Abstract

Introduction: Leishmaniasis is a complex disease with vast clinical and epidemiological diversity. It is in the entity of neglected tropical disease with a major impact on world health with a rising trend of burden, affecting the extreme poverty population globally without uniform diagnostics, treatment, or control for all. It is caused by protozoan parasites of more than 20 species of the genus *Leishmania*. The bite of an infected female sand fly belongs either to the genus *Phlebotomus* or genus *Lutzomyia* spreads the disease. Visceral leishmaniasis is in the pipeline for elimination from the Indian subcontinent achieving a successful reduction in VL incidence by 80%. Endemicity of the cutaneous leishmaniasis is, however, increasing with a lack of attention given to the long-term social and psychological burden to the affected. Sri Lanka is identified as a new endemic focus of CL. The limited data available on host-vector interaction in Sri Lanka hampers the planning of efficient disease preventive and vector control strategies. The study of vector bionomics forms the cornerstone in these efforts. The molecular confirmation of the sand flies as *P. argentipes* is an essential step due to the drawback of morphological identification. The molecular-based identification of sand flies, blood-meal source and infectivity rate of the *Phlebotomus argentipes* was done as an extension of the first vector-based island-wide study, conducted to understand the changing trend of bionomics among Sri Lankan sand flies.

Methodology: The representative population of sand flies from ten selected geographical locations covering all nine provinces, collected as part of a nationwide study and preserved in absolute ethanol were used for the current investigations. The morphologically identified 270 blood-engorged female *Phlebotomus argentipes* sand flies caught using CDC miniature light traps were used for the blood meal analysis by using conventional PCR targeting the vertebrate CYTB gene.

Infectivity rate among the *P. argentipes* female sand flies was detected using 1520 sand flies (206 pools and 490 individual flies) which included both fed females and non-fed females. For the pooling, five sand flies were incorporated for each pooled DNA extraction. A conventional PCR targeting the minicircle kinetoplast DNA (kDNA) of genus *Leishmania* was done with all the samples. The confirmatory nested PCR to amplify the *L. donovani* specific Internal transcribed spacer of ribosomal DNA (ITS-rDNA) gene was performed on sand fly DNA extract, which gave positive results for the first kDNA amplification PCR. The extracts of all 272 sand fly DNA, which included the positive amplicons of blood meal analysis and infectivity, were used for the molecular

confirmation of the sandfly as *P. argentipes* two conventional PCR protocols targeting the COI and CYTB genes of sandfly mitochondria.

The sequences were obtained for the positive amplicons of all the PCR experiments in the study. The sequence identities were determined by using the Basic Local Alignment Search Tool algorithm (BLAST) for nucleotides in NCBI-GenBank.

Phylogenetic analysis was done for the *P. argentipes* using the COI sequences and the *L. donovani* using the ITS-rDNA sequences with their relevant reference sequences from the GenBank repository.

Results: The successful sequence analysis with the positive PCR amplicons of blood meal identification PCR revealed that 153 fed female *Phlebotomus argentipes* has engorged with mammalian blood. Out of those, human was the most preferred host (n = 133; 86.9%) followed by cattle (n = 16; 10.4%), dogs (n = 2; 1.3%), rat (n = 1; 0.6%) and mongoose (n = 1; 0.6%). The neighbourjoining bootstrap tree topology of COI sequence of Sri Lankan *P. argentipes* demonstrated that sand flies from all the areas except Delft has the same genetic makeup without much diversity. *P. argentipes* females from Delft Island has demonstrated close relatedness with the Indian *P. argentipes*.

Parasite sequence analysis revealed the presence of 15 *Leishmania donovani* sequences within *P. argentipes* population used. From the infected sand flies, 13 were blood-fed female sand flies, the majority engorged with human blood. There were two *L. donovani* sequences identified in the non-fed sand fly DNA extracts. The difference in infectivity rate among geographical locations was not statistically significant. The infection rate of *L. donovani* in the Sri Lankan leishmaniasis vector is 1% (15/1520) throughout the country. According to the Neighbour-joining tree topology, Sri Lankan *L. donovani* is closely related to the visceralizing *L. donovani* in regional countries.

Conclusion: The anthropophilic nature of the *P. argentipes* in Sri Lanka is demonstrated in an environment where other hosts were also available. The vector potential of *P. argentipes* favours the view of the human as the potential reservoir for the *Leishmania donovani* in Sri Lanka. This strong human-sand fly relationship may explain the rapid spread of leishmaniasis in endemic areas in Sri Lanka emphasizing the importance of implementation of national infection containment strategies.