Entomological Survey of the Sand Fly Fauna of Kayseri Province: Focus on Visceral and Cutaneous Leishmaniasis in Central Anatolia, Turkey

Kayseri İlinin Kum Sineği Faunası Üzerine Entomolojik Çalışma: Türkiye'nin Orta Anadolu Bölgesi'nde Yeni Bir Kutanöz Leishmaniasis Odağı

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ABSTRACT

Objective: In Turkey, leishmaniasis occurs in two clinical forms: cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL). CL has been reported mainly from south-eastern Anatolia and the eastern part of the Mediterranean region, whereas VL is sporadic in almost all geographical regions of Turkey. Both clinical forms of the disease have been recorded in Kayseri province for decades, but no study has been conducted on the sand fly fauna in this area. Therefore, we determine the species composition and population dynamics of sand flies prevalent in Kayseri province and identify possible vector species.

Methods: Data related to the recent locations of CL and VL cases were obtained from the Ministry of Health, and sand flies were collected in 14 localities of different districts using CDC light traps. The specimens were transferred to the laboratory in 70% ethanol, and morphological identification to the species level was performed using previously written keys.

Results: A total of 1,636 sand fly specimens were collected. Morphological identification revealed eight species of the genus *Phlebotomus* (*P. perfiliewi* s.l., *P. halepensis*, *P. simici*, *P. major* s.l., *P. papatasi*, *P. tobbi*, *P. sergenti* and *P. mascittii*) and one species of the genus *Sergentomyia* (*S. dentata*). Among all specimens, members of subgenus *Adlerius* (54.58%) formed the dominant group, followed by the subgenus *Larroussius* (43.76%).

Conclusion: Our results showed that *P. halepensis*, belonging to subgenus *Adlerius*, and *P. perfiliewi*, belonging to the subgenus, are probable vectors of cutaneous and VL in the province, respectively.

Keywords: Kayseri, sand fly, Leishmaniasis, Central Anatolia, Phlebotomus

ÖZ

Amaç: Türkiye'de leishmaniasis, kutanöz leishmaniasis (KL) ve visseral leishmaniasis (VL) olmak üzere iki klinik formda görülür. KL ağırlıklı olarak Türkiye'nin Güneydoğu Anadolu ve Akdeniz Bölgesi'nin doğusunda bildirilirken VL hemen hemen tüm coğrafi bölgelerinde sporadik olarak görülmektedir. Kayseri ilinde hastalığın her iki klinik şekli onlarca yıldır kaydedilmiştir ancak bu alanda kum sineği faunası üzerine bir çalışma bulunmamaktadır. Bu sebeple Kayseri ilinde yaygın olarak bulunan kum sineklerinin tür kompozisyonu, popülasyon dinamikleri ve olası vektör türlerinin belirlenmesi amaçlanmıştır.

Yöntemler: KL ve VL olgularının son zamanlarda görüldüğü yerlere ilişkin veriler Sağlık Bakanlığı'ndan alınmış ve CDC ışık tuzakları kullanılarak farklı ilçelerde 14 lokaliteden kum sineği örnekleri toplanmıştır. Örnekler %70 etanol içinde laboratuvara getirilmiş ve türlerin morfolojik olarak teşhisleri mevcut teşhis anahtarları kullanılarak yapılmıştır.

Bulgular: Toplamda 1,636 kum sineği toplanmıştır. Morfolojik tür teşhisinde *Phlebotomus* cinsine ait sekiz tür (*P. perfiliewi* s.l, *P. halepensis*, *P. simici*, *P. major* s.l, *P. papatasi*, *P. tobbi*, *P. sergenti* ve *P. mascittii*) ve *Sergentomyia* cinsine ait bir tür (*S. dentata*) teşhis edilmiştir. Tespit edilen türler arasında *Adlerius* (%54,58) alt cinsi üyeleri en baskın grubu oluşturken bunu *Larroussius* alt cinsi (%43,76) izlemiştir.

Sonuç: Bu sonuçlara göre, ildeki kutanöz ve VL'nin muhtemel vektörleri sırasıyla *Adlerius* alt cinsine ait *P. halepensis* ve *Larroussius* alt cinsine ait *P. perfiliewi* olabilir.

Anahtar Kelimeler: Kayseri, kum sineği, Leishmaniasis, İç Anadolu, Phlebotomus



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INTRODUCTION

Phlebotomine sand flies (Diptera: Psychodidae) are the only proven vectors to transmit *Leishmania* parasites causing leishmaniasis. Approximately 1000 species of sand flies have been described in the Old and New Worlds (1,2). However, less than a hundred species belonging to *Phlebotomus* and *Lutzomyia* genera are proven or suspected vectors of human leishmaniasis in Old and New Worlds respectively (3).

Leishmaniasis is one of the endemic infectious diseases in Turkey and two clinical forms are present; visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL). CL became most important vector-borne disease in Turkey after the elimination of malaria. Around 2000 CL cases have been reported each year and 43, 600 new cases of CL were recorded between 1990 and 2010 (4). The influx of Syrian refugees has had significant effects on the epidemiology of CL in the south/southeastern part of Turkey (5,6). Leishmania tropica is main causative agent of CL but the cases caused by L. infantum, L. major and L. donovani have also been reported in Turkey (7,8). Ninety percent of CL cases have been reported from 6 provinces located in Southeastern and Mediterranean Regions (9). Between 30 and 35 cases of VL caused by L. infantum are yearly reported from almost all geographical regions of Turkey (10).

There are currently 28 species of phlebotomine sand flies identified in Turkey (11). *Phlebotomus tobbi* (*P. tobbi*) is the only proven vector of *L. infantum* causing CL in Southern Anatolia (12,13). *Phlebotomus sergenti* (*P. sergenti*) is probable vector of *L. tropica* in Southeastern Region (14,15) while *P. halepensis* was also incriminated as probable vector species of *L. tropica* in Central Anatolia (16). In addition, *Phlebotomus papatasi* (*P. papatasi*) has been implicated as a potential vector in some parts of Turkey where CL cases due to *L. major* have been detected (8). The members of *Larroussius* subgenus such as *P. tobbi* and *P. neglectus* can transmit main causative agent, *L. infantum* (MON-1), causing human VL (17,18).

Between 1995 and 2012, thirty six CL cases were reported from Yahyalı, Tomarza, İncesu, Develi, Melikgazi, Pınarbaşı and Akkışla districts in Kayseri province (19). In the same period, nine patients from Yahyalı, Felahiye, and Melikgazi districts were diagnosed with VL (20). However, to the best of our knowledge, there is no published data on the sand fly fauna of the province except for a collection of six sand fly specimens in the year of 2007 (21). It is essential to have an accurate and updated record of sand fly population dynamics as a starting baseline of formulating policies to control leishmaniasis. Therefore, we aimed to investigate the sand fly fauna in Kayseri province in order to determine the species composition and population dynamics of sand flies and to identify possible vector species for both diseases in the province.

MATERIALS AND METHODS

Kayseri is located in the Central Anatolia Region, Turkey (44' 5.2872" N and 35° 28' 4.7532" E) where the annual mean temperature is 10.6 °C. January is the coldest month with average temperature of -1.3 °C. August experiences the least amount of rain while April is the wettest month of the year.

Melikgazi, Hacılar, Kocasinan, Bünyan, Develi and Yahyalı districts were selected for sand fly collection according to the previous reports of CL cases obtained from Provincial Branch of Ministry of Health (Figure 1). Fourteen localities within these

districts were then selected for sand fly sampling. Table 1 shows altitudes, average temperatures, humidity and coordinates of the sampling sites.

Between 16^{th} and 21^{st} of July 2018 a daily sand fly collection was carried out in different localities of the study area. Centers for disease control (CDC) miniature light traps were placed 1-2 meters above the ground and left on each site from 18:00 PM to 06:00 AM. The traps were collected the following morning and then the specimens were put into 70% ethanol using mouth aspirators. They kept in the ethanol until morphological examination was performed.

CDC light traps were placed in suitable habitats for sand flies such as sheep farms, poultry houses, dog shelters, cow sheds, outside of human shelters and abandoned shelters rich in organic material. At least one representative of the household was informed before the traps were set up. They were also informed about the operation procedure of the light traps to avoid interference during sampling. Characteristics of the localities such as temperature and humidity were recorded. These factors greatly influence sand fly distribution and sampling.

The specimens collected from each location were separated as males and females under stereo microscope (Olympus SZ40). Subsequently, the total number of sand flies and the number of males and females were determined. Head and the posterior part of the abdomen including genitalia and other decisive characters of males and females were dissected using thin needles. For light microscopy identification of male species, the head and genitalia are mounted on a glass slide and covered with Swan solution (11). For clear observation of spermatheca, the female dissected parts were covered with a mixture of Marc-André and Swan solutions. The relevant literature on identification of species, diagnosis keys and drawings were used for morphological identification of the specimens (22-27).

Statistical Analysis

Tables and picture were used to display data. No statistical tools were used.

RESULTS

A total of 1.636 sand flies, 203 males and 1.433 females, were sampled. The female to male ratio was 7.06 (Table 2). Eight species

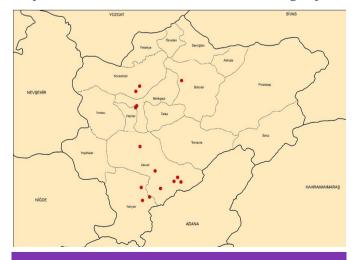


Figure 1. Map showing sampling localities in the study area

of phlebotomine sand flies belonging to genus *Phlebotomus* (*P. perfiliewi* s.l. 40.40%, *P. halepensis* 5.13%, *P. simici* 3.06%, *P. major* s.l. 3.06%, *P. papatasi* 0.79%, *P. tobbi* 0.06%, *P. sergenti* 0.06% and

P. mascittii 0.06%) and one species from genus *Sergentomyia* (*S. dentate* 0.55%) were identified (Table 3). Since it's not possible to morphologically differentiate females of subgenus *Adlerius*, only

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District	Locality	Altitude (M)	Temperature (°C) max-min	Humidity max-min	Coordinates
Melikgazi	Becen	1.115	22-11	78-37%	38° 40′ 36"N 35° 28′ 54"E
Hacılar	Erciyes	1.258	22-11	78-37%	38° 39' 52''N 35° 28' 22''E
Kocasinan	Boztepe	1.079	22-11	78-37%	38° 46′ 42′′N 35° 28′ 29′′E
Kocasınan	Elagöz	1.063	22-11	78-37%	38° 48′ 5.63"N 35° 30′ 14.85"E
Bünyan	Gergeme	1.303	22-11	78-37%	38° 51' 13''N 35° 49' 50''E
	Büyükkünye	1.371	24-12	73-28%	38° 10′ 30.07"N 35° 47′ 59.59"E
	Küçükkünye	1.421	24-12	73-28%	38° 8′ 35.23"N 35° 49′ 3.80"E
Develi	Yaylacık	1.461	26-15	63-23%	38° 8' 50.91''N 35° 46' 16.86''E
Deven	Yeniköy	1.468	35-22	80-19%	38° 5′ 49.48′′N 35° 39′ 59.50′′E
	Ayşepınar	1.316	24-12	73-28%	38° 13' 13''N 35° 37' 30''E
	Fenese	1.342	26-14	53-22%	38° 23′ 28.91"N 35° 30′ 27.44"E
	Taşhan	1.476	26-14	53-22%	38° 6′ 16.04′′N 35° 31′ 0.01′′E
Yahyalı	Dikme	1.468	34-22	87-28%	38° 0′ 49.09′′N 35° 31′ 37.22′′E
	Karaköy	1.306	34-22	87-28%	38° 2' 17.97"N 35° 34' 52.98"E

Table 2. Species an	d numbers of sand flic	es collected in	diverse areas of	Kayseri provinc	:e	
Subgenus	Species	Female	Male	Total	Female/male ratio	Degree of presence %
	Adlerius sp.	757	2	759	378.5	46.39
Adlerius	P. halepensis	-	84	84	0	5.13
	P. simici	-	50	50	0	3.06
	P. perfiliewi s.l.	627	34	661	18.44	40.40
Larroussius	P. major s.l.	34	16	50	2.13	3.06
Larroussius	Larroussius sp.	2	2	4	1	0.24
	P. tobbi	-	1	1	0	0.06
Phlebotomus	P. papatasi	5	8	13	0.625	0.79
Paraphlebotomus	P. sergenti	1	-	1	-	0.06
m 1111	Transphlebotomus sp.	-	2	2	0	0.12
Transphlebotomus	P. mascittii	-	1	1	0	0.06
S	S. dentata	7	2	9	3.5	0.55
Sergentomyia	Sergentomyia sp.	-	1	1	0	0.06
	Total	1.433	203	1.636	7.06	100

Table 3. Re	Table 3. Results of sand fly fauna investigation in different localities in Kayseri province	fly fauna inve	stigation	in different	localities in K	ayseri pr	ovince								
		P. halepensis	P. simici	Adlerius sp.	Adlerius P. perfiliewi sp. s.l.	P. major s.l.	P. tobbi	Larroussius sp.	P. papatasi	P. sergenti	P. mascittii	Transphlebotomus sp.	S. dentata	Sergentomyia sp.	Total
Melikgazi	Becen	1	9	7	1	1		ı	1	1	1	1		1	14
Hacılar	Erciyes	1	П	5	1	1	1	ı		1	ı	1	1	ı	9
1	Boztepe	-	1	2	-	-	,	-	-	1	1	1	1	1	2
посаѕіпап	Elagöz	-	1	-	-	-		1	-	-	1	1	1	1	1
Bünyan	Gergeme	-	1	1	93	-	-	-	-	-	1	1	1	1	94
	Büyükkünye	18	31	381	285	8	-	1	-	1	1	1	4	1	730
	Ayşepınar	13	1	22	126	2	_	1	2	-	-	_	-	1	167
-	Küçükkünye	13	3	88	51	8		1	1	1	1	1	1	1	164
Devell	Yeniköy	2	4	86	40	2	-	2	2	-	-	2	1	-	157
	Yaylacık	10	1	31	9	3	-	1	1	ı	ı	1	1	ı	55
	Fenese	1	1	5	-	-		-	-	-	1	1	1	1	9
	Karaköy	15	1	103	28	19	1	-	9	1	-	-	4	-	177
Yahyalı	Taşhan	11	1	5	15	1	,		1			1	1	1	34
	Dikme	1	2	11	14	1	,	1		1	ı	1	ı	1	29
Total		84	20	759	661	20	1	4	13	1	1	2	6	1	1.636

males were identified. Females of this group were identified at the subgenus level. Four damaged specimens belonging to Larroussius and two Transphlebotomus could only be identified at subgenus level and one *Sergentomyia* specimen at genus level. Among males, *P. halepensis* was found to be the most dominant species. Among females Adlerius sp. which was recorded in 13 out of 14 sampling localities was the most dominant (Table 3).

DISCUSSION

This study presents the first report on sand fly fauna in Kayseri province, which is a focus especially for CL in Central Anatolia, Turkey. Eight Phlebotomus and one Sergentomyia species were detected in total. P. halepensis (Adlerius) and P. simici (Adlerius) and P. perfiliewi s.l (Larroussius) were recorded as dominant species in the province (Table 3).

Only male P. halepensis and P. simici specimens could be identified in our study since it is very difficult to morphologically differentiate females of subgenus Adlerius. As they are always identified with associated males, it can be deduced that female P. halepensis the most and P. simici is the second most dominant female species in Kayseri province (Table 2). Phlebotomus halepensis has previously been detected in Mediterranean Basin countries including Turkey (23). Kavur et al. (16) reported P. halepensis as dominant species in Niğde province in Central Anatolia. In the same study, P. halepensis was incriminated as a probable vector species of L. tropica in the region. In a study of distribution and altitude structuring of sand fly fauna in the Southern Anatolia, Şimşek et al. (21) reported P. halepensis as the only species in Kayseri province even though very few specimens identified. P. simici was also the second most dominant species (3.83%) in a sand fly fauna study of a neighboring province, Niğde (16).

Adlerius species are competent vectors for Old World leishmaniasis across Europe and Asia. P. simici was found positive for Leishmania DNA in a VL focus in Greece (28) and the distribution of *P. halepensis* is reported to be in accordance with those of VL and CL. It was also reported to be highly susceptible to L. major and L. tropica with infection rates of ~90% and ~80% respectively in a Vectorial Competency test (29).

P. perfiliewi s.l. is a species complex composed of P. perfiliewi, P. galilaeus and P. transcaucasicus (30,31). Turkey is the only place where these three species were found in sympatry (21,32,33). It is very difficult to differentiate female members of this group and morphological features of the male aedeagus differ according to the geographical origin. Molecular techniques are necessary for better identification of this species complex. P. perfiliewi is one of the main vectors of L. infantum in Mediterranean Basin and Central Asia (31). Oshaghi et al. (34) reported P. transcaucasicus as the main vector for L. donovani and L. infantum in the Northwest Iran. Şimşek et al. (21) recorded P. perfiliewi s.l. (P. perfiliewi 0.01%, P. galilaeus 5.3% and P. transcaucasicus 13.3%) in Southern Anatolia in Turkey. In a previous study from the region, no P. perfiliewi s.l. was reported (16).

Phlebotomus major s.l. comprises morphologically similar species (P. major, P. wui, P. notus, P. wenyoni, P. syriacus and P. neglectus) (30). P. syriacus and P. neglectus have been reported in western, northern and southern parts of Turkey (33,35,36). In a recent study on sand fly fauna of Central Anatolia (Niğde Province) no P. major s.l. was reported (16).

P. papatasi has been previously identified in various regions of Turkey (33,37). In the present study area, 13 *P. papatasi* specimens were identified, representing 0.79% of the total collected sand flies. Only one specimen of *P. tobbi*, *P. sergenti and P. mascittii* were found in our study. This may be due to the small number of collected sand flies in some areas.

In previous studies of sand fly fauna in Turkey, *Sergentomyia dentata* has been reported in the Mediterranean Region of the country (30,36,38). Şimsek et al. (21) and Kavur et al. (16) did not found any member of genus *Sergentomyia* in sand fly studies in Central Anatolia Region.

CONCLUSION

Our results showed that *P. halepensis* belonging to *Adlerius* subgenus and *P. perfiliewi* belonging to *Larroussius* subgenus can be probable vectors of cutaneous and VL in the province, respectively. This study will provide data in designing vector control strategies and epidemiological prediction models aiming to control CL and VL in this area. However, more studies are required to confirm the suspected species' vectorial status. In addition, use of molecular markers in identification of species especially among females of subgenus *Adlerius* and *Larroussius* are needed.

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* Ethics

Ethics Committee Approval: Not applicable. Our work involved sand flies from the wild. No patients or animals were involved in the study. Therefore, we had no ethics committee and approval number.

Informed Consent: Not applicable. Our work involved sand flies from the wild. No patients or animals were involved in the study. **Peer-review:** Internally peer-reviewed.

* Authorship Contributions

Concept: Z.N.O., S.D., S.K.A., Design: Z.N.O., S.D., Data Collection or Processing: Z.N.O., S.K.A., Analysis or Interpretation: Z.N.O., S.D., S.K.A., Literature Search: Z.N.O., S.D., Writing: Z.N.O., S.D., S.K.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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