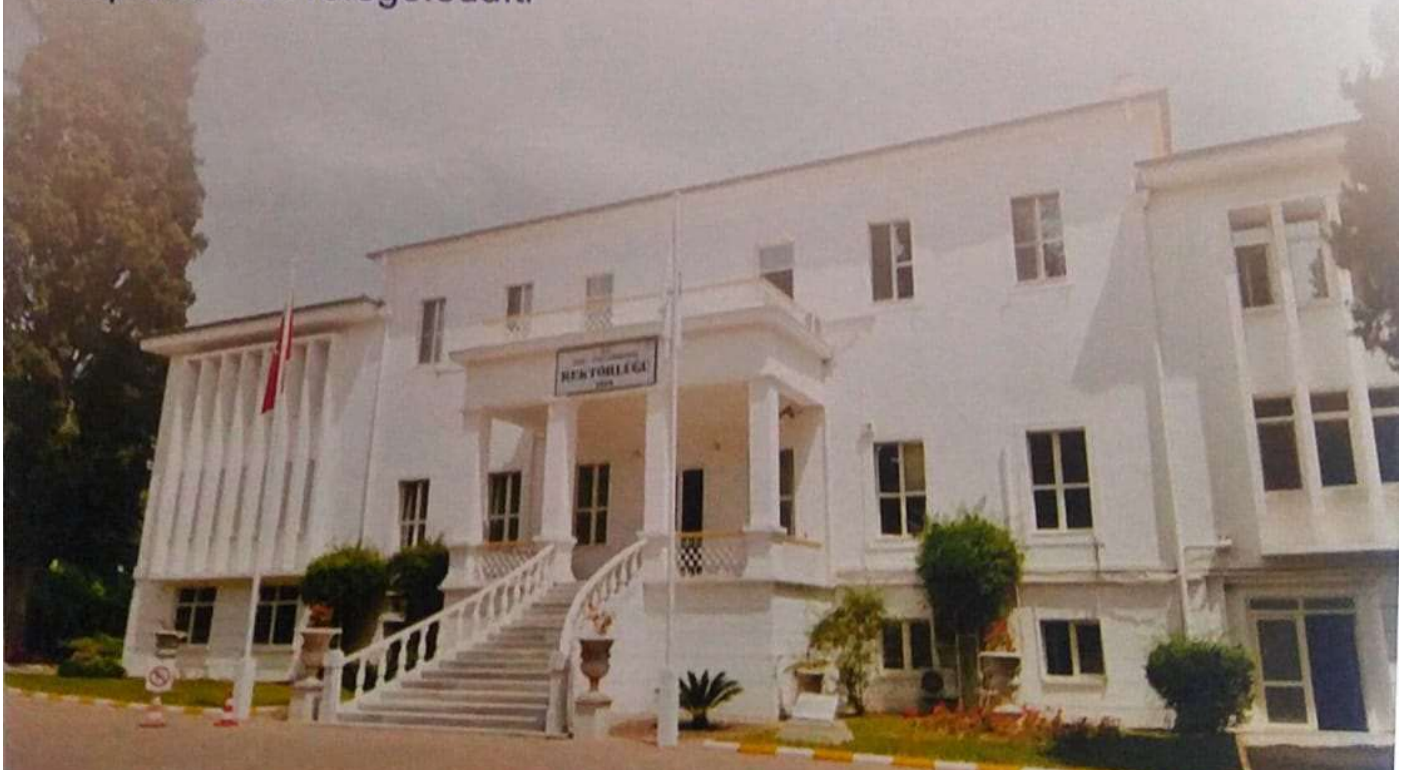
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Potential Role of *Phlebotomus halepensis* in the Transmission of Cutaneous Leishmaniasis

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Abstract- Leishmaniasis is prevalent in both cutaneous and visceral clinical forms in Turkey. The studies carried out on sand fly fauna, in different leishmania endemic provinces in Aegean, Mediterranean, Marmara, East, Middle East and Southeast Anatolia regions, have revealed a total of 21 different *Phlebotomus* and 4 *Sergentomyia* species. *L. tropica* is diagnosed as the causative agent and *P. sergenti* was identified as probable vector in the CL hyperendemic areas located in Southeast and East Mediterranean regions. However, in the preliminary studies, *P. halepensis* was found to be dominant species in two villages of Afyon and Niğde cities, where CL is endemic. In the present study, we aimed to determine the sand fly fauna in all endemic areas in Afyon and Nigde provinces and to investigate the situation of *P. halepensis* as a probable vector.

The field works were carried out in August 2008 and July 2009 in three villages of Nigde and in 5 villages of Afyon provinces where CL cases caused by *L. tropica* were seen. *P. halepensis* was found to be the dominant species in the range of 40% to 96%, indicating a strong probability of being the vector species transmitting *L. tropica* in these endemic areas. The other *Phlebotomus* species were as follows; *P. papatasi, P. neglectus, P. perfiliewi and P. simici* in Afyon province and *P. papatasi, P. sergenti, P. simici, P. mascittii, P. neglectus, P. alexandri* in Nigde province.

This work is a part of an ongoing PhD thesis and to incriminate *P. halepensis* as the potential vector of the disease, the study will further focus on isolating the *Leishmania* parasite in the sand fly specimens using microscopical examination and real-time PCR method.

Index Terms-Leishmaniasis, cutaneous, *Phlebotomus*, vector, Turkey

I. Introduction

LEISHMANIASIS, an infection caused by various species of *Leishmania* parasites, is transmitted by the bite of Phlebotomine sand flies. Leishmaniasis is present in both clinical forms, cutaneous and visceral, in Turkey.

Cutaneous leishmaniasis (CL) caused by *L. tropica* is hyperendemic in the Southeast and Western parts of Turkey while CL caused by both *L. infantum* and *L. tropica* is endemic in East Mediterranean Region of Turkey [5], [9]. The presence of 21 *Phlebotomus* species, 13 of them are probable vector species, was demonstrated in Turkey, so far. *P. sergenti* and *P. tobbi* was identified as probable vector in *L. tropica* hyperendemic areas [11], [12] and *L. infantum* endemic areas [10], respectively.

After 1998, cutaneous leishmaniasis caused by *L. tropica* is also recorded in two new foci, Afyon and Nigde provinces. The preliminary studies carried out in several villages where CL were seen demonstrated the absence of proven vector species, *P. sergenti*, and dominancy of *P. halepensis* in these two new foci (unpublished data), [2].

In the present study, we aimed to determine the sand fly fauna in all endemic areas in Afyon provinces where CL cases are reported and to investigate the vectorial situation of *P. halepensis*.

II. Materials and Methods

A. Study areas

Afyon (38°, 45', 20.88" N; 30°, 32', 30.48" E) is a 14.572 km^2 area, of plains, high mountains, streams and lakes. In the province, the summers (July and August) are usually hot in day-time (27-32 °C) and cooler in night time (20 – 22 °C) with low humidity; while the winters are cold (-14,9 – 2 °C) and wet (Figure 1).



Fig. 1. Study area.

During the present study, sand flies were collected in five villages, Aydın, Doğanlı, Yıprak, Kulak and Göçerli, where CL cases were reported.

B. Sand fly collections

The sand flies were collected at 5 villages using CDC miniature light traps, between 24-26th of July in 2009. CDC light traps were operated overnight in the animal barns, backyards or in the houses where active or treated CL case(s) was living (Figure 2). A total of 33 light traps were set in five villages (1-3 traps at each location) and left them for one night. After collection, in the field conditions, sand flies were anesthetized in the freezer for 5 min, placed in a sieve, washed briefly in a 1% detergent solution, and rinsed in distilled water. Female *Phlebotomus spp.* were separated and dissected in sterile saline under a stereoscopic microscope. The gut was covered for detailed examination under phase-contrast illumination for the detection of *Leishmania* promastigotes. The head and abdomen were removed, transferred to a separate slide [8]. Identification of the species was done using several keys and previous drawings [1], [4], [6].

III. Results

A total of 636 sand flies were collected belonging to 5 species in three catching nights. We determined 266 female and 362 male specimens (Table 1).

TABLE I
GROUPING OF SAND FLIES

Subgenus	Species	Male	Female			
			Gravid	Blood fed	Gravid-Blood fed	Normal
Phlebotomus	*P. papatasi*	143	35	30	6	58
Larroussius	*P. neglectus*	30	7	21	1	7
Larroussius	*P. perfiliewi*	6	-	-	-	1
Adlerius	*P. halepensis*	180	11	40	3	46
Adlerius	*P. simici*	3	-	-	-	-
TOTAL			53	91	10	112

Among all *Phlebotomus* specimens collected, the overall male:female ratio was 1.36 : 1. The specimens were identified as; *P. halepensis* (44.6%) (Fig. 2), *P. papatasi* (43.3%), *P. neglectus* (10.5%), *P. perfiliewi* (1.1%) *ve P. simici* (0.5%).

In 3 (Aydın, Kulak, Yıprak) and 2 (Göçerli, Doğanlı) villages of the 5, *P. halepensis* and *P. papatasi* appeared to be the most abundant species, respectively. In general, and in all individual settlements, *P. halepensis* and *P.papatasi* were found to be dominant species.

In total, 47 female sand fly midguts were checked for the presence of *Leishmania* promastigotes in the field conditions by direct microscopy method, but none of them were found to be positive.



Fig. 2. *P. halepensis* a: Abdomen ♂, b: Spermatheca ♀, c: Pharynx armature ♀

IV. DISCUSSION

In the preliminary studies from Afyon province, seven *Phlebotomus* species; *P. halepensis, P. simici, P. papatasi, P. perfiliewi, P. neglectus, P. similis, P. tobbi* and two *Sergenomyia*; *S. dentata, S. theodori*, recorded from Doganli, Iscehisar and Yaylabagi towns (unpublished data). Eight *Phlebotomus* species; *P. halepensis, P. neglectus, P. balcanicus, P. perfiliewi, P. syriacus, P. simici, P. tobbi* and *P. papatasi* reported from Cakir, Kucuk Cobanlar and Sulun villages [2]. Similar to our results, *P. halepensis* was also found most dominant species in these previous studies carried out in Afyon province.

Adlerius sand flies are associated with many foci of *L. tropica* causing cutaneous leishmaniasis. In Turkey, 6 *Adlerius* species, *P. halepensis, P. simici, P. balcanicus, P. brevis, P. kyreniae*, were reported, so far *P. halepensis and P. simici* are more common species among these five [3].

The laboratory experiments were shown that *P. halepensis* is highly susceptible to both *L. major* and *L. tropica*, supporting multiplication and development of both parasite species as in their natural vectors *P. duboscqi* and *P. sergenti*, respectively [7]. This study was demonstrated the feasibility of *P. halepensis* being a CL vector, but this species of Adlerius has not yet been proven to transmit *L. major* or *L. tropica* in nature. In the present study, in 3 out 5 villages in Afyon province, *P. halepensis* was found to be dominant species. In the present study, we also checked the presence of promastigotes in 47 female sand fly midguts in the field conditions by direct method, but none of them were positive. The preparations and remain bodies were also kept for later PCR analyses.

In conclusion, the present study was supported the feasibility of *P. halepensis* being a CL vector in CL endemic areas of Afyon province as well as, in the nature.

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