 to 2012.

Variables	Confirmed cases n=23 (%)	Co-inhabitants n=83 (%)	p value	Odds Ratio (OR) (IC-95%)
Gender[a]				
Male	17 (73.9)	25 (30.1)	0.0004	6.57 (2.31-18.63)
Female	6 (26.1)	58 (69.9)		
Knowledge of preventive measures[a]				
Yes	5 (26.3)	43 (52.4)	0.0464	3.09 (1.01-9.36)
No	14 (73.7)	39 (47.6)		
Whereabouts between 6 pm and 10 pm				
Indoors	12 (63.2)	65 (83.3)	0.057	2.91(0.96-8.81)
Outdoors	7 (36.8)	13 (16.7)		

[a]Excluding those without information.

group replied that there is no prevention. Within the Co-In group, there was no consensus just over half of respondents (52.4%) believed that the disease can be prevented. The main strategies for the prevention of HVL reported were: environmental cleanliness around the home (CC = 60% and Co-In = 76.7%); not leaving trash on streets, lots and backyards (CC=60.0 % and Co-In=74.4%) and use of insecticide inside the home (CC = 40% and Co-In =16.3%). The interventions that could be undertaken to prevent transmission of the disease are the following: the use of collars impregnated with insecticide on the dog (CC = 86.7% and Co-In = 88.7%); spraying insecticide indoors (CC = 100.0% and Co-In = 94.9%); spraying insecticide outside the home (CC = 93.9% and Co-In= 94.9%); cleaning around the home (CC = 100.0% and Co-In = 98.7%) and requesting the seizure of the dog by CCZ (CC=100% and Co-In=86.1%). Most of the individuals in both groups (CC = 63.2% and Co-In = 83.3%) said they try to carry out routine activities in the evening between 6 pm and 10 pm inside the home.

Evaluation of Risk Factors Associated With HVL

Risk factors associated with clinical cases were identified comparing the characteristics of cases and co-inhabitants, using the odds ratio (Table 3). Clinical cases were more likely to be male (OR = 6.57, CI95%=2.31-8.6) and occurred among those unaware of preventive measures (OR = 3.07, CI95%= 1.01-9.36). The proportion of cases that reported staying outside, in the backyard or away from the house, from 6 pm to 10 pm, was also higher than Co-In, showing a borderline level of significance and of the confident interval for the odds ratio (p = 0.057, OR=2.91, CI95%=0.96-8.81).

DISCUSSION

This study reported the increase of the occurrence of human visceral leishmaniasis in Governador Valadares/Brazil, in the last decades, and proposed to identify factors associated with clinical cases by comparing characteristics of cases and co-inhabitants. Clinical cases were more likely to be male and occurred among those unaware of preventive measures. A third characteristic suggested to be associated with the clinical disease regarded staying outside, in the backyard or away from the house, from 6 pm to 10 pm. However, this factor has to be reevaluated due to the borderline values of the level of significance and confident interval for the odds ratio. The greater occurrence of cases in male subjects has already been observed by Alvarenga et al. (2010) in residents of Campo Grande, Brazil, who found that 74.7% of cases of visceral leishmaniasis were male. Corroborating our data Ortiz and Anversa (2015) Gusmão et al. (2014) also found higher rates of HVL in males.

The chance of having the disease in males observed in the present study (OR = 6.57) is higher than the data reported on the literature. According Borges et al., (2008), men were 2.57 times more likely to contract HVL than women. Ali and Ashford (1994) observed that men were twice more likely to develop the disease than women; both seroconversion rates and the conversion of skin tests were higher in men than in women, especially in the elderly because they have a less resistant immune system. In the present study, however, we do not evaluate the incidence of cases or the presence of asymptomatic cases among the co-inhabitants, just the factors associated to confirmed cases. Recent studies have also shown that males are associated with disease, with an odds ratio (OR) of 1.30 when employing the skin test and an OR of 2.38 when considering the clinical cases (Belo et al., 2013). However, a study in Sabara, Brazil, did not find a significant difference between the number of asymptomatic cases among men and women (Moreno et al., 2005). Some authors do not rule out the

possibility of susceptibility to visceral leishmaniasis having genetic foundation (Góes et al., 2012; Oliveira et al., 2006; Nascimento et al., 1996). According to Góes et al. (2012), the greater exposure of men to infection may be due to the labor transit home at times that coincide with those of sand-fly presence.

In addition, it is noteworthy that in relation to lethality among the confirmed cases, the proportion of deaths was 17.4% for males. In a previous work, described by Vilas Boas (2011) the municipality of Governador Valadares had a significant lethality rate reaching value of 18.03% between 2008 and 2010. The average rate in the country was 5.7% in 2009 (Brasil, 2011), and 3.2 and 5.6% in Minas Gerais State in 2000 and in 2008, respectively (Brasil, 2010). In 2004, Belo Horizonte recorded the highest number of human cases and high lethality rate (18.7%) (Soares et al., 2010). In Campo Grande-MS lethality rate reached 18.4% (Alvarenga et al., 2010). In a recent study conducted in Bauru-SP, it was found that 8.1% of the reported cases died (Ortiz and Anversa, 2015). Regarding the age variable there is no significant difference between the mean or median age of confirmed cases and co-inhabitants. However, according to Vilas Boas (2011) study in Governador Valadares, children 0 to 9 years were the most affected by the disease in this municipality.

In Brazil, there is also a higher concentration of cases in this age group (Marzochi et al., 2009; Pedrosa et al., 2004). It has been discussed in the literature that the increased susceptibility of children under five years, of HVL may be related to a possible sharp picture of malnutrition (Bern et al., 2008). The study of Maia-Elkhoury et al., (2008) in Teresina, Brazil between 2001 and 2005 indicated that the most affected age group was 0 to 5 years. On the other hand, in Campo Grande /MS and Belo Horizonte / MG the most affected age group was 20 to 49 years. According to these authors, these changes probably occur because HVL shows in Brazil a distinct epidemiological profile resulting from climatic, geographical, biological and social conditions that vary according to each region.

Considering socioeconomic variables, most participants had not completed elementary school and received one to two minimum salaries. These individuals were mostly laborers. According Bevilacqua et al. (2001) health education practice for student training, contributes significantly to the epidemiological control of diseases. Thus, the limitation caused by the low level of education also seems to reflect the absence of preventive practice. Although some individuals have reported receiving more than two minimum wages, it was established that the living conditions were quite poor, which could indicate a false answer to this question because of embarrassment.

In our study we found no significant differences in relation to the variables education and occupation between CC and Co-In groups. Probably this result is because of a relatively small number of confirmed cases as well as the homogeneous character of the groups investigated. More recently, Belo et al. (2013) showed that low living conditions, lack of basic infrastructure services and low levels of education were closely associated with the development of HVL. It is noteworthy that in a study in Governador Valadares by Barata et al. (2013), it was found that the reported cases of HVL lived in homes with low sanitary conditions and inadequate garbage collection. According to these authors residents had low socioeconomic status, living with domestic animals and there was accumulation of organic matter in homes. These conditions have also been described in other places where transmission of HVL is endemic (Barata et al., 2004).

In general, socioeconomic status (poverty) and poor housing conditions are associated with increased risk in developing HVL (Felipe et al., 2011; Costa, 2008; Torres and Brandão-Filho, 2006; Moreno et al., 2005). While the dog is considered an important element in the epidemiological chain of HVL, no significant association was observed between the presence of dogs at home and infection with *Leishmania chagasi* in our study. However, it was reported by a large number of participants who despite not having dogs diagnosed with leishmaniasis in the household, there was the presence of dogs suspected of having the disease in the neighboring residences. The study of Barata et al. (2013) also showed an average prevalence of canine infection of 30.2% in Governador Valadares. However, in some neighborhoods the prevalence rate amounted to 53.4%, which reinforces that the presence of symptomatic dogs or seropositive asymptomatic ones plays an important role in maintaining infection. Furthermore, these authors evaluated the vector present in various neighborhoods, and found that there was a tendency for increased prevalence of canine infection in areas where the density of *L. longipalpis* was higher. Note that in our study the largest number of CC occurred in the same neighborhoods described by Barata et al. (2013), confirming the importance of the infected dog and vector presence in the maintenance of HVL. Knowing that between 6 pm and 10 pm, there is a higher concentration of sand flies outside the home, we evaluated the association between variables, getting in or out of home at this time and the risk of becoming ill.

Our results suggest that CC have a higher probability of being outside at this period than Co-In, but this association has to be confirmed in another study with a greater sample of participants, due to the borderline values for the significance (p value=0.056 and IC95%=0.96-8.81). This data corroborates the study of Barata et al. (2013), which indicated high density of sandflies in those neighborhoods where we found a higher number of CC. Thus, the identified factors seem decisive for the occurrence of HVL in Governador

Valadares, reinforcing the need for more stringent control measures such as the slaughter of seropositive dogs, the use of residual insecticide and environmental management in houses. Data reported by Brito et al. (2015) reinforce the importance of educational interventions in controlling the HVL. Moreno et al. (2002), despite having found an odds ratio of 0.4 to the variable to recognize dog with leishmaniasis, does not discard that the prior knowledge of the disease is considered a potential risk of infection. It is important to note that according to Borges et al. (2008), any knowledge of leishmaniasis was considered a protective factor, able to minimize the risk of visceral leishmaniasis in 2.24 times. Similar data have been reported by Dias (1998) and Cabrera et al. (1999).

In our study the immunoassay was conducted in 143 Co-In, of which two males, were positive. According Bern et al. (2005), the most important determinant for the presence of an asymptomatic infection is related to proximity to a previous HVL case. Lima et al. (2012) stress that family members and neighbors of patients with classic HVL history include groups of people more susceptible to acquiring the disease, since there are epidemiological, demographic and environmental characteristics that are common between these individuals like those of this case. In this sense, these individuals have a high likelihood of exposure to the parasite. Moura et al. (2012) analyzed factors associated with asymptomatic infection by *L. chagasi* in family and neighbors of patients with HVL. These authors found a proportion of 71.3% positives for intradermo reaction of Montenegro (IDRM) and 9.7% for the ELISA test. More recently, Chapman et al. (2015) demonstrated a strong association between elevated levels of rK39 antibodies in asymptomatic individuals and progression of the disease. Individuals with moderate seropositivity had 5 times higher risk of developing the disease compared to seronegative, suggesting that the rK39 ELISA test could be used as a predictor of HVL before the onset of symptoms. Although, the seropositivity rate between Co-In was low (1.4%), in our study, the positivity of the rapid test (immunoassay) indicates that the transmission of HVL is expanding in this endemic municipality.

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