Spatial distribution of phlebotomine sand flies in the Aydin Mountains and surroundings: the main focus of cutaneous leishmaniasis in western Turkey

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ABSTRACT: An entomological survey was conducted to determine the spatial distribution of phlebotomine fauna and understand the effect of environmental factors. The entomological survey was carried out during 2006-2007 in a study area in the rural area of Aydin province, near the Kusadasi town where VL, CL, and canine leishmaniasis (CanL) are endemic. In 2006 and 2007, 132 locations were sampled using sticky traps mainly on embankments. Detailed environmental and meteorological information was also collected for each location. The results of entomological studies indicated that the probable vectors are *Phlebotomus tobbi* and *P. neglectus* for VL and CanL, and *P. similis* for CL in this western leishmaniasis focus. The data revealed a correlation between their presence and spatial variables such as altitude, sampling site location, and humidity. The distribution areas of probable vector species in this study area allowed the identification of risk levels, which may provide useful information to guide the leishmaniasis research in endemic regions. ***Journal of Vector Ecology* 36 (Supplement 1): S99-S105. 2011.**

Keyword Index: Spatial distribution, phlebotomine, Turkey, cutaneous leishmaniasis, Aydin province, sand fly.

INTRODUCTION

Phlebotomine sand flies (Diptera: *Psychodidae*) are vectors of human and canine leishmaniases. Two clinical types of leishmaniasis, zoonotic visceral leishmaniasis (ZVL) caused by *Leishmania infantum,* and anthroponotic cutaneous leishmaniasis (ACL) caused by *L. tropica* and *L. infantum,* have been reported in Turkey. The ZVL is endemic in Aegean, Mediterranean, and Central Anatolia regions and has been found sporadically in other regions. The ACL is classically present in the southeastern and eastern Mediterranean regions endemically and epidemically and has been spreading to other regions because of the different risk factors (Ok et al. 2002). Canine leishmaniasis (CanL), caused by *L. infantum,* is one of the major veterinary problems in Turkey. So far, the epidemiological studies have been carried out in 22 different provinces and the prevalence ratios have been found to range between 2.4% - 46%, with the overall prevalence of CanL at 15.8% for Turkey (unpublished data). Several species of sand flies have been incriminated as vectors of ACL including *P. guggisbergi* and *P. aculeatus* in Kenya, but the majority of foci where ACL caused by *L. tropica* are found are transmitted by *Phlebotomus sergenti*. This species of sand fly has a wide distribution and has been incriminated as the vector of ACL in Yazd (central Iran), southwestern Saudi Arabia and in Sanliurfa, Turkey (Volf et al. 2002, Yaghoobi-Ershadi et al. 2002).

At least nine species of sand flies are potential or known vectors within Turkey (Killick-Kendrick 1990). To date there are twenty-one species of *Phlebotomus* (belonging to the subgenus *Phlebotomus, Adlerius, Paraphlebotomus,* and *Larroussius*) and four species of *Sergentomyia* known to be present in Turkey (Yaman and Dik 2006). Because of the location and geographic diversity in Turkey, there are various climate types and ecological conditions. Therefore, there are still more extensive surveys needed to determine the sand fly fauna there. The entomological surveys for leishmaniasis were mainly carried out in endemic areas (Dogan et al. 2006, Ertabaklar et al. 2005b, Ozensoy Toz et al. 2009), but there is limited information about the sand fly fauna in new foci, as in Aydin province.

Cases of human and canine visceral leishmaniasis have been reported from Kusadasi, a town in Aydin province, in western Turkey, since 1993. In Kusadasi, the seroprevalance of CanL among dogs is 16.6%, which is higher than the average ratio of 5.3% (range 3.6%-25%) in western Turkey. It is also reported that one-third of the dogs in an endemic area for CanL could be asymptomatic (Ozbel et al. 2000, Ozensoy et al. 2005a, b). Anthroponotic cutaneous leishmaniasis, which is generally reported from southeastern (Sanliurfa province) and the eastern Mediterranean (Adana, Osmaniye, Mersin, Hatay provinces) parts of Turkey, has been detected in increasing numbers in Aydin province. The number of ACL cases increased from four in the year 2000, to 151 and 110 in 2005 and 2006, respectively (Ertabaklar et al. 2005a). Aydin province has two important factors which may have assisted the emergence of ACL, a suitable ecological environment for the maintenance of a vector population and a susceptible human population to ACL infection. This region is therefore the first region in western Turkey to have indigenous ACL cases and is considered as a new focus for ACL within Turkey (Klaus et al. 1999, Ertabaklar et al. 2005a).

The work presented here was conducted under the Integrated Project of the European Commission, "Emerging Diseases in a changing European Environment; EDEN." The aim of this project was to identify and catalog European ecosystems and environmental conditions that determine spatial and temporal distributions. Because previous information related to sand fly fauna and environmental factors in this important endemic area located in western Turkey is not available, the present study examines the effect of environmental factors on the spatial distribution of phlebotomine fauna.

MATERIALS AND METHODS

Study area

The study area is located between Kusadasi on the Aegean coastline and Nazilli, including the Aydin Mountains and surroundings in western Turkey (Figure 1). The reasons for choosing this geographical area were that Kusadasi, located in the west sector, has been known as an endemic area for human and canine visceral leishmaniasis caused by *L. infantum* for a long time, the eastern part of Aydin province has been known as endemic area for ACL caused by *L. tropica,* and canine leishmaniasis has recently been found there.

Aydin province is located in southwest Turkey on the Great Menderes Basin, surrounded by mountains to the north and south and plains in the central and western regions. The climate is Mediterranean with summers being hot and dry and winters warm and rainy. The average temperature is 17.6° C, with an average of 80.6 rainy days per year, and an average of 677.5 mm of rain per year. The Municipal Ministry of Health has overall jurisdiction for health in the province including surveillance, diagnosis, and treatment of leishmaniasis. The study area is characteristic of the region, with a mountainous sector between two river basins in the north and south. There are about 80 villages and settlements in the area with populations ranging from 150 to 2,500. Sampling locations were chosen and sand fly collection was performed in 132 localities (Figure 1).

The cross-sectional field work was carried out in 132 localities using sticky paper traps. Seventy-six localities were sampled in July, 2006 and an additional 56 localities were sampled in July, 2007. During the field work, five to 20 A4-size sticky paper traps were placed into the holes on the embankment walls and left in the holes for four full days. The temperature and humidity were recorded during the set-up and recovery of the traps. A total of 1,119 sticky traps were set and 997 (89.1%) of them were recovered All collected sand flies were transferred to vials and each fly was identified to species, using the keys and descriptions of Theodor (1958), Perfil'ev (1968), Artemiev (1980), Lewis (1982), and Killick-Kendrick et al. (1991).

Data collection and statistical analysis

Data related to environmental variables for each location were collected using Pendragon v 5.0 software (PSC, Libertyville, IL, U.S.A.). At each location, a handheld GPS (Magellan Explorist 600) was used to record the coordinates. The collected data were related to habitat, wall and site characteristics. Variables included in the analyses are listed in Table 2. Normalized Difference Vegetation Index (NDVI) and Land Surface Temperature (LST) values were calculated from Landsat ETM+ (path 180, row 34) with 30 m spatial resolution. Sand fly density was calculated as the number of sand flies per m^2. Descriptive analysis was performed to determine the correlation between the density of the sand files and the altitude of each location.

Statistical analysis used the SPSS v.15 statistical package. A univariate binary logistic regression analysis was undertaken to determine the relationship between three probable vector species (*P. neglectus, P. tobbi, P. similis*) and environmental variables. Results of the analyses were transferred to ArcGIS v9.3 GIS software to produce maps.

RESULTS

In two collection periods in 132 locations, a total of 1,043 sand flies was collected and 554 and 489 of them belonged to *Phlebotomus* and *Sergentomyia* genera, respectively. A total of 11 *Phlebotomus* species and three *Sergentomyia* species were determined (Table 1).

P. similis, the probable vector of ACL, was the most abundant species (38.3%) among the *Phlebotomus* species. *P. neglectus* and *P. tobbi*, the probable vectors of human and canine visceral leishmaniasis, were also detected as the second and third most abundant species (26.6% and 12.1%). The male/female ratio was 4.03:1 for *Phlebotomus* species, 6.78:1 for *P. neglectus*, 4.14:1 for *P. similis,* and 2.35:1 for *P. tobbi*. A correlation was observed between the density of the three vector sand flies and the altitude of each location. Although found in every elevation range, including the highest location at 963 m, all vector species were found predominantly at elevations below 500 m (Figure 1).

The environmental variables at each sampling site are shown in Table 2. The minimum and maximum temperatures were between 20.1° C and 38.1° C; the humidity was between 23% and 58%. The general environment favored rural locations and forests for the distribution of all three vector species, especially for *P. neglectus* ($p<0.001$). The presence of *P. neglectus* is also related to elevation ($p<0.001$), NDVI ($p<0.05$), sampling site location ($p<0.05$) and type of wall ($p<0.05$). The presence of *P. similis* was related to elevation ($p<0.005$). A correlation was observed related to humidity for *P. tobbi* ($p<0.05$).

DISCUSSION

The distribution and incidence of leishmaniasis are both influenced by human behaviors and environmental variables affecting the vector and reservoir populations. Careful monitoring of environmental variables in relation to

Table 1. The sand fly species found in the area.

Genus/Subgenus	Species	Total	Male	Female	%
Phlebotomus					
Larroussius	*P. neglectus*	148	129	19	26.6
	P. tobbi	67	47	20	12.1
	P. burneyi	2	2	0	0.4
	P. perfiliewi	1	1	0	0.2
	P. mascittii	8	8	0	1.4
Adlerius	*P. brevis*	2	2	0	0.4
	P. simici	37	30	7	6.7
Paraphlebotomus	*P. alexandri*	6	3	3	1.1
	P. similis	212	170	42	38.3
	P. jacusieli	4	4	0	0.7
Phlebotomus	*P. papatasi*	67	48	19	12.1
	Total	**554**	**444**	**110**	**100**
Sergentomyia					
	S. minuta	389	137	252	79.5
	S. theodori	92	32	60	18.8
	S. dentata	8	2	6	1.7
	Total	**489**	**171**	**318**	**100**
Total number of sand flies		**1,043**	**615**	**428**	-

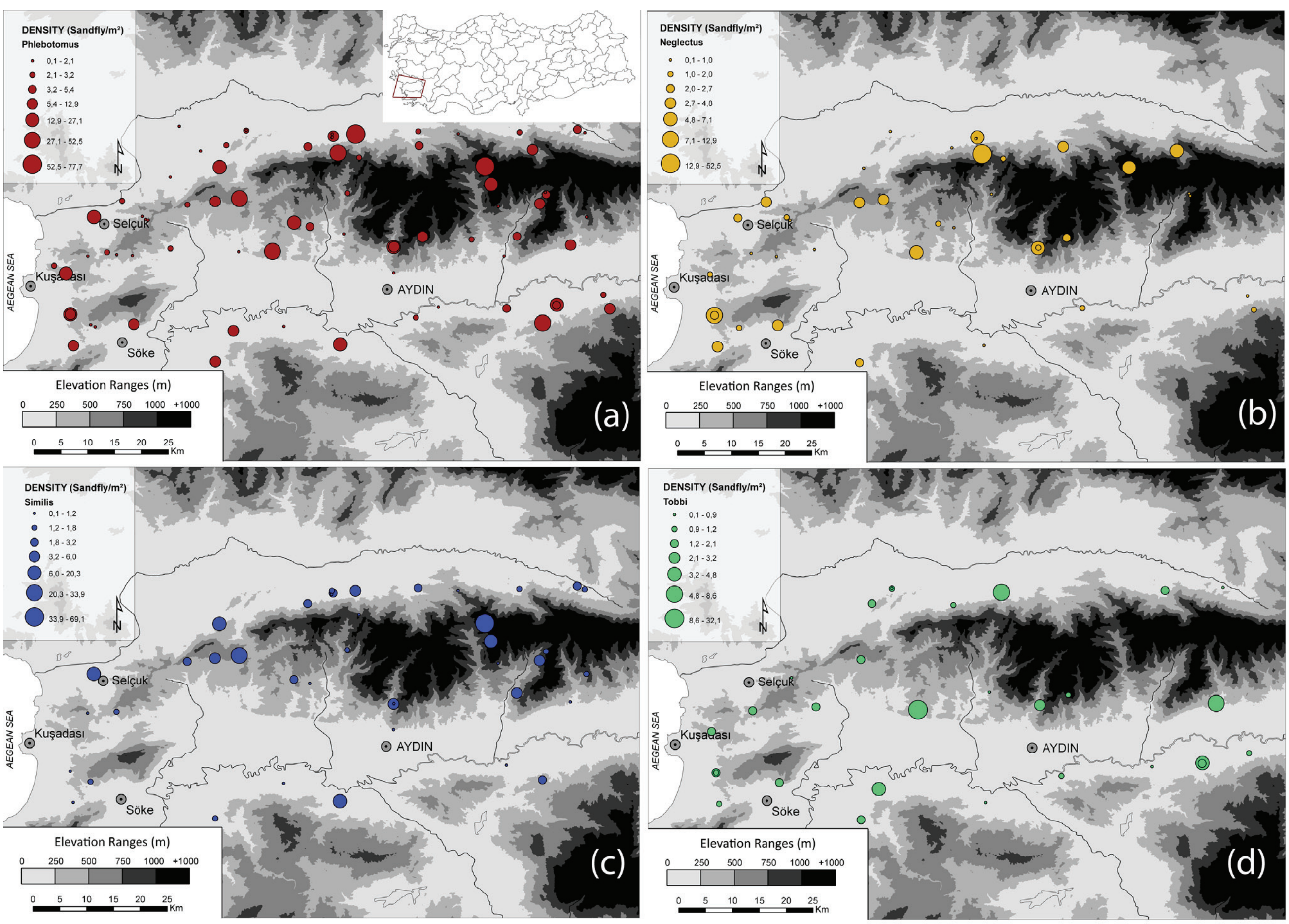

Figure 1. Vector densities shown on an elevation model a) all *Phlebotomus* species, b) *P. neglectus*, c) *P. similis*, d) *P. tobbi*.

Table 2. Localization characteristics and bivariate factors related to the presence of three major vector species.

Variable	*P. neglectus* p	*P. neglectus* CI (95%)	*P. tobbi* p	*P. tobbi* CI (95%)	*P. similis* p	*P. similis* CI (95%)
Sampling Site Location	**.177**		**.946**		**.539**	
within	1.0	ref	1.000	0.0	1.000	0.00
edge	.032*	(0.134 - 0.914)	.834	(0.283 - 2.769)	.221	0.663 - 5.939
between	.074	(0.117 - 1.104)	.779	(0.336 - 4.288)	.779	0.336 - 4.288
Type of Roadway	.680		.902		.354	
other		ref		ref		ref
garden	.788	(0.078 - 28.890)	.999	0.00	.648	0.102 - 39.076
paved drive	.410	0.220 - 40.931)	.814	0.096 - 19.643	.999	0.000
paved public	.705	0.169 - 3.327)	.369	0.065 - 2.766	.273	0.522 - 10.011
unpaved	.435	(0.209 - 1.962)	.665	0.219 - 2.632	.600	0.229 - 2.345
Type of Wall	.068		.211		.089	
other		ref		ref		ref
embarkment	.022*	(1.169 - 7.696)	.869	0.272 - 3.003	.266	0.667 - 4.349
wall	.374	(0.565 - 4.551)	.093	0.858 - 7.312	.085	0.056 - 1.205
Wall Aspect	.200		.356		.903	
E	.066	(0.063 - 1.093)	.130	0.063 - 1.427	.236	0.111 - 1.720
N	.495	(0.162 - 2.413)	1.00	0.250 - 3.998	1.00	0.262 - 3.820
NE	.682	0.190 - 2.964)	.897	0.215 - 3.843	.642	0.173 - 2.954
NW	.478	0.170 - 2.291)	.169	0.070 - 1.597	.497	0.165 - 2.399
S	.359	(0.093 - 2.369)	.166	0.021 - 1.950	.892	0.190 - 4.237
SE	.319	(0.469 - 10.210)	.732	0.144 - 3.903	.525	0.115 - 3.009
SW	.081	(0.036 - 1.214)	.104	0.016 - 1.471	.296	0.087 - 2.101
W		ref		ref		ref
Wall Construction	1.000		.989		.997	
Other		ref		ref		ref
Brick with mortar	1.000	0.000	1.00	0.00	1.00	0.00
Concrete	1.000	0.000	1.00	0.00	1.00	0.00
Stone mortar	1.000	0.000	1.00	0.00	1.00	0.00
Stone mortar plaster	.986	0.234 - 4.159	.452	0.270 - 18.910	.570	0.317 - 8.070
Stone without mortar	.999	0.000	.999	0.00	.999	0.00
Drain Hole Construction	**.317**		**.310**		**.601**	
Other		ref		ref		ref
Brick	.999	0.000	.999	0.00	.999	0.00
Cement	.204	0.110 - 1.603	.182	0.030 - 1.950	.389	0.537 - 4.945
Plastic	.078	0.019 - 1.239	.272	0.037 - 2.526	.999	0.00
Unlined	.826	0.412 - 3.037	.267	0.634 - 5.193	.206	0.142 - 1.522
General Environment	**.003****		**.043***		**.018***	
Rural Agr. & Forestry	.001***	3.138-71.693	.012*	1.860 - 162.741	.042*	1.071 - 37.810
Rural village	.015*	1.351-17.212	.033*	1.202 - 73.018	.005**	1.947 - 39.025
Urban (Town)		ref		ref		ref
Landcover			.999		.171	
Arable	.923	0.097-13.162	.682	0.050 - 7.068	.984	0.083 - 11.411
Orchard	.973	0.086-12.710	.862	0.065 - 9.844	.682	0.140 - 20.332
Urban park	.181	0.005-2.705	.998	0.00	.181	0.005 - 2.705
Forest	.522	0.165-34-928	.837	0.048 - 11.648	.577	0.026 - 7.666
Vineyard	.810	0.025-18.059	.999	0.00	.999	0.00
Pasture	ref			ref		ref
DEM	0.001***	1.001-1.005	.387	0.997 - 1.001	.003**	1.001 - 1.005
LST	.425	0.779-1.111	.592	0.862 - 1.297	.616	0.797 - 1.144
NDVI	.012*	1.983-245.459	.202	0.006 - 2.995	.191	0.459 - 49.232
Temp	.429	0.875-1.058	.724	0.915 - 1.136	.574	0.884 - 1.071
Humidity	.649	0.46-1.035	.032*	1.005 - 1.106	.099	0.911 - 1.008

leishmaniasis and vector biology is important for planning and implementation of control strategies (Galvez et al. 2010). The possibilities for the emergence of ACL in the Aydin region include a change in the demographic distribution due to migration from eastern to western Turkey. Other explanations have included the hypotheses that ACL may have been present in the region for some time and had remained undetected due to poor surveillance systems, or that the effects of global warming have increased both the vector populations and their ecological niches (Savk et al. 1999). Surveillance results of ACL in Aydin are inconsistent because of different surveillance methods.

Previous sand fly studies disclosed nine proven or probable vector species of the Old World leishmaniases (Killick-Kendrick 1990) in Turkey (Yaman and Ozbel 2004). In the study area, sand fly collection was carried out for the first time and 11 different *Phlebotomus* species were found including *P. tobbi, P. neglectus,* and *P. similis,* which are proven or probable vectors of *L. infantum* and *L. tropica* (Killick-Kendrick 1999). *Phlebotomus burneyi*, a rare *Larroussius* species, is reported for the first time in Turkey.

P. neglectus, the known vector of zoonotic VL in western and southern parts of Turkey (Tok et al. 2009, Yaman and Özbel 2004) was also reported from Greece (Chaniotis et al. 2000). Moreover, this species is also a probable vector in Adriatic countries such as Croatia (Bosnić et al. 2006), Albania (Velo et al. 2005), and Italy (Maroli et al. 2008). *P. tobbi*, a broadly permissive vector, is incriminated in the transmission of both *L. infantum* and *L. donovani* (Antoniou et al. 2009). *P. tobbi* was also previously reported as vector of canine leishmaniasis caused by *L. infantum* in Cyprus (Deplazes 1998, Léger et al. 2000). Among all *Phlebotomus* specimens collected, *P. neglectus* (26.6%) was the dominant species, followed by *P. tobbi* (11.17%) and *P. similis* (38.3%). These three most dominant species constituted 77% of the total *Phlebotomus* collected throughout the study. *P. neglectus* and *P. tobbi,* belonging to *Larroussius* subgenus, constitute potential vectors of VL and CanL in the study area, while *P. similis,* belonging to the subgenus *Paraphlebotomus*, is believed to be the potential vector of ACL, which is reported to be endemic in the area (Ertabaklar et al. 2005a). *P. neglectus* was also previously identified as a dominant species (43.52%) in a neighboring endemic site for zoonotic VL in western Turkey, Denizli province (Ozensoy Toz et al. 2009).

The diversity of the vector species have an equal distribution throughout the region in both sectors where visceral and cutaneous cases are present. Although Guernaoui et al. (2006) reported that altitude is one of the important factors in the distribution and structuring of sand fly species, they stressed that it is not an ecological factor by itself but it can act on the distribution by the diversity of habitats, relief, and by the gradient of climate that it offers. Density of sand flies is related to altitude.

The distribution of the suspected species must coincide in space and time with the distribution of infections in humans. In the study area, *P. neglectus,* which is considered as the probable vector species for zoonotic VL and CanL, is present in every altitude range with different densities and in the locations where CanL has reported to have a high seroprevalence rate in a part of study area (Özensoy et al. 2005a). The presence of *P. neglectus* increases with altitude in the Aydin Mountains, while *P. tobbi* is present between 0 to 250 m and no actual relation was found with previously reported CanL cases in the study area. *P. similis*, the probable vector species of ACL, was found mostly at elevations between 0 to 500 m. The presence of *P. similis* increased up to 750 m and decreased at higher altitudes.

It was previously reported that Turkey is the only country in which *P. sergenti* and *P. similis* are present, but *P. similis* should occur only west of Taurus and Antitaurus and *P. sergenti* only to the east (Depaquit et al. 2002). Both these species were found as sympatric in Central Anatolia where they were located in the west of Taurus and Antitaurus (Yaman and Dik 2006). In a previous study, carried out in a neighboring endemic site for zoonotic VL, in Denizli province, it was also noted that these two species exist in the area in the western part of Turkey. In the present study, *P. sergenti* was not found and it can be speculated that *P. similis* is the only probable vector species for this area (Özensoy et al. 2009).

Among specific environmental variables, site location is not an important factor for the presence of the vector species. In other studies, *P. neglectus* species has been collected both in sylvatic and domestic or peridomestic environments (Maroli et al. 2002). However, in our study *P. neglectus* had a higher presence at the edge of settlements.

The general environmental variable was found to be significant in favor of rural areas and forests for the distribution of all three vector species, especially for *P. neglectus* ($p<0.001$). This preference for natural, undisturbed habitats coincides with the findings of Galvez et al. (2010). This may be due to the insecticidal spraying in urban areas and the amount of asphalt and pavement covering the ground. The type of roadway, wall aspect, wall, and drain hole construction materials were found to be insignificant.

Sand fly distribution is also dependent on temperature and humidity, and species situated along altitudinal gradients have to adapt to a variety of climatic conditions (Telfer and Hassal 1999). Temperature is also one of the main factors preventing the spread of both visceral and cutaneous leishmaniasis (Kuhn 1999). This ecological factor varies with the altitude according to thermal altitudinal gradient (-0.6° C/100 m). Our data did not identify any actual significance related to temperature and humidity but this may stem from the lack of longitudinal data during the trapping process. Time punctual data (temperature, humidity, etc.) are more related to the level of activity of sand flies at the time of trapping, while data stable in time such as altitude or longitudinal data could be more related to the abundance of the populations over time.

Our survey indicated the importance of the diversity of the sampling locations and factors that may be effective during the collection process. Because of the reported cases of both CanL and ACL and the presence of a probable *Phlebotomus* vector species in the study area, we strongly recommend further analysis of the data using geographical information system tools that can provide an opportunity to produce risk maps or other analytical approaches and predict the potential risks in the areas where preliminary data has not been available.

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