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Distribution of *Phlebotomus argentipes* Annandale & Brunetti, 1908 in the Anuradhapura district, North Central Sri Lanka

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ABSTRACT

Background & objectives: *Phlebotomus argentipes* Annandale & Brunetti, 1908 (Diptera: Psychodidae) is the main vector responsible for the transmission of *Leishmania donovani* (Laveran & Mesnil, 1903) Ross, 1903 in the sub-continent of India. It is the potential vector of cutaneous leishmaniasis in Sri Lanka. The present study determined ecological factors that influence the abundance of *P. argentipes* in areas with high disease prevalence in the Anuradhapura district, North Central Sri Lanka.

Methods: CDC light traps and yellow sticky traps were used for sampling, and abundance was recorded throughout 12 months with selected environmental parameters namely, relative humidity, wind speed, and temperature. The relationships between the abundance of *P. argentipes* with mean temperature, % relative humidity, and wind speed were tested with regression analysis. The temporal distribution of the vector population was tested with a time series analysis.

Results: The study identified the most preferable microhabitats of *P. argentipes*: shrubs, unclear areas, gardening areas, wet soil areas with leaf litter, and termite hills. The results indicated that the abundance of *P. argentipes* was highly dependent on mean temperature ($P = 0.00$, $R^2 = 68\%$), and a high number of *P. argentipes* was recorded for a low mean temperature range of 24.7–27.3°C. Furthermore, the abundance of *P. argentipes* exhibited an increasing trend with high humidity levels of 72–88% ($P = 0.00$, $R^2 = 91.6\%$).

Interpretation & conclusion: These findings may help predict the temporal variation of the potential vector population with studied ecological parameters and contribute to a successful vector management strategy with thorough knowledge of the behavioral pattern of *P. argentipes*.

Key words *Phlebotomus argentipes*; relative humidity; wind speed; temperature; microhabitats

INTRODUCTION

Leishmaniasis is a potentially fatal mammalian disease caused by a species of parasitic protozoan *Leishmania*[1]. In humans, the disease clinically manifests in three clinical syndromes/forms: Visceral Leishmaniasis (VL), often known as Kala-azar in the Indian sub-continent, cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL)[2]. The local causative agent of the disease is *Leishmania donovani* (Laveran & Mesnil 1903)[1] MON-37, and it has an identical resemblance to the parasite causing VL in India; *L. donovani* MON-2[3].

Since 2008, cutaneous leishmaniasis has become a notifiable disease with an intensifying incidence trend in Sri Lanka. Even though occasional cases were reported from all the provinces, agreeing with Rajapaksa *et al.*,[4] most leishmaniasis cases were recorded in the North Central Province. The highest number of CL patients was recorded in Anuradhapura and Polonnaruwa districts during the last few decades. Nowadays, leishmaniasis has become an endemic vector-borne disease resulting in over 99% of cases of CL in the Anuradhapura district[5–6].

The female Phlebotomine sand flies (Diptera: Psychodidae) are the vectors of the leishmaniasis parasite. Facts about possible vectors of the leishmaniasis are limited, and only a few studies have pointed out the distribution of the potential vector of leishmaniasis, *Phlebotomus argentipes* Annandale & Brunetti 1908, in Sri Lanka[4]. Studies on parasitic aspects[7] and a few studies on vector aspects, including anthropophagic behaviour of *P. argentipes*[8], have been carried out so far in Sri Lanka. However, studies on ecological factors that may directly or indirectly influence leishmaniasis transmission and environmental factors that affect sand fly distribution are less in Sri Lanka[9].

High leishmaniasis transmission risk is related to the abundance of vectors which depend on environmental factors. Therefore, the objective of the present study was to determine the selected ecological factors that demand the spatial and temporal distribution of *P. argentipes* in Anuradhapura district, Sri Lanka. We sampled sand flies from distinct microhabitats within three different areas where a high disease prevalence of CL has been recorded.

MATERIAL & METHODS

Study area

The study was carried out in the Anuradhapura district, Sri Lanka. According to the studied data of the Medical officer of Health (MOH) office, Anuradhapura district, during the five years (2010–2015), three sampling localities were selected: Padaviya (80 52. 666° N, 800 47. 595° E), Thalawa (80 10. 786° N, 800 22. 633° E) and Wijeyapura (80 19. 433° N, 800 24. 464° E) where CL prevalence was the highest. Padaviya area is a highly pastoral area with a low population, while Wijeyapura is an urban area with a high population density. The Thalawa region is a vastly agricultural area and moderately occupied. A GIS map was created using ArcGIS 10.2.1 to indicate these sampling sites (Fig. 1).

Anuradhapura district belongs to the dry zone in the Northern part of Sri Lanka. The climate is tropical, and summers are much rainier than winters in Sri Lanka. The average temperature, rainfall, wind speed, and humidity are 29°C, 1368 mm, 12.1 miles per h (mph), and 67% relative humidity (% RH), respectively. The study area consisted of shrubs and semi shrubs, unclear areas,

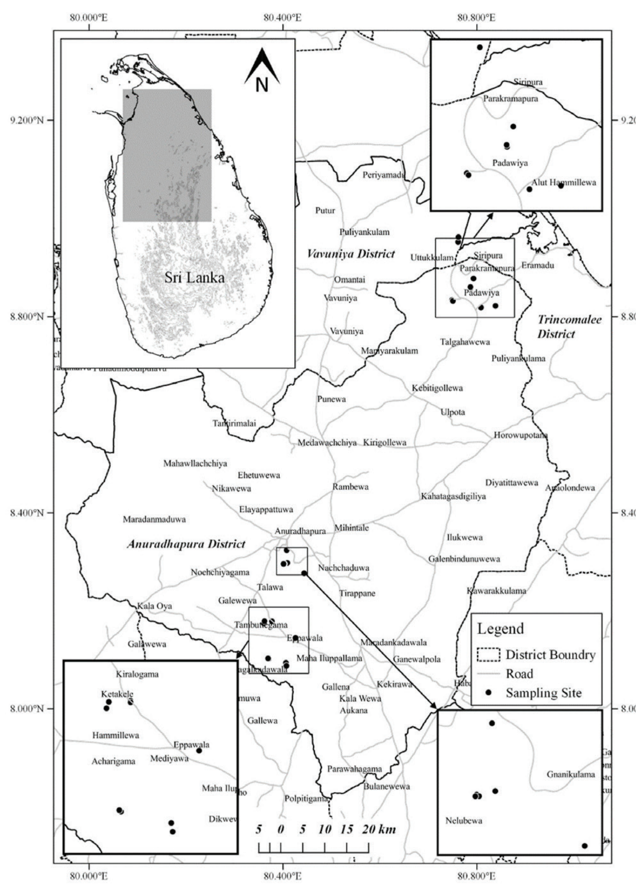

Fig. 1: Geographic Information System (GIS) generated map indicating the study sites in North Central province of Sri Lanka.

water streams, gardening areas, and wet soil areas. Tree cover was considerably less dense and covered with annuals, especially Maana grass (*Glyceria* spp.), with lots of decaying organic matter such as leaf litter. The Soil is comparatively ironic in nutrients. These kinds of microhabitats were more or less equally distributed in all three study sites while Thalawa and Padaviya were dominated by water streams and wet soil while they were less common in Wijeyapura.

Sand fly collection

An entomological survey was performed throughout the year (March 2016 to February 2017) using CDC light traps and yellow sticky traps. The collection took place once a month in each locality, Padaviya, Thalawa and Wijeyapura in the Anuradhapura district. Considering the most distinct features, eight habitats namely: shrubs, water streams, unclear areas, farming areas, wet soil areas with leaf litter, clearings covered with *Glyceria* and around termite hills were selected and 30 sticky traps and five light traps were set up. The light traps with a 5W bulb were operated for 12 h (16:30 to 06:30), and sticky traps were set up in the field for around 15 h. The temperature, humidity, and wind speed were recorded hourly for 5 h from the setup and for 2 h before the recovery of the traps to measure the average values.

The collected sand flies were transferred into 70% ethanol and were labelled accordingly. Specimens were cleared and mounted in Berlese medium for further identification. All the identifications in the present study were based on morphological characteristics using the identification keys of Lewis[9] and descriptions of Ozbel *et al*.[10].

Data analysis

The abundance of *P. argentipes* was calculated as a percentage.

$$\%\ \text{Abundance} = \frac{\text{No. of specimens of } \textit{Phlebotomus argentipes}}{\text{Total specimens of } \textit{Phlebotomine} \text{ species collected}} \times 100$$

The analysed data were used to illustrate the possible correlation between the dispersal of *P. argentipes* and the ecological factors studied (temperature, wind speed, and relative humidity).

Statistical analysis

Statistical analyses were performed using MINITAB 14 (Minitab Inc. 2004). All the data sets were subjected to the Anderson-Darling normality test. Once the data followed a normal distribution, they proceeded with regres-

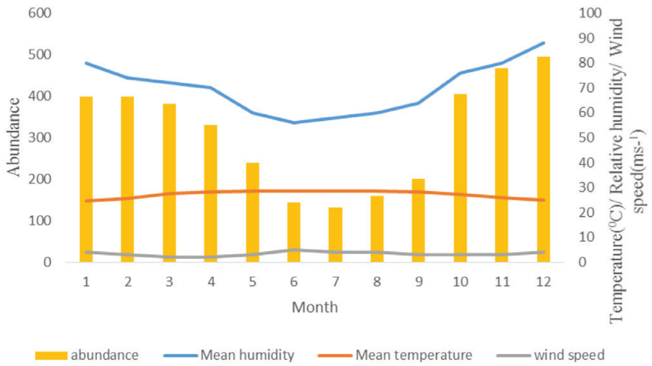

Fig. 2: Variation of the abundance of *Phlebotomus argentipes* with the temperature, relative humidity, and wind speed during a year. All these factors were obtained as monthly values.

sion analysis, one-way ANOVA and Tukey Kramer HSD test and time series analysis to determine the effect of the ecological factors on the potential vector population.

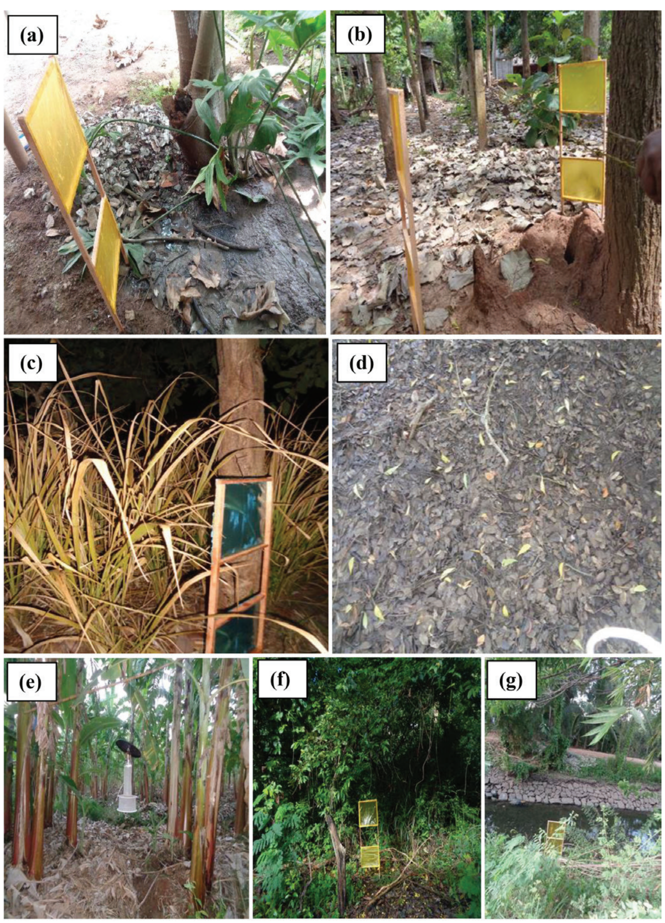

Fig. 3: Microhabitats identified in the Leishmaniasis prevalent areas in Padaviya, Thalawa and Wijeyapura A. domestic wet soil areas B. termite hills C. grass area D. decaying leaf litter E. gardening area F. shrubs and unclear area G. water stream.

Ethical statement: Not applicable

RESULTS

The present study determined the effect of ecological factors on the abundance of *P. argentipes* in the three study sites: Padaviya, Thalawa and Wijeyapura of the Anuradhapura district (Fig. 1). According to Figure 2, the relationship between the abundance of *P. argentipes* and the mean temperature was significant (P = 0.00, R^2 = 68 %), and an elevated abundance was recorded at a low mean temperature range of 24.7–27.3°C. Further, the abundance of *P. argentipes* exhibited an increasing trend with increasing humidity levels of 72–88 % (P=0.00, R^2=91.6%). However, the effect of the wind speed was not significant on the abundance of the vector (P=0.229, R^2=11.8 %).

Additionally, the current study identified five microhabitats in which the abundance of adult vectors is high: shrubs, unclear areas, gardening areas, wet soil areas of leaf litter, and around termite hills (Fig. 3A, B, D, E & F). However, water streams and grass areas did not support the population dynamics of *P. argentipes* (Fig. 3C & 3G). The present study identified 3754 *P. argentipes* specimens throughout the different environmental gradients studied

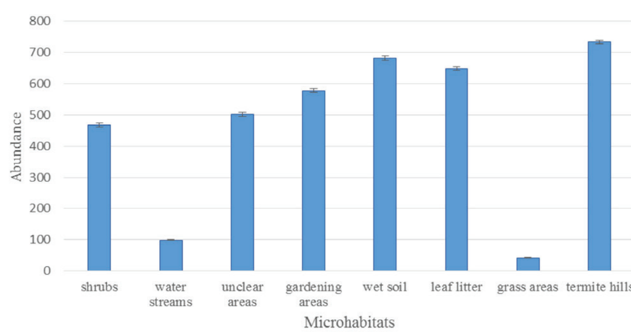

Fig. 4: The abundance of *Phlebotomus argentipes* in different microhabitats.

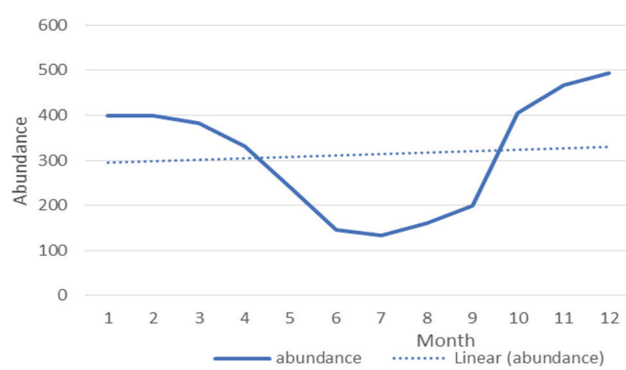

Fig. 5: Temporal distribution of *Phlebotomus argentipes* throughout the year (March 2016 to February 2017).

during the entomological survey. The results revealed a significant difference in the mean abundance of *P. argentipes* with different microhabitats (one-way ANOVA, $F_{7, 88} = 16.73$, P = 0.00; Fig. 4).

The abundance of *P. argentipes* exhibited a temporal variation during the study period, and the maximum population was recorded from October 2016 to February 2017. The time series plot (Fig. 5) exhibited a cyclical component following the upward linear trend model; abundance = 271.115 + 7.6648t.

DISCUSSION

In the current study, areas with the highest populations of *P. argentipes* were recorded. Hence, these sites are potential areas of high disease prevalence. Consequently, the present study identified potential environmental factors that predict the presence or absence of sandfly species. Also, it determined the relationships of ecological factors that deal with the abundance of *P. argentipes*. The situation revealed that low temperatures and high humidity levels support the abundance of vector populations. However, there was no correlation between the vector population and wind speed. These findings were in line with the temporal distribution of vector populations[9]. Therefore, *P. argentipes* abundance were high in the cold months of October 2016 to February 2017 while it was lower in the hotter months, June 2016 to August 2016. A previous study has shown no correlation between altitude and sand fly densities[9]. In addition, it revealed that main climatic factors such as rainfall, wind and temperature are the essential factors affecting the dispersal of sandfly species. It also reported that sand fly abundance is heavily dependent on temperature. Therefore, sand fly species along altitudinal gradients must acclimatise to the variability of climatic conditions[9]. However, the temperature has become one of the main factors preventing the spread of both VL and CL[11–13].

Phlebotomus argentipes is a very tiny delicate organism which is highly vulnerable to drying. According to Killick-Kendrick[14], sand flies usually prefer cool and humid resting places not directly exposed to sunlight. These include houses, latrines, cellars, stables, caves, fissures in walls, rocks, dense vegetation, tree holes, and caves of rodents and other mammals. Agreeing with Hugh-Jones[15] and Beck *et al*.,[16], the current study shows environmental variables correlated to disease occurrence with geoprocessing are crucial for landscape epidemiology of *P. argentipus*. The present study identified seven microhabitats preferred by *P. argentipus*: (i) domestic wet soil area, (ii) termite hills, (ii) grass area, (iv) decaying leaf litter, (v) gardening area, (vi) shrubs and unclear area (vii) water stream. The highest preference was for shrubs and unclear areas. Banana plantations were one of the highly engaged agricultural lands in the study area, and most CL patients were farmers associated with them. This microhabitat of banana plantations is usually dense vegetation with higher moisture levels and shadow areas with large leaves. Therefore, the vector may prefer associating with banana plantations, and it was observed that hanging dried leaves is the ideal resting place for adult sand flies. Figure 2 shows that the preference for termite hills is higher than in other microhabitats. It was observed that Phlebotomine sand flies were resting on the inner walls of termite hills. This observation may be due to constant moisture inside the termite hills.

Generally, the female sand fly lays eggs in burrows of certain rodents, cracks of walls, animal shelters, and household organic rubbish, where the emerging larvae can find organic matter, heat, and humidity necessary for larval development[17]. The study area was highly vegetative; thus, lots of decaying leaf litter was present everywhere, and this is one of the beneficial and ideal features of their oviposition. Decaying organic matter is the primary food source of larval stages of Phlebotomine sand flies, and gravid females usually prefer to lay their eggs in areas with high food availability to ensure the survival of their next generation[17]. This may be the main reason for selecting areas with decaying leaf litter as a microhabitat of *P. argentipes*. In addition, domestic wet soil areas are highly supportive of enhancing the abundance of vectors as oviposition preference of female adults in moist soil is the highest[17].

The present study revealed how the environmental factors affect the spatial and temporal distribution of the potential vector *P. argentipes* in selected study sites of the Anuradhapura district. It may assist and contribute to the current knowledge on the spatial and temporal distribution patterns of *P. argentipes* that would finally lead to a successful vector elimination strategy in managing CL in Sri Lanka.

CONCLUSION

The present study determined the distribution of *P. argentipes* in their microhabitats based on climatic and environmental factors. High humidity levels and low temperature enhance the abundance of the potential vector, *P. argentipes*. However, the wind speed does not influence the vector population. The preferred microhabitats of *P. argentipes* are shrubs, wet soil areas, decaying leaf litter, termite hills, and gardening areas. The temporal distribution of the primary vector exhibits cyclic components. Moreover, the trend model will help predict the seasonal changes in vector population with the changes in ecological parameters. Further, the present study contributes to the existing knowledge while on its way to a successful vector elimination strategy that may be a key component in eradicating this fatal disease i.e., CL from the country.

Conflict of interest: None

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