Simpósio Internacional
"Leishmaniose Visceral: Desafios para o Controle no Contexto da Diversidade dos Cenários"

Addressing the recent dispersion of urban visceral leishmaniasis in the border of Argentina, Brazil, Paraguay + Uruguay + Bolivia – Project IDRC

Abordando a recente dispersão da leishmaniose visceral urbana nas fronteiras da Argentina, Brasil e Paraguai + Uruguai + Bolívia - Projeto IDRC

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Oscar Daniel SALOMÓN[1]*

*Endereço para correspondência: Dirección, Instituto Nacional de Medicina Tropical, Neuquén y Jujuy s/n, Puerto Iguazú, Argentina, CP: 3370. Tel: 54 3757 425001. E-mail: dsalomon@msal.gov.ar
[1]Instituto Nacional de Medicina Tropical, Ministerio de Salud de la Nación, Puerto Iguazú, Argentina

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ABSTRACT

The territory located in the border of Argentina, Brazil and Paraguay is endemic for tegumentary leishmaniasis (TL). However, *Lutzomyia longipalpis* first report in the area was in 2010-Argentina, in 2012-Brazil, and no records in the Paraguayan border despite of reports of human visceral leishmaniasis (VL) cases. Therefore, we developed a research from 2014 to 2017 to study VL in the three-country border at locality level; Uruguay-2015, and Bolivia-2016 joined latter due to the alerts of VL in the Argentinean borders. The space-time distributions of vectors, infected dogs and environmental variables were recorded and associated at three progressive scales, while anthropological surveys were performed. Three scenarios were characterized based on canine VL prevalence, vector presence-abundance and the spatial distribution consistency between them: settled VL, incipient VL, and steady TL with imported canine VL. The vector abundance was clustered in 'hot spots' persistent in time that could act as 'source populations'. The clustering distribution was associated with environmental variables at the different scales studied. Therefore, the vector distribution (proxy of human-dog exposure) could be modeled in recent southern scenarios to focus the surveillance and interventions on predicted 'hot spots', in order to increase the effectiveness and efficiency of program activities.

Keywords. *Lutzomyia longipalpis*, canine visceral leishmaniasis, eco-epidemiology, modeling.

RESUMO

O território localizado na fronteira da Argentina, Brasil e Paraguai é endêmico para leishmaniose tegumentar (LT). Entretanto, o primeiro relato de *Lutzomyia longipalpis* na área foi em 2010-Argentina, em 2012-Brasil, sem registros na fronteira em Paraguai apesar dos casos de leishmaniose visceral humana (LV). Portanto, desenvolvemos uma pesquisa de 2014 a 2017 para estudar a LV na tríplice fronteira em nível de localidades; Uruguai-2015 e Bolívia-2016 aderiram mais tarde devido aos alertas de LV nas fronteiras argentinas. As distribuições espaço-temporais de vetores, cães infectados e as variáveis ambientais foram registradas e associadas em três escalas progressivas, enquanto se realizou o inquérito antropológico. Três cenários foram caracterizados com base na prevalência de LV canina, na abundância-presença de vetores, e a coerência da distribuição espacial entre eles: LV instalada, LV incipiente e LT estável com LV canina importada. A abundância de vetores foi agrupada em "pontos quentes" persistentes no tempo que poderiam atuar como "populações fonte". A distribuição de agrupamento foi associada a variáveis ambientais nas diferentes escalas. Portanto, a distribuição vetorial (proxy da exposição humana a cães) poderia ser modelada em cenários recentes do sul para focar a vigilância e as intervenções nos "pontos quentes" previstos, a fim de aumentar a eficácia e a eficiência das atividades do programa.

Palavras-chave. *Lutzomyia longipalpis*, leishmaniose visceral canina, eco-epidemiologia, modelagem.

INTRODUCTION

The first case of human visceral leishmaniasis (hVL) reported in the South Cone of South America was also the first record in the Americas. Luis Migone diagnosed in Paraguay hVL in a migrant from Italy that had been working in the railway Sao Paulo-Corumba. Further, in Argentina Salvador Mazza in 1923 also reported an hVL case in a worker from Italy working in Salta, a high endemic area of tegumentary leishmaniasis (TL). However, the rural autochthonous transmission was confirmed in the three countries only after the study of VL in Pará and Ceará in the 30`s by the Evandro Chagas team.

This rural-transmission scenario with sparse cases changed since the 70`s-80`s, when the 'VL great conquest of the American Southern frontier' started from NE Brazil, as epidemic urban outbreaks[1]. This event of dispersion is as complex as any eco-epidemiological event, involving: a) Human migration of workers from endemic areas correlated with changes in local demography; so the human clustering made more probable the diagnosis, but also increased the transmission due to bad planned urbanizations with deficient socio-sanitary conditions. b) Translocation of the parasite with the dog, its main urban reservoir, who travel with the migrants and usually has uncontrolled reproduction in new peri-urban settlements, with conditions that favor the transmission. c) Modification of the environment with primary forest encroachment, microclimate changes, and spread of the vectors/hosts more adaptable to anthropogenic habitats, as it happened with the main vector of VL in the Americas *Lutzomyia longipalpis*[2], and even pushing new species as potential vectors/reservoirs (Stockholm Paradigm) so additional agents in vector/reservoir communities could appear. In the environmental issues again the un-planned urbanization, deficient socio-sanitary conditions and health accessibility increase vector breeding sites and risk of human.-dog exposure.

In Mato Grosso do Sul the sequence of dispersion of hVL-canine VL (cVL)-*Lu. longipalpis* was associated in time and space with the Federal highway/rail-road building from SP State (as it was mentioned above the eventual infection scenario of the first hVL case in the Americas), and the Bolivia-Brazil gas pipeline since 1998. However, there were VL foci in Corumba reported in the 80´s, with *Lu. cruzi* as the main vector, and with up to 8.7% cVL prevalence in urban areas[3].

Since then, in Paraguay according to the Ministry of Health (Ministerio de Salud Pública y Bienestar Social, DGVS/SENEPA Paraguay) the cumulative hVL cases from 1995 (one case reported and the second one in 2000) up to 2017 were 1083 cases, distributed as 60 (2000-2005), 424 (2006-2010), 500 (2011-2015), 64 in 2016 and 35 in 2017. The reports of cases came mainly from the more populated jurisdictions (Distrito Asunción-Departamento Central) and surroundings, but also from the northern border with Brazil (Estado do Mato Grosso do Sul) and the eastern border with Argentina (Provincia de Misiones) and Brazil (Estado do Paraná), while the canine prevalence from 2005 to 2010 ranged between 20.9%-38.7%-69% in the foci.

In Argentina, the records since the first imported case mentioned above until 1989 showed only 16 cases with visceral symptoms, in places without vector captures, while *Lu. longipalpis* was found in forest locations close to the three-country border area in 1951 and again in 2000. However, as the urban VL was getting closer (Mato Grosso do Sul-Brazil and Asunción-Paraguay) the national program of leishmaniasis, focused up to then on TL, generated a yellow alert for VL since the year 2000. *Lutzomyia longipalpis* was found in urban environments on the border with Paraguay opposite to Lambaré (Departamento Central) in 2004 and then the alert was turned to orange. The first autochthonous hVL case was diagnosed in Posadas, city that also share a border with Paraguay, in 2006 and then the alert turned to red. The cumulative cases in Argentina from 2006 to 2017 were 182, more than 80% in the NE region of the country (Provincias Misiones and Corrientes), but during this period the vectors were reported in seven jurisdictions, including the border with Bolivia and Uruguay[4]. Two hVL-cVL foci away from the NE region were associated with *Migonemyia migonei*. Besides, cVL was reported thorough the country due to traffic and transit of dogs, and canine horizontal-vertical transmission. In urban *Lu. longipalpis*-related foci cVL reached a mean prevalence of 18.33% (8.5-26.2%).

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Further, modeling the potential distribution of *Lu. longipalpis* in Argentina, the gallery forest of the river Uruguay could be a suitable environment for the vector, even close to the mouth in the Rio de la Plata, according to the annual rainfall, the rainfall during the drier quarter and the mean temperature during the coldest quarter.

Visceral leishmaniasis further reports in the southern latitudes were: Brazil: Paraná, autochthonous hVL in SE region 1973/1974 and 1980; *Lu. longipalpis* in Foz de Iguaçu 2012 (after the alert about vectors in Puerto Iguazú in 2010 -AR)[5]. Santa Catarina without vector or cases in 2003, 29/2124 dogs with cVL in Florianópolis during 2011, 48/252 cVL 7:3 rural: urban 2014[6]. Rio Grande do Sul, Santa Maria 5 cVL in rural environments 1985, São Borja cVL, hVL 2008, *Lu. longipalpis* 2009; Uruguaiana cVL, 1 hVL 2009; Porto Alegre 3 hVL deaths 2016-2017[7]. Uruguay: *Lu. longipalpis* in Salto and Bella Unión 2010 (after the alert about vectors in Monte Caseros 2008-AR); Arenitas Blancas- Salto 11/45 cVL, *Lu. longipalpis, L. infantum* 2017[8]. Bolivia no records of hVL, cVL or *Lu. longipalpis* up to 2016 (alert about vectors in Tartagal 2013-AR).

Therefore, with these antecedents, we developed the project: "Addressing the emergence and spread of leishmaniases in the borders of Argentina, Brazil and Paraguay", supported by IDRC-Canada with the collaboration of PAHO from 2014 to 2017. After the starting of the project, due to the dispersion of the VL risk, Uruguay joined us in the year 2015, and Bolivia in 2017, the last with a small grant from PAHO.

MATERIAL AND METHODS

The project conceptualizes the multi-country border area as a common territory with biological symmetries, and socio-cultural asymmetries. Thus, the eco-epidemiology was the multidisciplinary theoretical frame selected to approach the problem, and so a common design was discussed at focus (locality) level. We selected critical sites for sampling, each one within cells of an urban grid of 400 x 400 m (census or stratified according to the city size) + ecotone transects (≈ 750 sites). We recorded the environmental variables at three successive progressive scales (spatial buffers): microscale-critical site, mesoscale-critical area around the site, and macroscale-landscape characteristics around the critical areas. At each critical site we performed vector captures (transversal sampling and seasonal sub-sampling) three-nights with mini light traps, we surveyed the five dogs closest to each vector-trap at least for VL and *L. infantum* infection (rapid test, ELISA, molecular biology of equivalent sensitivity between countries), and we evaluated synanthropic rodent presence with traps of activity. Simultaneously a medical anthropological study was performed developing a mapping of actors and then performed qualitative and quantitative surveys to collectives, key informants, and community.

RESULTS AND DISCUSSION

In Argentina, the study about the association between the spatial distribution of vector presence/abundance (proxy of human exposure) and environmental variables showed spatial clustering but segregated by species: *Nyssomyia whitmani*-vector of the agent of TL (45% of the sites) was close to forested areas and away of water bodies-more urbanized areas; *Lu. longipalpis* - vector of the agent of VL (47% of the sites) was close to water bodies-urban heterogeneous landscape areas (vegetal coverage and buildings intermingled). *Leishmania infantum* DNA was detected in 5 *Lu. longipalpis*, 1 *Ny. whitmani* and 1 *Micropygomyia quinquefer* (n:379). The spatial auto-correlation of *Lu. longipalpis* abundance had a range of 400m, and the abundance was better explained by land cover characteristics of 20.25ha (six classes evaluated for areas between 0.27 and 20.25 ha). The variables that better explained presence of this species were chicks (microscale) and NDWI (mesoscale), while for abundance the variables were the availability of different blood sources (hens, dogs or both) (microscale); and drinking water network, garbage collection, sewer (mesoscale). The abundance is higher in peridomestic than in indoor samplings, but inside the houses was higher in autumn (unpublished data). When *Lu. longipalpis* captures in Puerto Iguazu between 2011 (a year after the first record) and 2015 were compared, the VL vector kept 76.4% of its original distribution between dates. These data are consistent with studies

performed in other cities in Argentina, and with a 'hot spot'-source population hypothesis, as to an 80-20 relationship (20% of the sites are responsible for 80% of the abundance).

The canine infection prevalence at Puerto Iguazú according to rK39+ dogs was 21.2% (computed in dogs around the critical sites for vectors so it could be biased to higher values regarding the actual mean prevalence of the dog general population). The cVL abundance was spatially correlated negatively with the distance to vector traps and to human sleeping areas. However, the spatial distribution of abundance of cVL dogs are nor consistent with the VL vector abundance distribution, probably due to the traffic and transit of puppies/dogs within the city and between cities and international borders, based on the social networks of dog owners and transit of stray dogs. The anthropological study revealed the dynamics in a border territory between three countries of high porosity but different environment/urbanization social history. Regarding leishmaniasis the approach on knowledge, attitudes, perception and practices (KAPP) showed that the topic was focused almost exclusively in cVL, the KAPPs were fragmented, disparate and even contradictory or actively opposed between actors, and it depends on the human-dog interspecific distance according to dog uses/stray dogs, but almost ever delegitimizing the actions of the public agents and state policy but assigning all health-related responsibility to it. There are also gender bias due to domestic chores issues and assigned responsibility as family health and domestic area ´cleanliness' (unpublished data).

The Brazilian team developed the project in Foz do Iguaçu (FI), Santa Terezinha de Itaipu (STI) townships and along two transects between them (526 sites sampled) *Lu. longipalpis* was also the prevalent species. *Leishmania infantum* was found in *Lu. longipalpis, Ny. whitmani*, and *Ny. neivai*. The results suggested that according to the Stockholm Paradigm a probable shifting of potential vectors could be happening, and so the public health policies in international border areas should be aware of these eventual change of species in the transmission cycle. The spatial distribution of *Lu. longipalpis* is clustered in rural-urban mixed landscape areas (remnants of vegetation, debris and presence of synanthropic rodents and domestic animals). *Leishmania braziliensis* presence and abundance supports the need to develop public health policies to avoid TL outbreaks and TL urban spread in the area. Therefore, for an efficient control, it is essential to think in the aspects of urban planning and to coordinated it with the neighboring cities and countries[9].

The canine prevalence rate of *L. infantum* in the Brazilian border was 23.8% in FI, 4.7% in STI and 9.1% in the transect areas. Among the variables analyzed, the number of vectors and the presence of surrounding infected dogs were positively correlated with the occurrence of infected dogs. Dog size, an intrinsic variable, was positively correlated also with cVL infection, while the quality of the dog's nutrition affected cVL negatively. Once dogs are associated with humans, it maximizes the probability of VL transmission. As for TL, the record of dogs with *L. braziliensis* shows that the transmission in peri-urban areas is possible, while cVL is widely spread in FI with high prevalence, so sensitive differential diagnosis are required. These results also supports the hypothesis that the parasites have been present in the region for longer than previously believed, despite of the fact that the presence of VL in the region has only been recognized recently. Therefore, the control of the population of dogs infected with *L. infantum* (parasite and non-antibodies) is required to prevent the spread of the disease to other dogs and humans in the region[10].

The research in Paraguay to characterize knowledge, attitudes, and practices (KAP) regarding to leishmaniases was performed in neighborhoods with infected dogs and phlebotomine as it were reported by the project, characterized as transmission foci. Thus, households of San Francisco and Santa Rosa neighborhoods were visited to perform the qualitative-quantitative survey. Seventy-one percent of respondents stated that they had heard about the disease, however they have little knowledge about the different leishmaniases, the transmission cycle, or the symptoms. The attitudes and practices were associated and focused on dogs as pets, and so there were many topics of confusion between human and canine leishmaniasis. The results obtained by the team of Paraguay suggested the importance of educational interventions in populations at risk, to improve conditions for prevention and control of leishmaniasis in the triple border, taking into

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account that the flux of people is very intense. One point that should be highlighted is that the people surveyed in Paraguay stated that animals (dogs) have rights (95.2%), feelings (98.4%), but interestingly also a human exclusive cultural construct as it is morality (74.2%)[11]. This issue shifts the human-dog interspecific distance to an issue of distance between subjects of equal values, so a dog could be conceptualized as closer than a human neighbor, while the focus is again in cVL instead of on VL or leishmaniasis. Further, even scientific presentations on cVL have many pictures of babies and puppies playing together, as a subliminal reminder to the audience on the human character of the dogs. These results, consistent between countries could not be avoided or despised and less judged if we need to develop a strategy for cVL control that show efficacy but also effectiveness in the actual world.

When a similar study was performed as case-control study in and endemic focus of cVL in Argentina the knowledge resulted to be a risk factor, because a previous positive diagnosis of cVL in an current urban environment generated in the dog owner a surfing in internet about the disease, more than that generated by a general alert about VL transmission in the city among the general public[12]. Therefore, the case-control studies designs should be interpreted with caution in times of internet, mainly when urban populations are involved. On the other hand, taking into account the community reception of controversial issues about cVL, despite any personal position a basic common discourse should be shared among the legitimate voices, so the risk to have a dog infected in an area with vectors should not be despised[13]. In this sense, in Argentina a workshop and agreement act was promoted by the project researchers between public and private practitioner health agents for cVL management.

Paraguay reported *Lu. longipalpis* for the first time in the three-country border with the methodology of the project, mainly in the shore opposite to Argentina also in rural-urban mixed landscapes, and together with Uruguay showed a very clustered and spatially consistent distribution of the vector and *L. infantum* infected dogs. In the Bolivian border with Argentina *Lu. longipalpis* was also reported for the first time. These results draw attention to the fact that TL-related surveillance routines may overlook *Lu. longipalpis* unless the urbanized habitat of the vector of the VL agent is taken into account.

Regarding the dispersion in larger scales than a locality, although the parasite spread is fairly explained with the migration of infected hosts, the vector spread from NE Brazil to Uruguay and Argentina it is difficult to explain yet by the proposed passive and active mechanisms, and still require better hypothesis[2]. On the other hand, not all the pheromone types of *Lu. longipalpis* has the same dispersive potential, landscape plasticity and vectorial capacity, as the (S)-9-methylgermacrene-B seems the spreader one responsible to colonize new environments[14]. This pheromone type is also the one present in three-country border area[15], but preliminary results seems to indicate at least two ways of dispersion in the region, one from Mato Grosso- Paraguay- Argentina, and another one related to Rio Grande do Sul-Argentina shared VL foci (unpublished data).

CONCLUSION

Looking at the whole the results in the three countries and the preliminary results from Uruguay and Bolivia, allowed us to characterize different scenarios: A) VL settled: Urban distribution of *Lu. longipalpis* in 'hot spots', cVL prevalence rates 22%-26% increasing along time; Ar- Puerto Iguazú, Br- Foz do Iguaçu. B) Visceral Leishmaniasis incipient: *Lu. longipalpis* and cVL restricted to small clusters, cVL general prevalence up to 4%, spatial consistency between cVL and vector distribution; Br- Santa Terezinha de Itaiupu, Pr- Presidente Franco, Ur- Salto, Bo – Pocitos. C) Cutaneous Leishmaniasis steady: *Ny. whitmani Ny. neivai* in ecotones, without *Lu. longipalpis*, but it could be cVL imported cases; Ar – Puerto Iguazu urban periphery, Ar, Br, Py – transects. D) No risk up to now: Uy-Paysandú 32°19'S, 58°04'W.

Therefore, regarding the spatial clustering, time persistence and environmental variables associations with *Lu. longipalpis* presence-abundance at the different scales studied, we propose as a next step to build up a model of models to be validated in the field. This operational statistical model would identify in a given city potential

risk areas (microscale-macrohabitat) by satellite and census data. After this selection, weighting environmental, biological, and social risk drivers with secondary and primary data, probable risk areas (critical area) could be selected among the potential risk areas, and risk sites (microscale-microhabitat) chosen inside the probable risk areas. These 'hot spots' identification will allow to focus the monitoring and interventions activities only in few small areas, where it is more probable the initial colonization or the reproduction of 'source populations', increasing both the effectiveness and the efficiency of the surveillance and control strategies.

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