Natural *Leishmania* infection of *Phlebotomus sergenti* (Diptera: Phlebotominae) in an endemic focus of cutaneous leishmaniasis in Şanlıurfa, Turkey

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Introduction

Sand flies (Diptera: Phlebotominae) were surveyed for *Leishmania* in various villages of Şanlıurfa which is a provincial capital near the Syrian border and the largest focus of anthroponotic cutaneous leishmaniasis (ACL) in Turkey. It is expected that the number of CL cases will increase in this area for years to come as a result of the breakdowns in public health infrastructure due to the conflicts in the neighbouring countries. In order to determine the vector sand fly species molecular biological methods which are sensitive and require less time and effort were used. This information is important for designing focused control methods.

Material & Methods

Sampling was performed at 12 sites in 6 villages (Fig.1) most of Which have had ACL cases in the last two years (Fig. 2a)

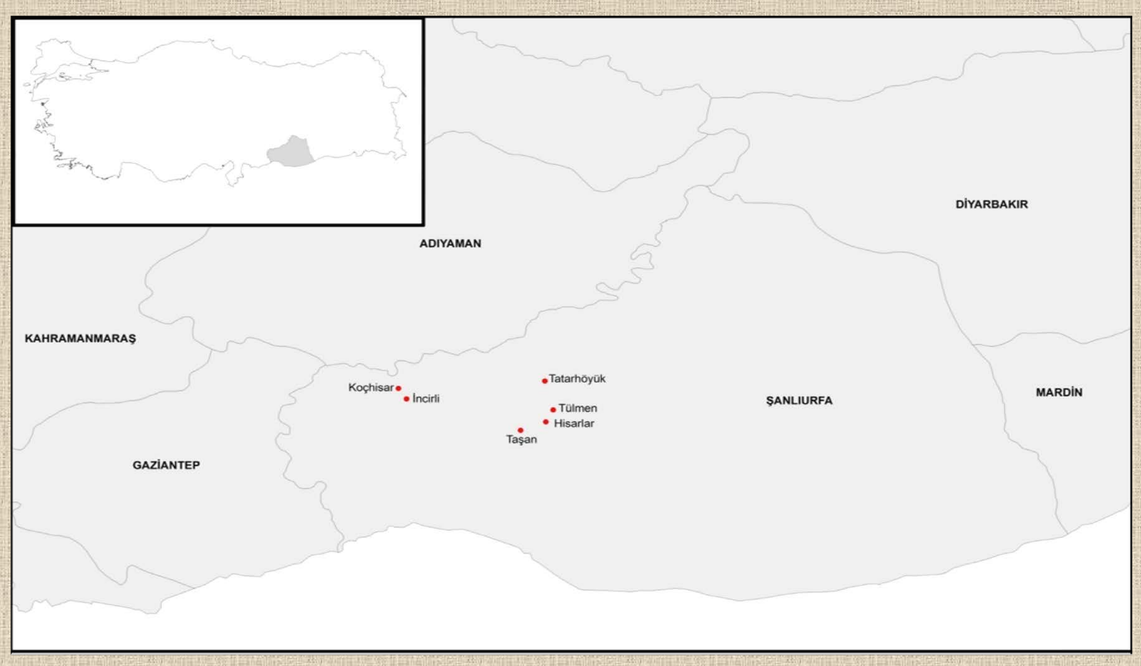

Figure 1. Map of the collecting sites in 6 villages of Şanlıurfa.

Sand flies were collected with CDC miniature light traps (Fig. 2b).

The head and genitalia were mounted on permanent microscope slides for species identification. The body of the females were kept for molecular investigation. A total of 34 tubes and one negative control using male bodies were generated. DNA extractions were made using Qiagen®DNeasy kit by following the manufacturer's instructions. Ready-to-use PCR master mixture (Helixamp®T500N) was used with ITS-1 primer set (LITSR/L5.8S) and amplification was carried out as described by El Tai et al. (2000).

PCR products were analyzed by electrophoresis in 1.2% agarose gel stained with GelRed™ nucleic acid stain mixture and visualized under UV light. 100-bp DNA size marker was used to determine 297-bpTS-1 amplicon in agarose gel.

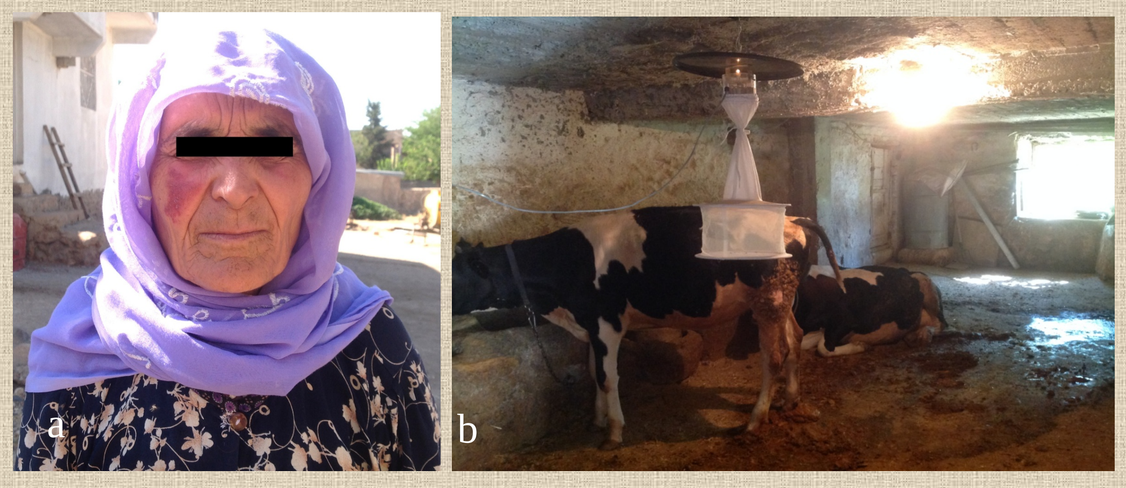

Figure 2. a) A healed lesion in one of the patients in the study area b) CDC miniature light traps set in the collecting sites.

Results & Discussion

A total of 474 sand flies were collected. Six species belonging to 5 subgenera were identified morphologically (Table 1). *P. sergenti* was the most abundant species (49.57%) followed by *P. papatasi* (48.10%).

Table 1. Species and numbers of sand flies collected from villages of Şanlıurfa and their relative abundance (%).

Species	Female	Male	Total	%
P. (Paraphlebotomus) sergenti	111	124	235	49.57
P. (Phlebotomus) papatasi	91	137	228	48.10
P. (Paraphlebotomus) alexandri	3	2	5	1.05
P. (Larroussius) perfiliewi	2	0	2	0.42
P. (Adlerius) sp.	1	0	1	0.21
S. (Sergentomyia) theodori	3	0	3	0.42
Total	211	263	474	100

Thirty four monospecific sand fly pools were screened for molecular detection of *Leishmania* using the target ITS1. Four pools belonging to *P. sergenti* were found to be infected by *Leishmania tropica* (infection rate 11.76%). 15 pools of *P. papatasi* and 3 pools of *P.alexandri* showed no PCR positives.

In conclusion the relative abundance and high infection rate with *L. tropica* suggests that *P. sergenti* is the most probable vector in the region and the abundance of *P. papatasi* indicates a great potential for flourishing of new agents such as *L. major*. The knowledge provided by this study could be used for designing focused control methods.

References

El Tai N.O., Osman O.F., El Fari M., Presber W., Schönian G., 2000. Genetic heterogeneity of ribosomal internal transcribed spacer in clinical samples of *Leishmania donovan i*s potted on filter paper as revealed by single-strand conformation polymorphisms and sequencing. Trans. R. Soc. Trop. Med. Hyg. 94, 575–579