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A one health approach to leishmaniasis in a slum: another piece of a global scenario

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ABSTRACT

OBJECTIVE

Visceral leishmaniasis (VL) is a severe vector-borne and zoonotic disease transmitted by phlebotomine sand flies. In the New World, is caused by the protozoan *Leishmania infantum*, having a great burden on human and animal health. Besides, environmental, and socioeconomic factors are significantly associated with the epidemiological pattern of the disease. VL continues to expand worldwide and in Brazil this situation is no different, reaching territories where it did not occur before, especially among marginalized populations in peri-urban areas. From a one health perspective, this paper details the first environmental health survey in one of the Brazilian marginalized communities, considered a new focus of VL.

METHODS

In a qualitative approach, we combine entomological collections, records, and field observations to provide a comprehensive assessment of environmental conditions.

RESULTS

The results highlight the need for monitoring the sand fly species found, as well as further studies for the real delimitation of their roles in the transmission of *Le. infantum*. The findings also suggest that bad housing conditions, lack of sanitation and the presence of arthropod vectors were associated with the occurrence of VL. Yet, we argue that many of the results found are quite similar across regions, and that the findings are not just a regional report but can reflect the reality of different parts of Brazil and the world.

CONCLUSIONS

A one health approach is essential to truly face VL, addressing the health risks at the animal–human–ecosystems interface, without ignoring the social context involved.

DESCRIPTORS

One health, Environmental health, Neglected tropical diseases, Health inequalities, Leishmaniasis, Vectors, Sand flies.

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INTRODUCTION

Leishmaniasis is a complex of zoonotic diseases caused by more than 20 protozoan parasites *Leishmania* (Kinetoplastida: Trypanosomatidae) and transmitted through the infective bite of a female phlebotomine sand fly (Diptera, Psychodidae). The disease can present different clinical manifestations, with visceral leishmaniasis (VL) the most severe and often lethal form[1,2]. In the New World, in addition to the Mediterranean basin, and West and Central Asia, VL is caused by *Leishmania infantum*[3], having a great burden, not only on human health, but also on animal health, especially dogs, reservoirs of the parasite[4]. Furthermore, environmental changes are strongly associated with epidemiological pattern of VL[5], and just like other neglected tropical diseases (NTD), its burden is disproportionately distributed, affecting the poorest sections of the population[6].

Following a global trend[7], in Latin America leishmaniasis continues to expand, with 96% of cases of the disease reported exclusively in Brazilian territory[1,8]. With regard to VL, at least 90% of the world's cases are concentrated in developing countries, including, again, Brazil[9]. Notably, areas of irregular occupation and proximity to forested areas have been major sites for the spread of the disease, especially among marginalized populations in peri-urban areas[10], reaching territories where it did not occur before[11,12]. In face of this scenario, it is evident that control strategies for LV require integrated approaches such as one health[13,14], which focuses on balancing the animal, human and environmental health, based on an equity and inclusion perspective[15]. Importantly, this concept also proposes to develop control measures for zoonotic diseases in a flexible way, so that they can be applied in local contexts, respecting cultural and economic particularities[16,17].

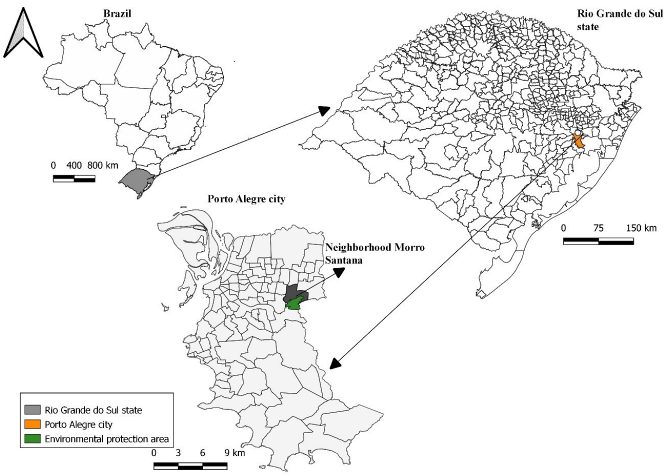
From a one health perspective, this paper details the first environmental health survey in one of the Brazilian marginalized communities "favelas", considered a new focus of VL[18], examining the environmental characteristics related to the public health conditions to which residents are exposed.

METHODS

The study area is Porto Alegre, capital of the State of Rio Grande do Sul (RGS). The city is in the humid subtropical climate zone and has an estimated population of 1,409,351 people and a territorial unit area of approximately 495,390 km^2 [19]. Data were collected in the slum region of the Morro Santana neighborhood (Figure 1). The neighborhood is in the eastern region of the city, has about 19,000 inhabitants, with an area of 5,745 km^2. It is estimated that about 1600 people live in the slum region of the neighborhood, however, it is complex to determine the real number of residents, since there is no official delimitation of informal settlements, which are located on the edges close to an environmental preservation area[20]. The average income in the Morro Santana neighborhood is below the national minimum wage and presents a Human Development Index and a Social Vulnerability Index below the values indicated for Porto Alegre[20]. This location was selected for the study because it had recorded autochthonous human cases and deaths due to VL, in addition to continuing to be one of the locations with the highest density of dogs diagnosed with canine leishmaniasis in the city[21, 22].

In a qualitative approach, this research combines biological collections, records, and field observations[23] carried out during the summer of 2021, to provide a comprehensive assessment of environmental conditions, relating health and social vulnerability. Focusing on environmental health, we empirically examine environmental characteristics that reflect precarious living conditions[23]. Fieldwork occurred in a non-systematic manner, and the main areas of focus were presence of vector arthropods; basic sanitation (garbage collection, water, and sewage treatment); and housing conditions.

Figure 1. Geographical localization of the study area, Morro Santana neighborhood, Porto Alegre, Rio Grande do Sul, Brazil.



Vector collection and identification

Sandflies collections were performed in the peridomicile of 2 dwellings that already had a history of human or canine VL. The sites shared the similar characteristics, such as precarious basic sanitation, absence of regular garbage collection, proximity to native forest, presence of domestic dogs and backyard with leaf litter covering the soil. Captures were carried out non-systematically over 8 days between February and March, the period of highest sand fly activity in this region. Two CDC (Centers for Disease Control and Prevention) light traps were installed at each sampling point for a period of 12h (7 p.m. to 7 a.m.). The specimens found were identified according to the taxonomic classification proposed by Galati (2018)[24]. Mosquito larvae were collected in breeding grounds and identified according to the key described by Forattini (1996)[25].

DNA extraction and *Leishmania* identification

After identification, the female sand flies were stored dry at -20 °C in pools containing up to five specimens from the same species. The DNA extraction was performed according to the instructions and guidelines of the manufacturer of the PowerSoil Kit (MOBIO). The DNA was analyzed for purity, using a NanodropLite spectrophotometer (Nanodrop Thermo Scientific, Wilmington, DE, USA), and quantified with a Quantus™ fluorimeter (Promega Corporation, Wisconsin, USA), using the QuantiFluor® ONE dsDNA kit according to the manufacturer's instructions. The total DNA extracted was used as a mold to investigate the presence of *Leishmania* DNA by its ITS1 polymerase chain reaction (PCR) using the primers and amplification conditions described by El Tai et al., (2000)[26] and Schönian et al., (2003)[27]. All negative ITS1-PCR samples were retested by kDNA-PCR using the same conditions described by Passos et al., (1996)[28]. Molecular weight aliquots of 100 bp and amplicons were submitted to electrophoresis in 1.5%–2% agarose gel, heated with ethidium bromide (10 mg/ml). Positive controls were used on all PCRs, such as: *Leishmania amazonensis* Lainson & Shaw, 1972 (IFLA/BR/67/PH8), *Leishmania braziliensis* (MHOM/BR/75/M2903), *Leishmania infantum* (MHOM/BR/ 74/PP75), and *Leishmania guyanensis* Floch, 1954 (MHOM/BR/75/M4147). In addition, an internal negative control containing all PCR components without DNA and a negative control of the DNA extraction step was also used in all reactions.

RESULTS

Sanitation

Through field visits, it could be observed that in the study area there is no regular sewage collection. Residents try to adapt some structures to facilitate the disposal of waste, but they are clearly not functional. Feces and urine are left in the open environment, making the movement of the residents difficult.

Another adaptation that some residents tried to make was in relation to the bathroom. Attempts at constructions like septic aisles were carried out, but the stony character of the soil does not allow proper excavation without the use of specialized machines. In this way, the overflow of waste occurs again. In addition, many adapted bathrooms do not provide a minimum structure regarding the comfort and safety of their users (Figure 2). In this same environment, the presence of children, usually playing without shoes, and the transit of stray animals of various species (such as dogs, cats, ducks, and chickens), which also defecate and urinate freely around the site, were observed. The dogs were often harboring ectoparasites such as ticks and fleas.

Figure 2. Type of improvised bathroom commonly found in the neighborhood.

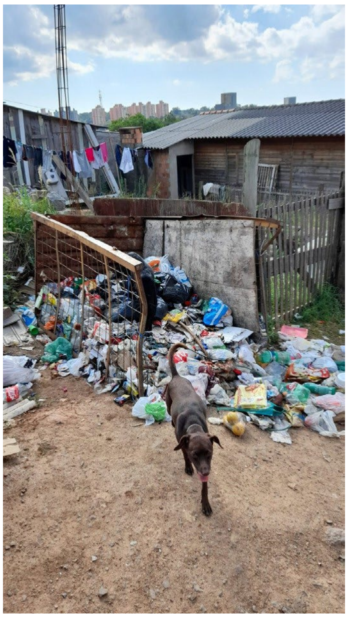
Drinking water has also proved to be quite problematic, since there is no regular supply. Thus, residents do not know when they will have water either for consumption or hygiene. The water boxes of collective use (Figure 3), supplied irregularly by the Municipal Department of Water and Sewage, present a series of problems due to lack of maintenance. The lids of the water boxes have large cracks, holes, and high porosity, allowing the entry of rainwater, dust, insects, and other animals.

The water has an unpleasant odor and the presence of dark particles, already indicating that it may be unhealthy for human consumption. Another important point is that there is an irregular adaptation of hosepipes at the water outlets so that there are access points closer to other dwellings. However, these hoses are exposed in the environment by crossing the sewage in the open air. Since they are often crushed by the crossing of cars and are the target of bites of stray dogs, it is possible to observe cracks and large tears in the hosepipes. There are also secondary water supply points fueled by improvised hosepipes. At these points, many close to animal husbandry, water is stored in water barrels that do not have adequate sealing, allowing the entry of insects and other animals, which is already observed by the presence of slime and dark particles in the water.

Figure 3. Water barrel for collective use without maintenance.

In addition, in this region, there is no regular garbage collection. Thus, many residents' resorts to using an improvised community recycle bin (Figure 4), causing a massive accumulation of garbage. When the amount of accumulated garbage becomes intolerable, without assistance, residents see the elimination of this material via fire as the only option.

Figure 4. Community containers (Open trash with free access of animals).



Housing

Many houses have an improvised structure, being built informally in forest invasion regions. Without sewage collection and lack of adequate toilets, in some houses, waste is free in the yard. With the uncertainty of the arrival of water, many residents prefer to put it in inappropriate places, such as improvised water barrels. The structure of the houses is constructed of various materials, such as wood and masonry, usually with several openings allowing the entry of animals, especially insects. Some houses are also very close (less than 100 m) to the shelters of animals, such as birds and pigs that circulate freely through the environment. Another major problem with housing is that some of the homes are in regions considered at high risk of collapse being subjected to falling rocks[20].

Arthropod vectors

Sand flies were collected in the peridomiciles of dwellings in a region of proximity to native forest. In total, 30 sand flies were captured (20 females and 10 males). Four species were identified: *Migonemyia migonei* (63.3%), followed by *Pintomyia fischeri* (16.7%), *Lutzomyia gaminarai*, and *Brumptomyia* sp. (both 10%). Females were separated into pools containing up to five specimens from the same species and tested for the presence of *Leishmania* DNA (2 pools of *Mi. migonei* containing 5 specimens each; 1 pool of *Pi. fischeri* containing 5 specimens; 1 pool of *Lu. gaminarai* containing 3 specimens; and 1 pool of *Brumptomyia* sp with 2 specimens). No pool tested was positive for the presence of *Leishmania* DNA. In the same region, mosquito larvae were found, all identified as *Aedes aegypti*. Without exception, all collected larvae were found in improvised water storage sites, such as containers of water.

DISCUSSION

There is a strong link between leishmaniasis and poverty[10]. Brazil have experienced epidemics in the fast-growing peripheries of large- and medium-sized cities[11]. The circulation of sandflies has exceeded geographical limits due to the change in human exposure factors; informal urbanization is one of the most important changes, causing domestication and expansion of natural transmission cycles[29].

The limit of south Brazil was previously considered a region without risk of *Le. infantum* transmission[30]; however, this situation is changing. RGS has registered an increase in the number of cases since 2009, when the first outbreak occurred[31]. Since then, the RGS has had 43 autochthonous cases of human VL where at least seven have evolved to death[32]. Porto Alegre, considered a new focus of VL in Brazil[18], has a significant mortality rate[33] and records more than half of the cases in the state, which occur mainly in peri-urban and socially marginalized areas, such as the slum region of the Morro Santana neighborhood[32], where the first human cases of VL were registered in the city[22]. However, the expansion of leishmaniasis in urban and peri-urban environments is not a phenomenon restricted to Brazil or tropical areas[34,35]. Very similar scenarios have been reported in different parts of the world, as in Cyprus[36], Europe[37], Middle East[38], Central America[39], Asia[40] and Africa[41].

Arthropod vectors

In this work, four different species of sandflies were found; although none were positive for PCR tests or have been previously proven as a biological vector of *Le. infantum*, the epidemiological importance of these species should not be underestimated. In Brazil, *Lutzomyia longipalpis*[42] and *Lu. cruzi*[43] are considered the only vectors of the parasite. However, in Porto Alegre, both are absent[18,44], as well as in many regions with VL foci in Brazil, making other species found in these localities highly suspect to participate in the transmission cycle[45-49]. This does not seem to be an isolated case, as other Latin American countries, such as Colombia[50], Venezuela[51] and Argentina[52], also reported a similar scenario indicating other sand fly species as a putative vector of *Le. Infantum*.

Pintomyia fischeri is an example of this case, having a wide geographical distribution where *Lu. longipalpis* is absent, although its vector competence has not been truly demonstrated[48,53]. In other entomological surveys in the same region and proximity, this species has previously been found containing DNA from *Leishmania* parasites, including *Le. infantum*[44,54-56], being pointed out as a putative vector. *Pintomyia fischeri* was also found in marginal areas of cities with small forest pockets, maintaining a significant degree of anthropophilic behavior, especially where there are human dwellings and shelters of domestic animals[57,58]. *Migonemyia migonei*, found in this study, is also an anthropophilic species that has been associated with the transmission cycle of *Le. infantum*[46,48,59] in this same area[44,54]. *Lutzomyia gaminarai* is an endemic species in southern Brazil[24,54,60] and together with *Mi. migonei* is the most common species found in Porto Alegre[22]. This species has also been found with the presence of *Le. infantum* and *Le. braziliensis* DNA in the same region in native forest and peridomicile areas of social vulnerability, corroborating with our findings[44,55]. Some other facts draw attention, such as the close phylogenetic proximity to *Lu. longipalpis*, with the females of both species being morphologically indistinguishable, in addition to the presence of *Lu. gaminarai* in areas of focus of VL where *Lu. longipalpis* is absent[44,60].

The large presence of stray animals, especially dogs, with a lack of any type of assistance or surveillance, represents an important risk to public health. Poor sanitary and animal health conditions can increase the risk of VL propagation by this group of animals that has been involved as the main reservoir of *Le. infantum*, not only in the Brazilian slums, but in the whole America[61], the Mediterranean basin[62] and even China[63]. Besides that, these animals may be involved in the cycle of other zoonotic diseases, such as rabies[64,65]. In addition, the remarkable presence of ticks largely affects canine health, as they can act as vectors of important pathogens[66].

In our survey, in storage sites unsuitable for water, we also found larvae of the *Ae. aegypti*, a mosquito with a strong presence in the Americas and widely distributed on the planet[67]. This species is involved in the transmission of several arboviruses, such as dengue (DENV 1–4), Zika and chikungunya[68], and is responsible for an enormous burden to the Brazilian's health population[69], as well as on global public health[70].

Compared to the rest of Brazil, RGS has fewer cases of arbovirus transmission, but the number of cases, deaths, and infestation by *Ae. aegypti* have been increasing every year[71]. In 2022, the State Department of Health announced a high alert against dengue, registering infestation by *Ae. aegypti* in almost 90% of the municipalities[72]. In Porto Alegre, the distribution of dengue also seems to be related to social and environmental determinants; until July 2019, it was observed that of all registered dengue outbreaks, more than 80% occurred in regions with areas of social vulnerability[73]. It is already well established that socioeconomic factors can be important components for the spatial distribution of *Ae. aegypti* [74,75], since the life cycle of these mosquitoes can be provided by environments that permeate the precariousness of peri-urban dwellings. Due to the scarcity of access to basic sanitation, poorer populations store water in residential gallons, increasing the number of breeding sites, causing an increase in the population of vector mosquitoes and, consequently, intensifying the risk of transmission[76,77]. Many arboviruses have appeared or reappeared in several countries around the world in recent decades[78,79] proving to be complex to determine the risk of emergence of pathogens, which often involves multifaceted interactions between biological, ecological and socioeconomic factors[74]. But even in continents with different development realities, arboviruses seems to demonstrate a propensity to reach the most socioeconomically vulnerable population[74,75,80].

Housing

In this research, very bad conditions were observed regarding the type of housing. VL affects the poorest layers of society, which is reflected in the characteristics of their homes[40,81].

Still, there are large gaps of knowledge on the subject, but it is known that characteristics, such as the material type of walls, the presence of open areas, lack of screens in the windows, and moisture from the ground, can play a fundamental role in the transmission cycle, meeting the appropriate needs for the survival of the vector[82,83]. In Argentina[84] and northeastern Brazil[85], household characteristics that reflect bad living conditions were also important factors in VL foci. As in India, poor quality houses are associated with an increased risk of infection[86]. The construction site of the houses is also inadequate in the context of health and well-being of the population, as many of them are occupying forested areas. All cases of human LV cases in Porto Alegre are associated with border areas with native forest[22]. The proximity to forest can be a significant risk factor for VL, especially when the vegetation is related to peri-urban areas[87]. These characteristics were associated with VL incidence in the south-eastern France[88] and central Spain[89]. The same in northeastern Brazil, where peripheral neighborhoods with high rates of VL incidence were close to vegetation[90].

It is not simple to determine how a change in the environment can affect the spread of infectious diseases in a specific area, mainly due to the variation in vulnerability of exposed populations and the complicated relationships between pathogen transmission and habitat modification[91]; however, socioeconomic inequalities correlated with environmental factors can determine the occurrence of leishmaniasis[92]. Significant changes in the environment, such as a closer approximation of urban areas to forest regions, are correlated with the presence of zoonotic diseases in a general sense[93,94]. For leishmaniasis, changes, such as the emergence of irregular settlements, allow the establishment of new transmission cycles near homes. In addition, the increase in the number of people in a risk area, exposing residents to bites of vector insects, will facilitate the transmission of this pathogen[95], causing the occurrence of new human cases[96,97]. Besides that, the proximity of houses to the breeding of animals, such as pigs and chickens, also observed in some dwellings in the region, directly influences the size of the sand flies population intra- and peridomicile[98].

Sanitation

The sanitation situation in Brazil is very precarious and even worse in the RGS, where more than half of the population does not have access to sewage services, about 30% do not have regular garbage collection, and at least 13% of people do not have access to potable water[99]. The risk of acquiring VL becomes higher for those who live in homes with lack of sanitation and sporadic garbage collection[87], mainly because it has the potential to increase sand flies breeding sites and to attract animal reservoirs[100-102]. In Brazil, high mortality by VL has a strong relationship with indicators of social vulnerability, including lack of access to basic sanitary services[103]. In the northeast, houses with adequate sanitation infrastructure were inversely associated with the incidence of VL[102], as well as their absence, caused a high incidence in the same region[104] as in the southeast[103].

Beyond leishmaniasis, the inadequate infrastructure of water supply, sewage, and garbage collection, puts residents and even stray animals, to a risk of exposure to various pathogens. Environmental degradation and the regular absence of garbage collection led to a favorable environment for the appearance of other animals with health importance, such as *Rattus norvegicus*, which is implicated in the transmission of rat-borne pathogens associated with NTD[105,106]. The lack of sanitation creates environments contaminated with several pathogens, such as bacteria[107], helminths[108], and protozoa[109], increasing the risk of human and animal infection. Flies are also commonly found in regions with unhealthy conditions where sanitary measures are not met. Sewage and open-air waste are important attractions, since decomposition of organic matter, such as human and animal excrement, are fundamental for its proliferation[110]. Equally associated with this same type of unhealthy environment, cockroaches (*Periplaneta americana*), commonly observed in the studied community, also act as mechanical vectors of pathogens, such as endo and ectoparasites[110,112].

CONCLUSION

The results presented in this work highlights the need for monitoring the sand fly species found, as well as further studies for the real delimitation of their roles in the transmission of *Le. infantum*, better understanding their impacts on the epidemiology of VL and assisting in the development of effective vector control strategies. The findings also suggest that housing conditions, lack of sanitation and the presence of arthropod vectors were elements associated with the occurrence of VL in this marginalized area, since so many risk factors can exacerbate the incidence of the disease. The observed environmental factors linked to human and animal health do not appear to occur in isolation. On the contrary, they are related and feed each other. The control of insect vectors, the improvement of the conditions of the houses, the access to basic sanitation and the stray dog care are of extreme importance to reduce, not only the risk to the health of this population, but also the culture of negligence on those who suffer daily from the exclusion of their most basic rights. Yet, when there are such similar transmission patterns across regions, it seems clear that these findings are not just a regional report but can reflect the reality of different parts of Brazil and the world. A one health approach is essential to reach the layers that permeate VL, addressing the health risks at the animal–human–ecosystems interface, without ignoring the social context involved. Regardless of whether it affects a Brazilian slum or elsewhere, recognizing the equal importance of the elements that impact the epidemiology of VL, is an important step towards finding truly effective tools to face this disease.

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