Original Research

Ecological Dynamics of Sand Flies (Diptera: Psychodidae) in Two Leishmaniasis Endemic Foci in Sri Lanka

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Abstract

Background: Leishmaniasis has emerged as a significant global public health concern and has been notable in Sri Lanka since 1992, particularly in the Anuradhapura district, where Cutaneous Leishmaniasis is prevalent. The disease is caused by the Leishmania parasite, which is transmitted by sand flies of the genus Phlebotomus.

Objective: This study compared the diversity, dominance, and density of sandfly species in selected locations within the Anuradhapura and Kegalle districts.

Methods: Sand flies were collected monthly from January to March 2024 from locations identified through patient records from the Regional Epidemiologist's unit. Collection was conducted using cattle-baited traps (CBT), and identification was performed morphologically and through DNA analysis. Ecological indices, including Simpson's Dominance Index for species dominance and Shannon's diversity index for species diversity, were calculated using the collected data.

Results: A total of 154 sandflies were collected from ten locations in both areas. Among the collected samples, 87.6% (n=135) were P. argentipes and 5.19% (n=8) were P. stantoni, both of which belong to the genus Phlebotomus. Additionally, 7.4% (n=11) were Sergentomiya zelanica belonging to the genus Sergentomiya. P. argentipes predominantly dominates locations in the Anuradhapura district, where Simpson's dominance index is one (D=1), highlighting its high-risk status. In contrast, the Kegalle district showed higher species diversity with uneven distribution patterns. The Shannon Diversity Index reflected the highest diversity in Kegalle (H=0.6365), likely due to multiple species assemblages and ecological complexity.

Conclusions and Recommendations: This comparative study underscores the importance of understanding sandfly population dynamics for targeting integrated vector control strategies. Tailored vector control is essential to prevent the annual emergence of new endemic foci of leishmaniasis in Sri Lanka. This study also emphasizes the integration of environmental, entomological, and epidemiological approaches for effective leishmaniasis control in Sri Lanka.

Keywords: Leishmaniasis; Phlebotomus argentipes; Species Diversity; Vector Control; Ecological Indices

Background

Leishmaniasis is a serious public health concern worldwide. This parasitic disease is caused by a protist belonging to the Leishmania genus. The first local transmission in Sri Lanka was reported in 1992, and the Epidemiology Unit of Sri Lanka officially recognized it as a notifiable disease after 2008 [1]. This has become a major public health issue in the country. Among the three main forms (cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL), CL is the prominent, locally acquired form in Sri Lanka (2). Anuradhapura District is recognized as one of the high-risk districts in Sri Lanka. From 2009 to 2016, the Anuradhapura district reported the highest number of leishmaniasis cases (n=2671), comprising 31.5% of all identified patients. Although the disease has been mainly concentrated in specific areas, such as Anuradhapura, Hambantota, Polonnaruwa, and Matara, new foci emerge annually in Sri Lanka [2].

Understanding the transmission mechanisms of Leishmania is crucial for elucidating its lifecycle and role in disease transmission, and Phlebotomus argentipes is considered the sole vector of Leishmania donovani, the identified causative agent in Sri Lanka [2]. Recent studies have indicated that the population dynamics of this vector species are highly influenced by seasonality and associated risk factors [1]. Despite this significant finding, a comprehensive understanding of the ecological factors related to transmission dynamics remains incomplete because of the limited exploration of sand fly species diversity and species dominance in the area.

Therefore, it is important to identify the principal sandfly species, their diversity, and dominance in leishmaniasis-endemic areas of the country to inform future research and strengthen vector control strategies through integrated environmental, entomological, and epidemiological approaches.

Objective

The objective of this study was to identify the principal sand fly species and compare species diversity and dominance between the two leishmaniasis-endemic districts in Sri Lanka

Methods

study setting

Two endemic foci in the two districts were selected as the study setting based on past epidemiological data obtained from the Regional Epidemiologist's units (RE Units) in both Anuradhapura and Kegalle districts. After analyzing the data, the Anuradhapura district was recognized as a high-risk district, whereas the Kegalle district was classified as low-risk.

Trapping locations were randomly selected and varied in biotopes, including cow stables, chicken pens, and suburban jungles.

Data related to land coverage of both districts were obtained from the ArcGIS Living Atlas of the World website (https://livingatlas.arcgis.com/).

Collection of sandflies

Sandflies were collected using cattle-baited traps (CBT). Traps were placed where the patients were reported during the time period and in areas where the known vector control interventions were not being carried out (e.g., fogging and cleaning programs). The above data were extracted from RE units in both Anuradhapura and Kegalle districts.

The CBTs were set up at 6.00 p.m., and sand flies were collected between 11.30 p.m. and 2.00 a.m. in the day. This timing was selected to maximize the capture of sand flies during their peak activity period, which is considered the period between dusk and dawn (2). The total number of CBTs that were set up in Anuradhapura was six (n=6) and that in Kegalle was four (n=4).

The captured sand flies were kept in paper cups and transported to the laboratory at the Department of Bioprocess Technology, Faculty of Technology, Rajarata University, Sri Lanka. Subsequently, the samples were stored in 70% ethyl alcohol until further analysis.

Identification of sand flies

Morphological identification of the collected sandflies was performed using published taxonomic keys [3]. Male and female sand flies were differentiated based on the presence of genitalia.

Measurements such as the wing index (R2/R2+3), wing overlap (R1 overlap/R2), ratios of the length of the labrum, length of the wing, length of antennal segment 3, and length of antennal segments 4+5 were measured using a Nikon Stereo Microscope SMZ18.

Cryptic species were identified using molecular identification methods.

Data analysis

The data were analyzed using the following ecological indices:

1. Species dominance

This is a measure of diversity that considers both the richness and evenness of sand flies in the area. Ranging from 0, all taxa are equally present, to 1, which is one taxon that dominates the community completely. This was determined using Simpson's Dominance Index [4].

Simpson's Dominance Index = ∑ (ni * (ni - 1)) / (N * (N - 1))

Where:

D = Dominance Index

ni = number of individuals in the ith species

N = total number of entities in the dataset.

2. Species diversity

$$H' = -\sum_{i=1}^{s} p_i \log p_i$$ [5]

The diversity of each sand fly species was determined using the Shannon Diversity Index (H). Diversity increased with an increase in the index value. The higher the number, the higher the species diversity. The Shannon Diversity Index had a maximum value of 5 [5].

Where:

H' = Shannon Diversity Index

Pi = fraction of the entire population made up of species

S = numbers of species encountered/species richness

∑ = sum from species 1 to species S

All diversity-related data was calculated by using the Paleontological Statistic Software Package for Education and Data Analysis (PAST) software version 4.03.

Results

A total of 154 flies belonging to two genera were collected during the study period. Among the collected samples, 87.7% (n=135) were P. argentipes and 5.2 % (n=8) were P. stantoni, both belonging to the Phlebotomus genus. Additionally, 7.1% (n=11) were Sergentomiya zelanica belonging to the genus Sergentomiya. However, only P. argentipes was found at the selected study locations in the Anuradhapura district during the study period.

The species dominance and diversity of the captured sandflies are presented in Table 1. Simpson's Dominance Index indicated that P. argentipes overwhelmingly dominated the study area in Anuradhapura district (D=1). In contrast, Simpson's Index for the Kegalle district was 0.3395, indicating uneven distribution among the species, with P. argentipes being the most prominent species (n=76, 80%) (Figure 2). The Shannon Diversity Index revealed high species diversity in the Kegalle district (H=0.6365), whereas in the Anuradhapura district, it was 0.

Given that P. argentipes is considered the sole vector for leishmaniasis in Sri Lanka and completely dominates the Anuradhapura district, it is noteworthy that the male-to-female sex ratio for sandflies is 7:1. In contrast, Kegalle district exhibited the lowest ratio of 18:1 (Table 2).

Discussion

This study compared two leishmaniasis-endemic focus districts, where ecological and meteorological factors differ significantly. The study results offer valuable insights into the dominance and diversity of sandfly species in these areas, underscoring the importance of understanding their distribution and abundance in relation to disease transmission.

Kegalle district is classified as a low-risk district for leishmaniasis. The Shannon Diversity Index recorded the highest diversity in this area, which was higher than that in the Anuradhapura district. The presence of multiple species, although P. argentipes remains the most abundant species, may indicate more complex ecological conditions in the area. These multiple species assemblages could lead to competition among anthropophilic sand fly species for hosts, thereby diluting pathogen transmission.

Studies have demonstrated that the species richness and evenness (species diversity) of arthropods decrease with increasing agricultural fields and an altered landscape structure [6]. This explains the lower sand fly diversity in Anuradhapura, where 29% cropland coverage occurred (Figures 3 and 4). It has been suggested that degradation of forest cover and/or the proliferation of secondary vegetation may lead to the disappearance of sand fly species due to competition among species for microhabitats [7]. Simpson's index showed that P. argentipes predominantly dominated the Anuradhapura district, which is considered a high-risk district for leishmaniasis in Sri Lanka.

In addition, although some studies have shown that the infection biting rate is independent of land use and depends only on the availability of the mammalian community [8], this study shows that it is indirectly linked to land coverage. Kegalle district and Anuradhapura have the same construction area coverage of 32%, except for a similar mammalian community. However, Kegalle has a more concentrated construction area, whereas Anuradhapura has a wide dispersal build area (Figure 4). This must dilute the available infected and host communities. However, land coverage in the Anuradhapura area favors sand fly species that are closely related to human activities, such as P. argentipes. Simpson's index showed that P. argentipes dominated the Anuradhapura district. However, the Kegalle district shows unevenness in species distribution, with high species richness. This may be due to the low crop land coverage (4%) and high vegetation/forest coverage (51%). These patterns of prevalence of P. argentipes are noteworthy, given their role as disease vectors.

The observed differences in sex ratios among the study areas were intriguing. Even though the highest number of samples was collected from the Kegalle district, it had the lowest male-to-female sex ratio (18:1), in contrast to Anuradhapura (7:1). These variations may be indicative of different mating behaviors related to habitat or ecological dynamics. For example, male sandflies are attracted to plants as a source of sugar meals. Plants also provide suitable resting, breeding, and mating sites for males [9]. This ultimately results in disease transmission in the area.

Conclusion and Recommendation

From an epidemiological point of view and considering the vector-parasite relationship, we consider that multiple assemblages of sand fly species with high density could create competition within and between the species, thereby diluting disease transmission. As this study was conducted in a narrow time frame, this factor can be correlated with underlying factors, such as host species density [10] and patient availability. However, understanding the distribution, dominance, and diversity of sandfly species in a particular area is crucial for implementing area-specific control measures and mitigating the risk of disease transmission.

The current study utilized only CBTs to collect sand flies from outdoors. This decision was based exclusively on its effectiveness in capturing sand flies, especially Phlebotomus argentipes sp., which has been proven to be the sole vector for leishmaniasis transmission in Sri Lanka.

As the objectives of this study were to compare species diversity and dominance between different ecological settings in endemic areas, CBT provided valuable insights into outdoor sand fly populations, which is also important for understanding disease transmission.

However, the authors believe that future studies should incorporate indoor collection methods, such as human landing, double traps, hand collections, and CDC light traps. Using these approaches would provide a more comprehensive understanding of both indoor and outdoor sand fly compositions. This information is essential for developing targeted prevention and control strategies for leishmaniasis.

Further research into the ecological factors influencing sandfly populations in these areas would also be valuable for developing targeted intervention strategies and preventing the annual emergence of new endemic foci.

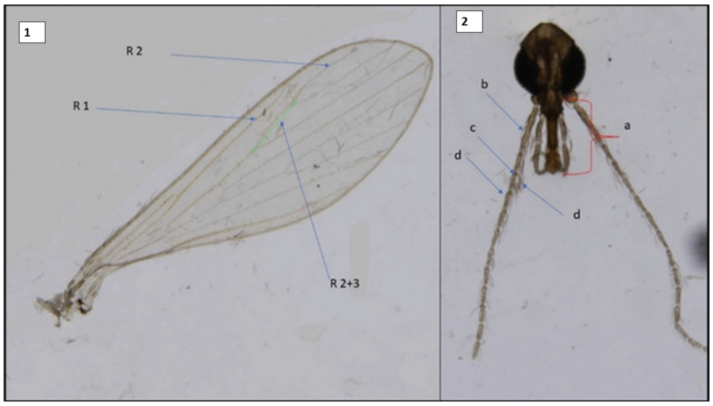

Figure 1 : Important measurements for the identification of the sand flies; (1.) Wing measurements (2) Head measurements (a) Length of the Labrum (b) antennal segments 3 (c) antennal segments 4 (c) Antennae 5 (d) Ascoid on antennal segments 4

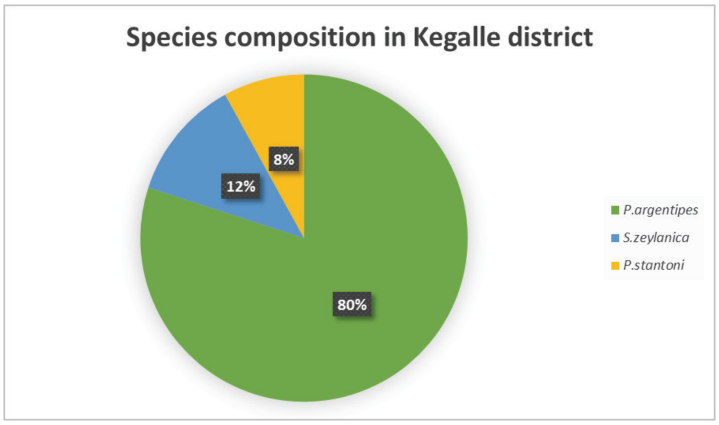

Figure 2 : Species composition in the Kegalle district

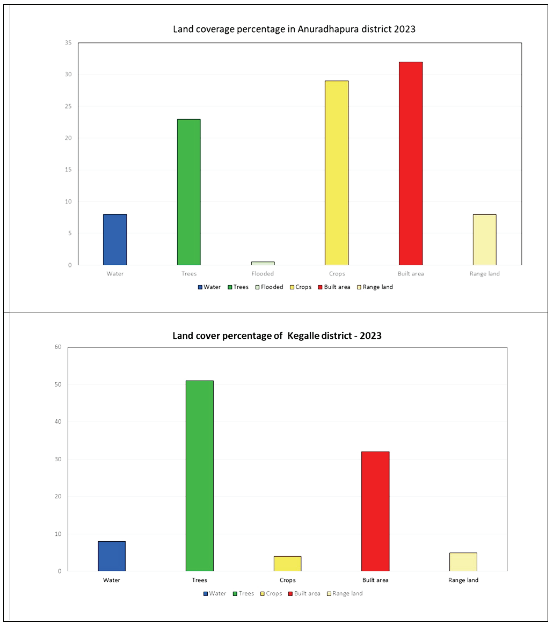

Figure 3 : Land cover percentage of Anuradhapura and Kegalle District 2023 (https://livingatlas.arcgis.com/)

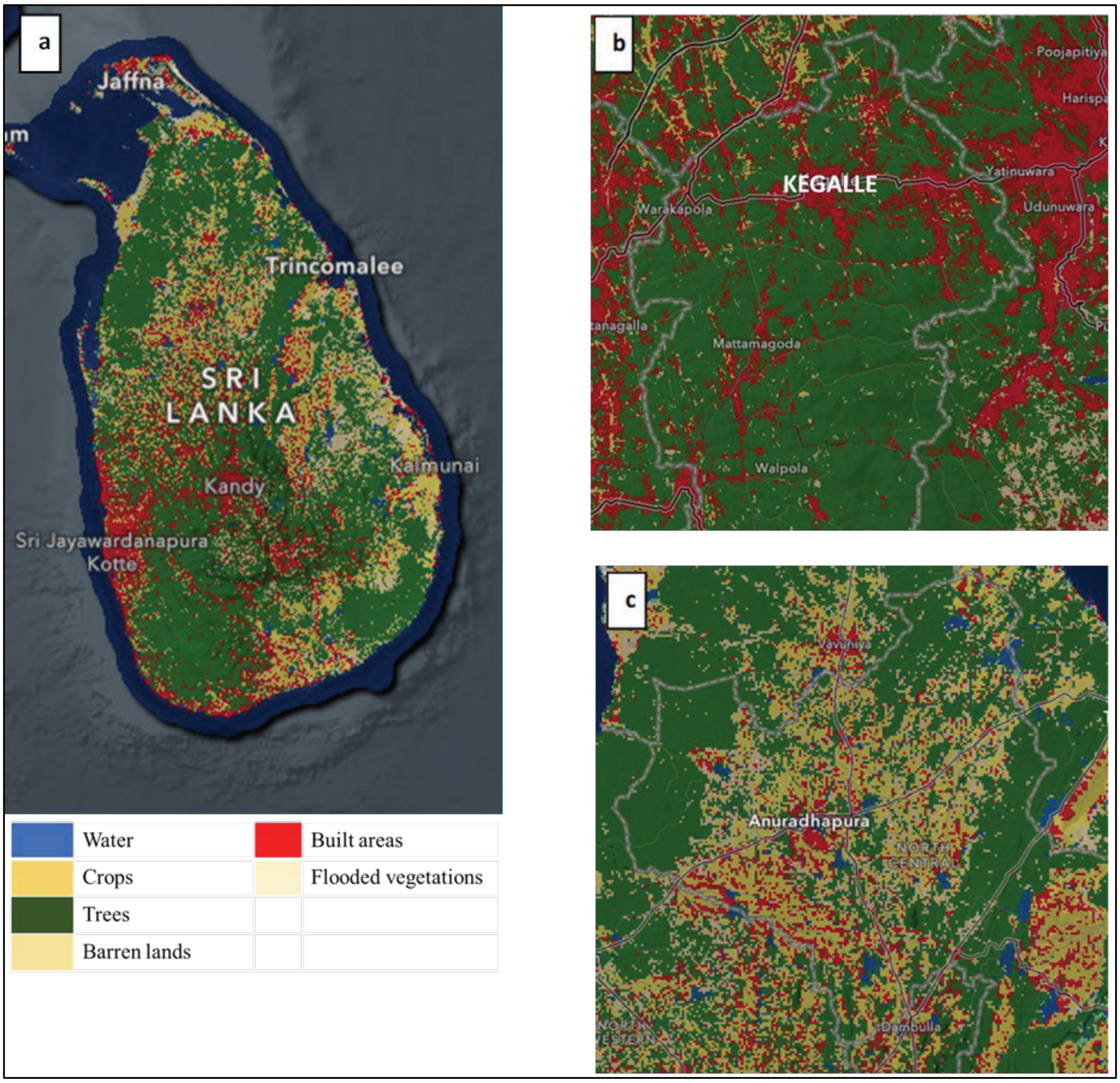

Figure 4: Dispersal patterns of the land cover in Sri Lanka (a) Land cover dispersal pattern in Sri Lanka (b) Kegalle district (c) Anuradhapura district (https://livingatlas.arcgis.com/)

Table 1: Species dominance and the species diversity of the study areas

	Anuradhapura	Kegalle
Number of taxa	1	3
Number of Individuals	59	95
Dominance index	1	0.3395
Shannon Diversity index	0	0.6365

Table 2: Sex ratio details of the P. argentipes in the study areas

Study area	No. of males	No. of females	Sex ratio (Male: Female)
Anuradhapura	49	7	18.1
Kegalle	50	5	3.1

Author declaration

Author contributions: All authors contributed to the conceptualization and design of the study. MM, KW and NDADW contributed to the acquisition of data and conducted the data analysis. MM, NDADW and PK contributed to data interpretation and writing the manuscript. All authors read and approved the final manuscript.

Conflicts of interest: The authors declare no conflicts of interest

Ethics approval and consent to participate: The study was exempted from the review process by the Ethics Review Committee, Faculty of Applied Sciences, Rajarata University of Sri Lanka.

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