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Risk Factor Analysis of Cutaneous Leishmaniasis in Sri Lanka through a Nationwide Survey

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Abstract. Leishmaniasis in Sri Lanka was first reported in the early 1990s. Cutaneous leishmaniasis (CL) cases have markedly increased in recent years, demanding due attention from health authorities. The spatial distribution of CL is not homogeneous. This case-control study investigated factors that may contribute to this heterogeneous distribution through a nationwide study. Information on sociodemographic, economic, and environmental characteristics was collected from study participants (cases, $n = 303$; controls, $n = 2,762$). All individuals were followed up for 3 years, and signs of CL or associated complications were recorded. Differences in possible risk factors between cases and controls were analyzed. Individuals $<$18 years old, electricity supply, spending $>$2 hours outdoors, visiting jungles/water bodies, and living near CL patients were identified as risk factors. Household members of 1.3% of cases, 2.3% of controls residing within a perimeter of 500 m from a patient, and 0.8% of controls living beyond 2 km from a case developed CL. Thus, CL in Sri Lanka appears intertwined with living environment and host behavior. Common environmental factors may be responsible for the higher risk of CL in individuals living in close proximity to CL patients. This may at least partly explain the clustering of CL cases in selected areas of the country.

INTRODUCTION

Leishmaniasis is a vector-borne disease caused by the protozoan parasite *Leishmania* spp. and transmitted by the sand fly (*Phlebotomus* spp. and *Lutzomyia* spp.). It is a global health problem affecting approximately 90 countries in the world, with 700,000 to 1 million new cases and 70,000 deaths reported annually.[1] There are three main forms of leishmaniasis, namely cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL). An estimated 50,000–90, 000 new cases of VL occur annually.[2] Cutaneous leishmaniasis also poses a major threat worldwide, with more than 200,000 new cases reported in 2020.[3] The predominant form of leishmaniasis in Sri Lanka is CL, with MCL and VL reported sporadically.

The first locally acquired CL case was reported in 1992.[4] However, administrative records of leishmaniasis date back to the early twentieth century.[5] There has been a steady increase in the burden and spread of disease since 2001, with CL cases reported by all 24 administrative districts in Sri Lanka, with five districts reporting $>$10 cases per 1,000 population per year from 2001 to 2019.[6] Although the cases have spread across the island, the distribution is not homogeneous,[6,7] and according to spatial statistical and risk factor analyses, differences in social, economic, and environmental characteristics in different areas may contribute to these differences.[8–11]

The causative agent is *Leishmania donovani* MON-37, which is better known as causing VL in East Africa and India.[12] Furthermore, the same zymodeme of *L. donovani* was also reported from autochthonous VL in Sri Lanka.[13] Although *L. donovani* MON-37 is known to cause both VL and CL in Sri Lanka, it makes distinct genetic clusters in molecular analysis[14,15] and is speculated to be a result of mutations and/or amplifications of selected parasite genes with resultant phenotypic differences.[16]

Disease surveillance is important in determining the disease burden and distribution, monitoring of disease transmission trends, and forecasting of outbreaks. Early detection of outbreaks is important to enable effective preventive measures, such as planning and implementation of control methods and resource allocations, in a timely manner.[17] A major part of surveillance constitutes cases and foci investigation, which involves active case detection after reporting of an index case (proactive surveillance).[18] Reactive case detection (usually referred to as "contact tracing") is an equally important technique that helps track down more cases after detection of an index case. Reactive case detection is conducted within a specified area, usually within a predetermined radius around the index case, with the goal of detecting further transmission by identifying additional infections.[19] This is based on the assumption that individuals living in close proximity to an index case are more likely than a randomly selected individual residing away from the disease focus to get the infection. Transmission hot spots with clustering of cases in space and time can feed disease transmission throughout the year.[20] Similarly, risk factor analyses remain important to enable planning for an effective disease control program. The risk factors of leishmaniasis are known to be multiple, interconnected, and diverse.[11,21] These include environmental (e.g., rainfall, foliage, water bodies), socioeconomic (e.g., education, occupation), and demographic (e.g., gender, age, ethnicity) as well as genetic (both host and parasite) factors. Although studies have been conducted previously in Sri Lanka to identify the risk factors associated with CL, the geographic coverage has been limited (i.e., limited to a province or a district) or confined to a certain group of individuals (e.g., army soldiers serving in the north).

This study aimed to explore the risk factors associated with CL through a nationwide study with the assessment of the magnitude of their effect on disease acquisition and spread. This study investigated the risk of acquiring the

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infection by comparing those living in the vicinity of an index case with those living at a distance (i.e., disease risk of neighborhood versus non-neighborhood individuals).

MATERIALS AND METHODS

Ethics approval. Ethics approval for this study was granted by the Ethics Review Committee, Faculty of Medicine, University of Colombo, Sri Lanka (Ref. no. EC-17-962).

Recruitment of study subjects and data collection. The study was carried out as a case-control study. Sample size of the index cases was calculated to detect an odds ratio (OR) of 1.5, using a two-tailed type 1 error of 5% and a power of 80% with assumption made as to 20% of controls having a higher risk or exposure. The controls (1,500 neighborhood controls and 1,500 non-neighborhood controls) were recruited using a 1:5:5 case-to-neighborhood-to-non-neighborhood control ratio.[22] The minimum case number needed (minimum sample size) per calculation was 300.

To select 300 cases (index cases) from across the country (Figure 1), a method based on sampling intervals with consideration of the number of cases reported from each national health administrative unit was used. For administrative purposes, the country is divided into health units that include provincial health units (largest level), units at the regional level (medium), and the Medical Officer of Health (MOH) level (smallest health administrative unit), which operates within the health care system. All MOH areas (in all nine provinces) on the island were initially included in the sample frame. The MOH areas to be selected and the number of index cases to be included from each MOH area were calculated proportionate to the case prevalence of each of the MOHs per patient data recorded at the Epidemiology unit, Ministry of Health in 2018. The list of selected MOH areas is included as supplementary information (Supplemental File 1).

After the number of index cases to be included from each MOH area was determined, individuals were randomly selected from a list of laboratory-confirmed (either microscopically or by polymerase chain reaction) CL patients (maintained by the MOH office) reported within the past 3 months of the visit. The patients selected were contacted with the aid of the Public Health Inspector of each MOH. For each index case, five controls residing in the neighborhood (i.e., residing within <500 m) and five controls residing in a non-neighborhood area (i.e., residing >2 km away) were selected, with a total of 10 controls for each index case. The distances of the controls from the index cases were decided based on the approximate flight range of the sand flies.[23] A total of 303 index cases were included.

The residences of selected individuals were visited by the investigator teams, and a validated case reporting form (CRF) was completed by trained research assistants. Individuals with a previous history of CL were not included in the study sample. The information obtained through the CRF was uploaded into the RedCAP (research electronic data capture) software and electronically stored securely.[24]

Reactive case detection and follow-up of index cases. The index cases, and near and distant controls were followed up by contacting them via telephone at 6 monthly intervals for up to 3 years. The individuals listed on the control list were contacted 2–3 years after the first data collection; information regarding their health status was gathered (i.e., whether they had acquired new skin lesions and, if yes, whether they had been clinically confirmed as having leishmaniasis). Index cases were also questioned regarding likely symptoms of visceralization, such as prolonged fever, loss of weight, and loss of appetite. The same information was collected from the household members of the index cases, who were not initially included in the study sample. The information was used to determine whether any of the associated controls had acquired the disease within the past 2–3 years. The information was cross-checked through records maintained at each MOH office by public health officials.

Data analysis. The total number of records collected was $N = 3{,}303$ (cases, $n = 303$; controls, $n = 3{,}000$). The data were transferred to an Excel worksheet (Microsoft Excel 2016), and all the records were checked for any obvious errors, duplicates, and incomplete records. These records were cross-checked with the hard copies of the CRFs, and errors were corrected. The records, which cannot be used for further analysis, were removed from the database ($n = 238$). The cleaned database (cases, $n = 303$, and controls, $n = 2{,}762$; total, $N = 3{,}065$) was transferred to an SPSS V20.0 spreadsheet for further analysis.

Differences between cases and controls (both neighborhood and non-neighborhood) in demographic characteristics, travel, house types, peri-domestic environment, economic factors, behavioral factors, and contact with animals were analyzed in detail using the χ^2 test. Univariate analysis (binary logistic regression) was carried out to determine the ORs and the 95% CI limits of each variable compared with a baseline characteristic to determine the effect of the assessed variables on disease outcome. Variables that indicated a significant impact on disease outcome were used in a final model (using multiple logistic regression) to assess their collective impact.

The χ^2 test to determine the differences between the cases and controls, binary logistic regression, and final multiple logistic regression, were also repeated in a gender-stratified manner to separately determine the effect of these risk factors on males and females.

RESULTS

The study sample comprised 303 index cases and 2,762 controls (total study sample size, $N = 3{,}065$). The samples were collected from 74 MOH areas in 14 administrative districts covering all nine provinces of the island (Figure 1). The highest numbers of cases were from the southern province (36.1%), north central province (25.8%), and northwestern province (15.6%).

The number of females ($n = 1{,}937$; 63.2%) was nearly double compared with the number of males ($n = 1{,}128$; 36.8%) in this study sample. Almost all the study participants (99.2%) belonged to the ethnic group "Sinhala." The age of the study population ranged from 1 to 93 years, with a mean of 45.23 years.

Reactive case detection and patient follow-up. All individuals included in the cases and control groups were contacted via telephone between 2 and 3 years after the first interview to check for the development of CL lesions. All the index cases remained contactable even 3 years after the initial data collection. Of the 2,762 controls whose data were used for further analysis, 788 (28.5%) were uncontactable.

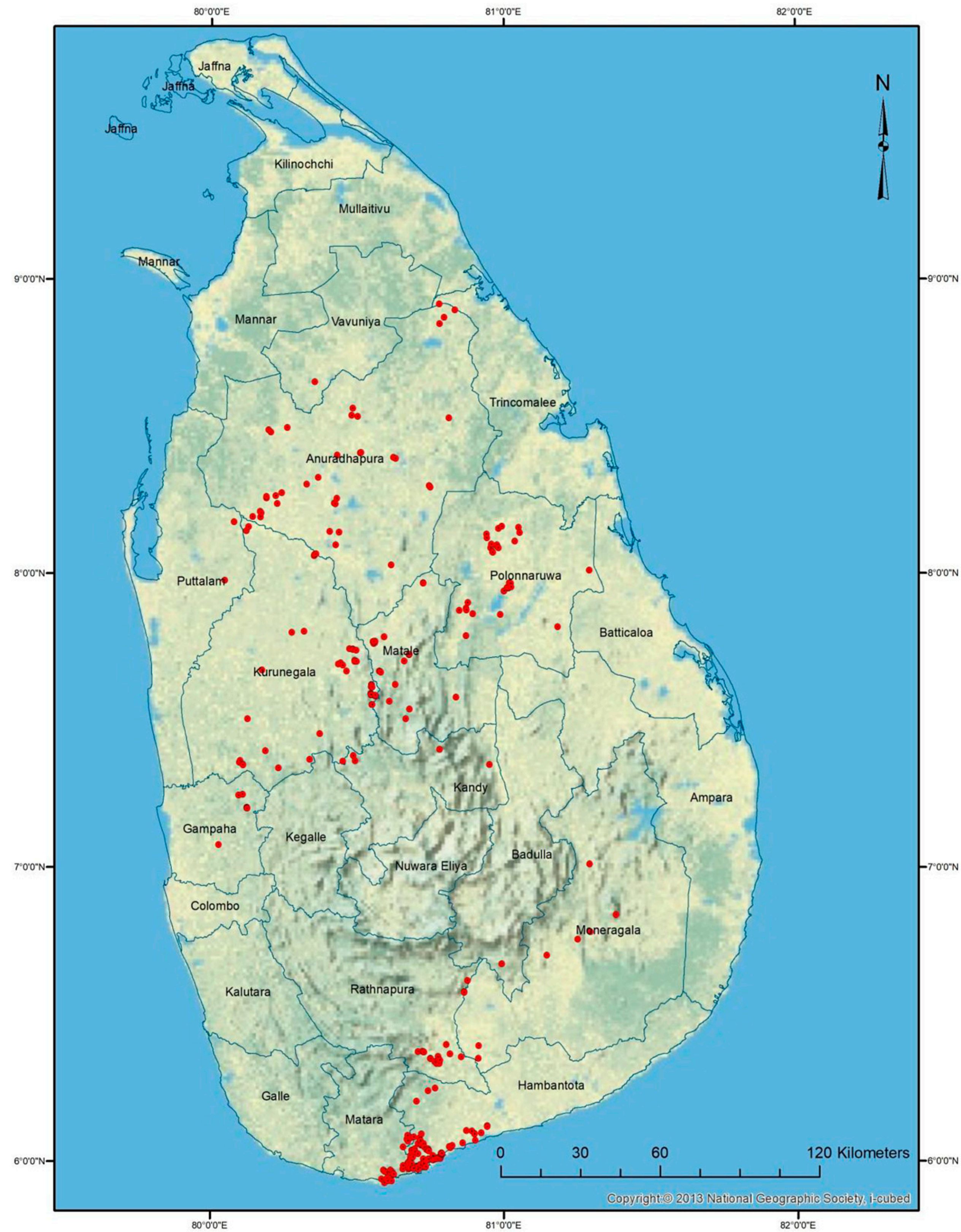

FIGURE 1. Locations of residences of the patients included in the study.

Upon inquiry, four (1.3%) index cases indicated that individuals from their households had become positive for leishmaniasis within the past 3 years. Similarly, 31 (2.3%) and 11 (0.8%) neighborhood controls and non-neighborhood controls, respectively, reported *Leishmania*-positive skin lesions during the follow-up period.

Most of the household members/controls (both near and distant) who acquired the disease during the 3-year follow–up period were reported by the Kurunegala district ($n = 13$; 28.3%). Nine new cases were reported by the Hambantota district. The Polonnaruwa and Matara districts each reported six new cases in exposed household members and control groups. None of the index cases developed recurrences or new lesions or reported any symptoms of visceralization during this 3-year follow-up period. Further information on reactive case detection is summarized in Supplemental File 2.

Differences in risk factors between cases and controls and binary logistic regression to assess the effect of risk factors on disease outcome. Differences between cases, neighborhood controls, and non-neighborhood controls were seen in gender, education, occupation, age, duration of outdoor activity, and behavioral activities (e.g., visits to the jungle, visits to water bodies, use of bed nets). Significant differences between the three groups are summarized in Supplemental File 3.

Binary logistic regression analysis revealed that males (compared with females), students (compared with unemployed males), children <18 years old (compared with individuals over 60 years of age), individuals living in households with an electricity supply, and individuals spending more time in outdoor activities had higher odds of acquiring the disease (Supplemental File 4). Differences were seen between males and females with the use of gender-stratified analysis. All occupational groups of males considered were at significant risk of getting CL compared with unemployed ones ($P < 0.05$), whereas only students and government/private sector or retired employees had a risk of being CL positive in the female group. Wearing protective clothing during outdoor activities was a significant factor that determined disease outcome for males but not for females. Furthermore, the risk of having a CL infection was different between males and females in different age groups as well as depending on time spent outdoors. (Supplemental File 5).

Multiple logistic analysis for assessing the collective effect of predictor variables. Multiple logistic regression analysis was carried out to assess the collective impact of predictor variables, which had a significant effect on disease outcome (i.e., gender, educational group, occupational group, age group, roof type, sleeping location, duration of outdoor activities, visits to the jungle and water sources, livestock-related activities, and contact with CL patients [Table 1]).

Of these, individuals in select educational level groups (i.e., studied up to grades 6–11 and ordinary level [O/L] passed), occupational groups (self-employed, government or private sector workers, or retired), students, individuals younger than 18 years, and individuals who made weekly visits to jungles or weekly or monthly visits to water sources were at significant risk (Table 1).

Individuals with lower educational levels had higher odds of acquiring the disease (for individuals who had studied up to grades 6–11, OR = 3.049, $P = 0.007$, and for the O/L passed group, OR = 2.179, $P = 0018$) than individuals with higher educational attainment (advanced level [A/L] and above).

People with an occupation had more than twice the odds of being a case compared with an unemployed person (OR > 2, $P < 0.007$). The highest odds were for the student group, with a 4 times higher risk of getting the disease compared with the unemployed group (OR = 4.014, $P = 0.009$). Interestingly, individuals who lived in houses with asbestos roofing had a marginally significant but negative risk ($B = -0.374$, $P = 0.056$; OR = 0.688, 95% CI = 0.468–1.01).

The combined effect of risk factors on males and females separately were also assessed in multivariate analysis. The risk factors for males were similar to the ones that had a significant effect on the whole study group (Table 1); however, for females, "other" occupations (i.e., self-employed, family worker, government worker, private sector worker, or retired) except being a student did not pose an increased risk of acquiring the disease. Female students had a high risk of acquiring CL similar to that of male students (OR = 8.018, 95% CI = 4.161–15.44). All other risk factors considered remained the same for males and females.

DISCUSSION

This countrywide study was conducted to assess infection risk due to living in close proximity to leishmaniasis-infected individuals and to identify other possible risk factors (sociodemographic, environmental, economic, and behavioral) associated with CL in Sri Lanka. Data were collected based on a nationwide survey that was conducted across all nine provinces of Sri Lanka, including 14 administrative districts (out of 25) in 74 MOH areas.

As suspected, reactive surveillance indicated that neighborhood controls had a higher risk of acquiring the disease

TABLE 1
Multivariate analysis (the combined effect) of the characteristics with significant effect on CL

Characteristic/Risk Factor	*B*	*P*	OR (95% CI)
Educational group (grades 6–11) **(compared with A/L/degree or above)**	1.115	0.007	3.049 (1.365–6.810)
Educational group (O/L passed) **(compared with A/L/degree or above)**	0.779	0.018	2.179 (1.141–4.162)
Occupational group (self-employed/unpaid family worker) **(compared with unemployed group)**	0.769	0.004	2.158 (1.279–3.642)
Occupational group (government/private sector/retired) **(compared with unemployed group)**	0.859	0.006	2.360 (1.285–4.334)
Occupational group (student) **(compared with unemployed group)**	1.39	0.009	4.014 (1.408–11.44)
Age group (< 18 years old) **(compared with > 60-year-old group)**	1.209	0.015	3.352 (1.259–8.923)
Roof (asbestos) **(compared with tiled roofs)**	−0.374	0.056	0.688 (0.468–1.010)
Visits to jungle (weekly) **(compared with the group that never visited the jungle)**	0.798	0.016	2.221 (1.158–4.258)
Visits to water sources (weekly) **(compared with the group that never visited water bodies)**	0.95	0.001	2.586 (1.451–4.607)
Visits to water sources (monthly) **(compared with the group that never visited water bodies)**	0.883	0.007	2.418 (1.270–4.602)

A/L = advanced level; CL = cutaneous leishmaniasis; O/L = ordinary level; OR = odds ratio. The baseline factors which each risk factor is compared with is indicated in boldface within brackets.

than did non-neighborhood controls. The main reason could be shared risk factors, such as the presence of vector-breeding places in the vicinity (i.e., jungle areas, areas with water accumulation).[10,25–27] Interestingly, household members of an index case did not appear to be at higher risk of acquiring the disease compared with others. This might indicate the multifactorial nature of risk factors that coexist. Furthermore, such household members may be better informed about the disease and may follow preventive/control methods that may have kept the disease at bay. A survey done in parallel with this study, which was conducted to assess knowledge, attitudes, and practices regarding leishmaniasis in the same study sample, revealed that knowledge of the disease and disease transmission was significantly high in index cases compared with the control samples,[28] which supports the above assumption. Similarly, the odds of being a case were significantly higher when the contact was a coworker rather than a household member, a relative, or a neighbor (Supplemental File 4). Although having a CL patient as a close contact was recognized as a risk factor for acquiring the disease in a previous study conducted in a limited geographic area and based on patient history records,[29] the current analysis that involved nationwide survey data reveals more descriptive and confirmatory details.

Individuals with an occupation (i.e., self-employed, employed by the state or private sector, or retired) and students had higher odds of being patients in the univariate analysis (Supplemental File 4), and these occupational groups/students imposed a significant influence on disease outcome in the final model compared with the unemployed group of individuals (Table 1), with the latter group being the least affected. This could be due to the fact that people who are employed spend more time outdoors, exposing themselves to sand flies. Furthermore, in rural areas self-employed, small-scale family business owners have more interactions with the environment (e.g., chena cultivators amid the jungle, people involved in small-scale pottery business, sand mining from riversides) than people who are unemployed (mostly women in this study). This assumption holds true for students, who are likely to spend a considerable amount of time outdoors during school hours as well as in the evenings while playing. This result, however, contradicts the outcome of the study described by Iddawela et al.,[29] where they found that unemployed persons were the most affected. The assumption that occupation is not a significant risk factor for females (who may not participate in outdoor work as much) was further strengthened in the final model of the gender-stratified analysis, which demonstrated the effect of interactions with the surrounding environment on disease acquisition in an individual.

The proportion of children under 18 years of age among cases was significantly high compared with the proportion within the control group (21% in cases and 4.4% in controls, respectively; Supplemental File 3). This group had a >6 times higher risk of having CL compared with the older age group, those >60 years (Supplemental Files 4 and 5, Table 1). Similar observations were made previously in Sri Lanka,[8,29] as well as in other countries.[30,31] A possible cause for children to be at higher risk of acquiring the infection (and subsequent disease manifestations of CL) could be the higher proportion of time that children spend outdoors, probably playing, during evening hours, subjecting themselves to sand fly bites.[29] Similarly, the relative immaturity of their immune systems as well as malnutrition could play a role in their susceptibility to the disease.[30] Similar observations have been made elsewhere as well.[32]

Individuals who have studied up to grades 6–11, as well as those who have passed the O/L, were at higher risk of getting the disease compared with individuals who had studied up to the A/L and had achieved more qualifications, which was demonstrated in univariate and final multivariate analyses (Supplemental Files 4 and 5, Table 1). This indicates that education and being informed and aware of the disease may play a role in protecting exposed communities.

None of the housing characteristics had an impact on disease outcome according to the final regression model, except for asbestos roofing (Supplemental Files 4 and 5, Table 1). Having asbestos roofing sheets had a negative impact on having the disease compared with those with clay tile roofs (Supplemental File 5; $B = -0.422$, $P = 0.001$; OR $= 0.656$, 95% CI $= 0.511–0.842$). Asbestos sheets heat up in the sun compared with clay tiles and could reduce the vector density within houses, whereas clay tiles provide a much cooler environment for the sand flies to rest in. This has been observed in other geographical areas as well.[33] However, a previous study done in the Matara district of southern Sri Lanka indicated that the type of walls in the households (e.g., unplastered brick walls) are a significant risk factor for CL.[10]

Individuals visiting water sources or jungles at least monthly or more frequently are at higher risk than individuals who do not engage in such practices. Sand flies usually inhabit jungle areas or are in the vicinity of water bodies; therefore, they would be abundant in such environments.[23] The importance of these environments as breeding sites for sand flies was indicated previously.[25,34,35] Kariyawasam et al.[10] previously made the same observations with noticeable levels of case clustering observed near jungle areas in the southern province of Sri Lanka. However, contradicting observations were reported in another study done in Sri Lanka, where exposure to scrub jungles did not play a significant role in acquiring the disease.[29]

Although not significant in the final model, electricity supply to households seems to play a role in disease development, indicating a higher risk of CL occurrence in households with electricity in the univariate analysis. It could be assumed that illuminated houses can attract more sand flies inside compared with relatively darker households with a weaker source of light (i.e., candles and oil/kerosene lamps), thereby increasing the risk of infection. In contrast, some studies revealed that having electricity in households reduced the risk for the disease owing to the likelihood of electric lights attracting sand flies away from the humans compared with candles or lamps, which are generally kept on tables or shelves close to where people sit or work.[36]

Similarly, in the univariate analysis individuals who spent more time outdoors were at higher odds of getting the disease than those who did not, especially at dawn, dusk, or during the daytime. This is understandable, as in rural areas, where foliage is abundant, individuals who spend more time outdoors are likely to have a higher chance of being bitten by sand flies. Similar observations have been made in previous studies as well.[10]

There were more males than females in the index case group (Supplemental File 3), and in the univariate analysis, males had more than twice the odds of becoming infected with leishmaniasis compared with females (Supplemental File 4). This could be due to the fact that males spend more time outdoors where sand flies are abundant (i.e., jungle areas, rivers) compared with women. Furthermore, men in rural areas remain bare chested, particularly when engaged in manual labor, owing to hot and humid weather, which exposes them more to sand fly bites.[8,29,37] The proportion of males in the case records maintained by the relevant MOH offices is also higher than the proportion of females recorded, which further confirms that males have a higher risk of acquiring the disease. However, gender did not play a significant role in the final model (multivariate analysis; Table 1). Furthermore, the number of females in the control groups was significantly higher than the number of males. The survey occurred during the daytime, when data collection teams visited the households to complete the questionnaires, which may have led to this outcome. During these hours, most males are away at their workplaces with only the females left at home. The majority of the work force in rural communities in Sri Lanka tend to be males, with females either functioning as housewives or engaged in small-scale businesses near their residence.[38] The case and control groups therefore comprised unequal proportions of males and females. This can be considered a major limitation of this study. However, gender-stratified analysis was carried out to minimize the effect.

The fact that none of the treated index cases developed recurrences or new lesions of CL, in spite of the likely persistent risk of exposure, favors the view of lasting immunity after cure of CL.[39,40]

In conclusion, individuals in the younger age groups (children <18 years old) and those engaging in more outdoor activities, (i.e., employed individuals, schoolchildren, those who visit water sources and jungles) had higher odds of acquiring the disease according to the final regression model, indicating that probable exposure to sand fly bites through outdoor activities is a critical risk factor for disease acquisition. These findings can be used by health authorities in designing and implementing elimination strategies (i.e., prevention of man-vector contact especially while outdoors). Furthermore, having a CL patient in the neighborhood imposes a higher risk of acquiring the disease compared with individuals without a close contact. However, those living in the same household with patients are not at such high risk of infection, possibly because of better awareness of preventive and control measures. This finding highlights the importance of public awareness programs that impart knowledge of disease transmission and avoiding vector contact in containing the disease in Sri Lanka. Educating young people, especially children, on infection transmission and its prevention through the school system may be an effective way of achieving this end.

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Data availability: The data presented in this study are available from the corresponding author on request. The data are not publicly available owing to the fact that the dataset contains personal identifiers.

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