RESEARCH ARTICLE

Peri-domestic risk factors associated with transmission of cutaneous leishmaniasis in selected areas in Hambantota District, Sri Lanka

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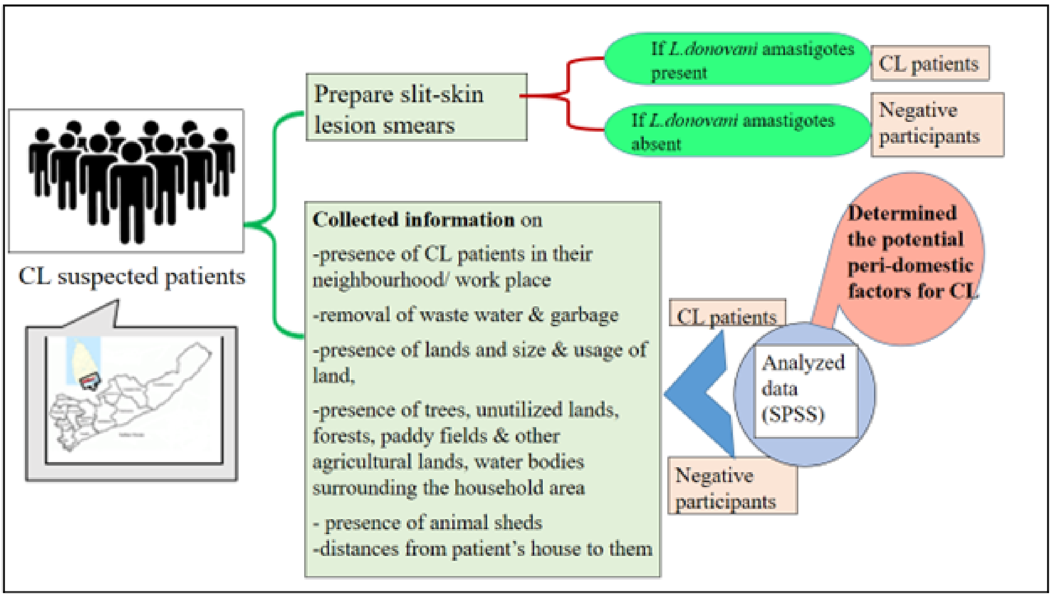

Highlights

- Cutaneous leishmaniasis (CL) cases are increasing in Hambantota district, Sri Lanka.
- There is a significant difference between CL patients and negatives neighbouring CL patients
- Open dumps, sewerages and vector-friendly habitats are potential peri-domestic factors

Ceylon Journal of Science 52 (1) 2023: 99-105
DOI: http://doi.org/10.4038/cjs.v52i1.8109

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Peri-domestic risk factors associated with transmission of cutaneous leishmaniasis in selected areas in Hambantota District, Sri Lanka

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Received: 09.09.2021; Accepted: 11.02.2023

Abstract: Cutaneous leishmaniasis (CL) is a notifiable disease in the Hambantota District. Aim of this study was to identify the peri-domestic factors which attributed to increase CL cases in the Hambantota District. CL suspected patients who attended the Tangalle Base Hospital were interviewed using a structured questionnaire. Information on the presence of CL patients in neighbourhood, and distance to their houses, land ownership and presence of vector habitats were recorded. CL patients were identified by examining them using slit- skin lesion smears. A total of 151 participants were CL positive. Among patients, 52.3% had family members/ neighbours / co-workers that infected with CL during last five years period. The size of the land ownership of 53.6% patients were >1,000 m^2 ($p<0.05$). Most of their gardens were covered with vegetation. Majority of patients (45.0%) dump their garbage in their own yards within <50 m distance from their houses ($p<0.001$). Eighteen percent of patients threw wastewater into their own yards within <100 m distance from their houses. Present findings revealed that distance of houses of noticeable number of patients were <100 m to unutilized lands (31.1%), shrub jungles (39.1%), agricultural lands (31.8%), forests (18.5%) and water bodies (16.6%). It is concluded that CL patients in this study profile are living very close to previously infected CL patients, vegetated lands, garbage sites and vector-friendly habitats such as shrub jungles, agricultural lands, forests and water bodies.

Keywords: Cutaneous leishmaniasis; Peri-domestic factors; *Leishmania*; Lesion smears

INTRODUCTION

Leishmaniasis is a protozoan parasitic disease which is caused by *Leishmania* spp. The disease is prevalent in South Asian countries such as India, Bangladesh and Nepal where it remains as a major public health problem (Joshi *et al*., 2008). In Sri Lanka, the first autochthonous case of cutaneous leishmaniasis (CL) was reported in 1992 (Athukorale, 1992). At present, CL is an established disease in the country affecting almost all districts of the island (Iddawela *et al*., 2018; Karunaweera *et al*., 2021).

Hambantota District is considered as a CL endemic area because there was a gradual increase in CL cases from 2001 to 2010, followed by Anuradhapura and Matara districts (Karunaweera *et al*., 2020). Meanwhile, clustering of CL cases had been reported until 2011 in Sri Lanka, mainly in Hambantota and Polonnaruwa districts. In 2011, 940 cases of CL were reported in Sri Lanka out of which 723 cases were confirmed through field investigations. In 2018, a rising number of CL cases (3,271 CL cases) were documented from nine districts of the country (Karunaweera, *et al*., 2020). Spatial distribution of CL cases is still increasing in the country with a southern hotspot (Karunaweera *et al.,* 2021). Meanwhile, a few cases of visceral leishmaniasis were also reported from Anuradhapura district in 2005 and 2007 (Abeygunasekara *et al*., 2007). The main clinical characteristic of CL in Sri Lanka is cutaneous lesions which develop at the site of the sand fly bite (Reithinger *et al*., 2007). The aetiological agent of local CL is *L. donovani* MON-37 which has been reported to cause human visceral leishmaniasis (VL) in the Indian subcontinent (Karunaweera *et al*., 2003; Siriwardana *et al*., 2007). VL is the clinical form that leads to the potential death of humans (Ranasinghe *et al*., 2015).

Intracellular protozoan parasite, *Leishmania* species requires two different hosts to complete its life cycle where humans and some other mammals act as definitive hosts and sand fly species acts as intermediate host or a vector. Humans are accidental host and they infect by contacting with vectors at their habitats or habitats of reservoir hosts (Desjeux, 2004). Sand fly *Phlebotomus argentipes* is considered as the possible vector of CL in Sri Lanka (Surendran et al., 2005a; Wijeratne *et al*., 2021)) and these *Phlebotomus* species can be found in desert or semi-arid eco-systems (Colacicco-Mayhugh *et al*., 2010). Three sibling species of *Argentipes* complex such as *Phlebotomus argentipes sensu stricto*, *Phlebotomus annandalei* and *Phlebotomus glaucus* are reported from the northern area of the country (Gajapathy *et al*., 2011). Alternatively, *P. argentipes* may feed on blood meals of cattle, goats, dogs, or rodents (Poche *et al*., 2018) and Senanayake *et al*., (2015) also reported that *P. argentipes glaucus* showed the zoophilic behavior. The finding of Nawaratne *et al*. (2007) indicates that the presence of *Leishmania* parasites in dogs in Sri Lanka. Siriwardana *et al*., (2010) reported that the transmission of *Leishmania* species was mainly zoonotic in the northern part while the transmission of the south of the country appeared to be peri-domestic. CL lesions may result in scars which can make mental or emotional problems in

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infected patients especially in Younger populations. The increase in CL incidence and prevalence is mainly attributed by risk factors associated with environmental conditions, socio-demographic status and human lifestyle activities. Research group in Brazil on American CL (Chaves *et al*., 2007) indicated that many factors involve in transmission of the diseases such as the diversity of causative agent, variety of vector species, existence of wild and domestic reservoirs, differences in environmental conditions (including climate, vegetation, rainfall and altitude) and differences in human factors (such as individual habits, housing conditions, work conditions and leisure activities). Other two studies also (Votypka *et al*., 2012; Reveiz *et al*., 2013) reported that environmental conditions, socio-economic status, demographic and human behaviors are major risk factors for human leishmaniasis. Keeping domestic and livestock animals in the house or surrounding areas attracts sand flies into human settlements known to increase the transmission of the disease to human (Bern *et al*., 2010). Furthermore, the risk of cutaneous and visceral leishmaniasis has been found to be associated with environmental factors such as land use, temperature and vector sand fly density etc. (King *et al*., 2004; Elnaiem *et al*., 2003; Chaves & Pascual 2006; Valderrama *et al*., 2010). Therefore, the current study was performed to identify peri-domestic factors which can be attributed to increased incidence of CL in the Hambantota district, Sri Lanka.

MATERIALS AND METHODS

Study location

Hambantota district is the largest administrative district in the Southern Province of Sri Lanka (6° 7' 28.5348'' N and 81° 6' 3.8664'' E) with a land area of 2, 609 km^2 and total population of 599,903. Annual average rainfall is 1,000-1,250 mm whereas annual average temperature is 30 ^{0}C (Department of Census and Statistics, Sri Lanka, 2012). About 90.7% of the populations of the Hambantota district live in rural areas and agriculture is the main income generating occupation among the people there (CEA, 1989).

Data collection

Data collection on possible peri-domestic factors was performed using an interviewer-administered questionnaire that has been used previously (Siriwardana *et al*., 2010). After clinical examination done by the Dermatologist, CL suspected patients were interviewed using a structured questionnaire. Before collecting information, participants were informed about the research clearly and their consent of the participation was obtained. If there were children, consent of their guardian was obtained. Information on the presence of any other CL patients in their neighbourhood or work place, peri-domestic factors such as presence of lands around their houses and size and usage of the land, presence of trees, unutilized lands, forests, paddy fields and other agricultural lands, water bodies surrounding the household area and presence of animal sheds and distances from patient's house to them were recorded from each patient.

Slit-skin lesion smears

After getting the written consent from each participant and under the supervision of the clinician slit-skin lesion smears were prepared from the suspected lesions on the day of enrolment of participants to the hospital. To prepare this thin smear, at the beginning, the lesion was cleaned with surgical spirit. Then, a cut was placed at the margin of the lesion using a sterile scalper blade and tissue substances were collected from the edges of the cutting place. Tissue substance was spread over a cleaned glass slide and it was left to air-dry. Prepared dried smears were stained with Giemsa and examined under a light microscope using an oil immersion for *Leishmania* amastigotes at the Department. Participants who had thin lesion smears containing *Leishmania donovani* amastigotes were considered as CL patients, whereas the participants having lesion smears without *L. donovani* amastigotes were considered as negative individuals. Slit-skin smear samples were not collected from participants who had lesions on their faces.

Statistical analysis

Information collected from CL patients and CL negative participants was analysed using the SPSS (Version 20.0). Participants whose slit-skin smears were free of *Leishmania* amastigotes were considered as negative individuals in the data analysis. Significant differences were tested with Non-parametric Pearson chi-square test at $p<0.05$ and $p<0.001$ levels.

RESULTS AND DISCUSSION

Tangalle Base Hospital and Hambantota General Hospital, two major hospitals in the Hambantota district, provide the services of clinical dermatologists. CL suspected patients from other hospitals in the Hambantota district were referred to these two hospitals for the correct diagnosis and proper treatments. Based on the preliminary data, it was observed that more CL patients were presented to the Tangalle Base Hospital more than to the Hambantota General Hospital. Therefore, the study was focused on the CL suspected patients who presented to the Tangalle Base Hospital within the limited study period from June, 2016 to January, 2017.

In total, 266 CL suspected patients were recorded. Out of them, 151 (56.77%) participants were positive for *Leishmania donovani* amastigotes (Figure 1) and a few displayed CL lesions (Figure 2). Participants with slit-skin lesion smears in which *L. donovani* amastigotes were not detected, were considered as CL negative participants. There were 115 negative participants in the present study. The data collected from CL patients and negative participants was given in table 1.

The CL patients were reported from District Secretary Divisions (DSDs) of Katuwana, Okewela, Walasmulla, Weeraketiya, Beliatta, Tangalle, Ambalantota and Angunakolapelessa in the Hambantota district. More CL patients of this study profile were reported from rural areas than urban areas of the district (Sudarshani *et al*., 2019). Generally, Tangalle, Ambalantota and Hambantota DSDs are considered as urban areas while the remaining DSDs

(Katuwana, Okewela, Walasmulla, Weeraketiya, Beliatta, Angunakolapelessa, Tissamaharamaya, Sooriyawewa and Lunugamvehera) as rural areas with patchy forests and agricultural lands.

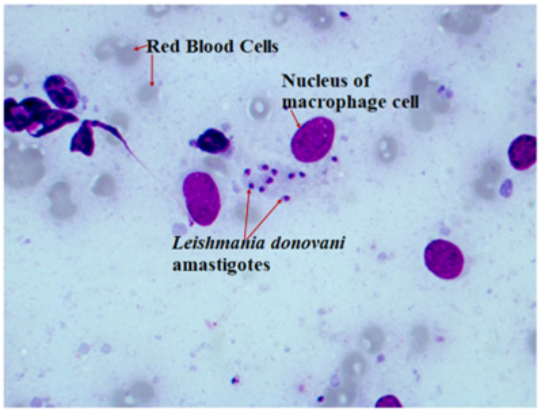

Figure 1: Presence of *Leishmania donovani* amastigotes in slit-skin lesion smears of patients (×1000).

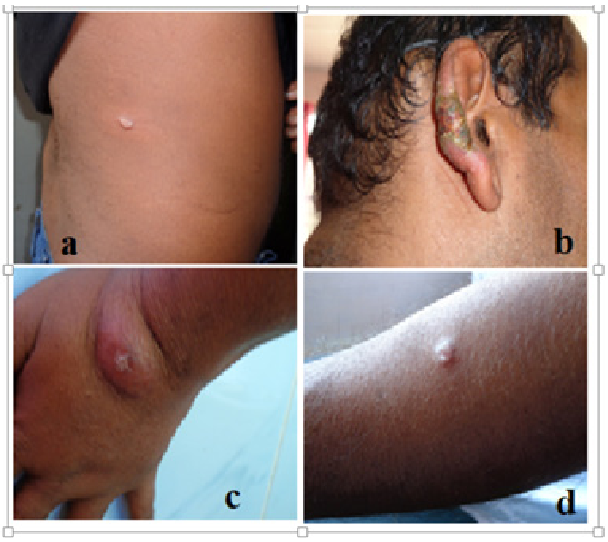

Figure 2: CL lesions in different body parts of the identified CL patients of the current study, **a**: on trunk, **b**: on ear lobe, **c**: on hand and **d**: on arm.

Out of all participants, 52.3% (N=79) of CL patients and 26.1% (N=30) of negative participants had family members or neighbours or co-workers that were infected with CL during the last 5-years period. However, there is a statistically significant difference between participants with and without CL having family members or neighbours or co-workers with CL (χ^2 (3)= 29.557 p=0.001). The protozoan parasite, *L. donovani* needs an insect vector and a mammalian host to complete its life cycle. The possible vector of Sri Lankan CL is *Phlebotomus argentipes* sand flies, which is found in different parts of the country (Lewis and Killick-Kendrick, 1973; Surendran *et al*., 2005a & 2005b). Another *Phlebotomus* species, *P. salehi* Mesghali which has been recorded from rural areas of Sri Lanka, is a proven vector of CL in India (Gajapathy and Surendran, 2012). The females of sand flies feed on mammal blood for their egg development and they are zoophilic in many areas (Lewis and Killick-Kendrick, 1973), but mainly anthropophagic in other countries (Lane *et al*., 1990). CL transmission is anthroponotic (ACL) (*via* human) or zoonotic (ZCL) (*via* wild or domestic mammals). Humans are the sole source of *Leishmania* infection for the sand fly vector in ACL and mammals such as cattle, rodents and dogs are reservoirs which maintain the transmission cycles and disseminate the *Leishmania* parasites in ZCL (Desjeux, 2001). Reithinger *et al*., (2010) reported that ACL risk is strongly associated with the presence of other CL infected members in the vicinity. As sand flies have short flight range, it is believed that sand flies get infected with *Leishmania* sp. when biting CL infected people in peri-domesic area indicating active ACL transmission.

Being a district with a majority of people rely on agriculture, a higher percentage of CL patients owned lands larger than 1,000 m^2 (CL patients: 53.68%, N=81; negative participants: 45.22%, N=52; χ^2 (3)=11.110, p=0.011). A majority of these lands were covered with tall trees such as coconut (*Cocos nucifera*), mango (*Mangifera indica*), teak (*Tectona grandis*), Jak (*Artocarpus heterophyllus*) and kohomba (*Azadirachta indica*) as well as other plants such as banana (*Musa* spp.), vegetables, ginger (*Zingiber officinale*) and pepper (*Piper nigrum*). The vector, phlebetomine sand fly adults feed on vegetable sugars and females require vertebrate blood for the development of their eggs (Cameron *et al*., 1995), while the larvae feed on small organisms in the soil (Hanson, 1968). Memmott (1991 & 1992) reported the importance of having large trees for their survival as sand flies use tree bases for their mating and resting, swarming, feeding and waiting for preferable vertebrate hosts. A study carried out by Poche *et al*. (2017) stated that in Bihar, India a high numbers of *P. argentipes* were captured from banana plants and palm trees. Poché *et al.* (2012) also indicated that *P. argentipes*, despite being a weak flyer, is capable of vertical dispersal even up to 18.4 m from the ground level.

Present findings indicated that 82.1% of CL patients and 31.9% of negative participants discard wastewater through pipelines to a place away from their houses, while 18% (18%, N=27) of CL patients and 64.9% (N= 74) of negative participants discard wastewater to the immediate vicinity of their houses (less than 100 m distance). Sixty-eight CL patients (45%) and seventy-four (64.4%) negative participants were dumping garbage on their homegardens and most of these open dumps (92.1% of CL patients and 85.2% of negative participants) were located less than 50m away from their houses (Table 1). Out of them, 175 patients (55.6% CL patients and79.1% of negative participants) are burning collected garbage daily (CL patients: 8.6%, negative participants: 26.1%) or weekly (CL patients: 69.5%, negative participants: 41.7%). As a result, wastewater and garbage that are rich with organic matter provide a good food source for larval stages of the vector, sand flies, as well as for domestic (dogs) and wild mammals (rats) which are considered as reservoir hosts of zoonotic cutaneous leishmaniasis. Dawit *et al*., (2013) described that poor sanitary conditions such as lack of waste management and open sewage could increase breeding of sand flies as they prefer moist soils rich in organic and nitrogenous matter to breed and feed their larvae (Napier and Smith, 1926).

Table 1: Statistical parameters following chi-square test in factors tested among CL patients in Hambanthota, Sri Lanka.

Factor	No. of CL patients (N=151)	No. of negative participants (N=113)	Pearson Chi-value	df	P value
Presence of other CL patients					
Family members/relations	39	26			
Neighbours/Co-workers	38	3			
Both co-workers & neighbours	2	1			
No any other CL patient	72	85	29.557	3	0.000
Land area					
<1000m^2	70	63			
>1000 <2000m^2	15	3			
>2000 - <4000 m^2	28	11			
>4000 m^2	38	38	11.110	3	0.011
Presence of tall trees within 30m distance					
Yes					
No	109	63			
	42	52	8.652	1	0.003
Removal of waste water					
Via pipe line	124	41			
To surface of land	23	19			
Both	4	35			
Others (throw to a pit)	0	20	83.429	3	0.000
Garbage removal by					
Burning	82	34			
Surface dumping	66	16			
Both burning and dumping	2	58			
Others (to MC..etc)	1	7	104.152	3	0.000
Garbage discharge distant					
<50m	139	98			
Between 50m – 100m	7	6			
>100m	5	11	4.632	2	0.099
Unutilized land distant					
≤100m	47	26			
>100m	1	4			
No	103	85	4.780	2	0.092
Shrub jungle distant					
≤100m	59	52			
>100m	1	1			
No	91	62	1.086	2	0.581
Agricultural land distant					
≤100m	48	30			
>100m	2	1			
No agricultural land	101	84	1.199	2	0.549
Forests distant					
≤100m	28	24			
>100m	1	2			
No forests	122	89	0.947	2	0.624
Water bodies distant					
≤100m	25	22			
>100m - <500m	3	3			
>500m	1	0			
No water bodies	122	90	1.171	3	0.760
Presence of Animal shed within 30m distance					
Yes	3	2			
No	148	113	0.022	1	0.883

Leishmania donovani transmits by the insect vector, sand fly species. Sand fly species prefer to live in shady, damp areas and researchers described that vegetative areas like shrub jungles and agricultural lands, as well as water bodies are suitable habitats for them to thrive. Hence, the current findings indicated that noticeable number of CL patients were living very close (≤ 100m) to ecosystems such as unutilized lands (CL patients: 31.1%, N=47; negative participants : 23%, N=26), shrub jungles (CL patients: 39.1%, N=59; negative participants: 45.2%, N=52), agricultural lands such as paddy fields and coconut, banana and pepper cultivations (CL patients: 31.8%, N=48; negative participants : 26.1%, N=30), forests (CL patients:18.5%, N=28; negative participants: 20.9%, N= 24) and water bodies (CL patients: 16.6%, N=25; negative participants: 19.1%, N=22) (Table 1). Pedrosa and Ximenes (2009) and Ranasinghe *et al*., (2013) reported that living at the edge of paddy fields or near forests less than 200m from the house are risk factors for the infection of CL. In Sri Lanka, pepper bushes, termite mounds and cattle huts were identified as the resting sites for adult sand flies, and immature stages of sand flies have been detected from rice paddies, irrigation tanks, decaying hay, cattle huts and canals (Wijeratne and Gunathilaka, 2020). The findings of the current study indicate that there is no any statistically significant difference between the number of CL patients and negative participants living nearby the vector preferred habitats. Generally, to get infected by this disease, it is essential to contact between a *Leishmania* parasite infected vector and a human. Lifestyle of the people in Southern Sri Lanka and increased outdoor activities during the evenings increase the human–sand fly contacts (Kariyawasam *et al*., 2015). Asgari *et al*., (2007) and Valderrama *et al*., (2010) also indicated that environmental factors such as forest coverage, proximity to woodlands, new agricultural projects, irrigation and storage of waste products close to urban areas increase sand fly, vector populations by providing breeding and feeding places which are associated with risk factors of CL in old world.

Presence of animal kennels or chicken cages within 30m distance from houses is also important when peri-domestic transmission of CL is carrying out at the study area. However, a rather low percentages of CL patients (2%, N=3) and negative participants (1.8%, N=2) had dog kennels or chicken cages in their homesteads in the present study. Dogs are considered as hosts in canine leishmaniasis and other domestic animals such as cats, cattle and goats and wild animals such as rodents have been identified as reservoirs in other CL infected countries (Akhoundi *et al*., 2016; Palatnikde-Sousa & Day, 2011; Rohousova *et al*., 2015; Dereure *et al*., 2000).

A previous study using the same study profile reported that males were more prone for the infection compared to females, while house-wives and students were the most affected occupants in the study area (Sudarshani, *et al*., 2019). This may happen due to the risk factors that CL commonly includes the household proximity to forested areas or other areas where sand flies are known to aggregate, and migration of household members in CL prevalent areas (Hewitt *et al*., 1998; Pedrosa & Ximenes, 2009)

CONCLUSION

The study suggested that the transmission of the parasite in the study area is mainly through humans and sand fly vectors. On the other hand, immediate peri-domestic areas of CL patients provide ideal habitats for CL vectors, *Phlebotomus argentipes*, increasing of vector populations. The combination of these peri-domestic factors and infected humans may have led to the high prevalence of CL in the study area.

ACKNOWLEDGEMENT

Authors would like to acknowledge the financial support provided by the University Grants Commission in Sri Lanka for the completion of the research work (Grant No.: UGC/DRIC/PG/MAY 2014/RUH 02) and Medical staff of Tangalle Base hospital for their support to collect data from study participants.

CONFLICT OF INTERSTS

The authors declare no competing interests. Necessary ethical approval for this study was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Ruhuna, Sri Lanka.

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