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Sand Flies (Diptera: Phlebotominae) in Sanliurfa, Turkey: Relationship of *Phlebotomus sergenti* with the Epidemic of Anthroponotic Cutaneous Leishmaniasis

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ABSTRACT Sand fly (Diptera: Phlebotominae) fauna were surveyed in various districts of Sanliurfa in southeast Turkey for 3 yr immediately after an epidemic of cutaneous leishmaniasis (*Leishmania tropica*). Sticky papers and CDC light traps collected a total of 10,937 sand flies, of which 10,919 (4,158 females and 6,761 males) were identified as *Phlebotomus* and 18 (11 females and seven males) as *Sergentomyia* (*S. theodori* Parrot; *S. adleri* Theodor). Eight *Phlebotomus* spp. were identified: *P. sergenti* Parrot (72.3%), *P. papatasi* (Scopoli) (27.2%), *P. brevis* Theodor & Mesghali (0.20%), *P. neglectus* Leger & Pesson (0.13%), *P. perfiliewi* Parrot (0.05%), *P. mascitti* Grassi, *P. halepensis* Theodor, and *P. alexandri* Sinton (0.01%). *Phlebotomus mascitti* and *P. neglectus*, along with both *Sergentomyia* sp., have not been previously described from the study area. Similar results were obtained when both trapping methods were applied in the same houses, indicating that local *P. sergenti* and *P. papatasi* populations were equally attracted to the light. *P. sergenti* was consistently abundant, agreeing with the general view that this species is the vector of leishmaniasis in the region. There was no apparent decrease in the relative abundance of this vector versus the other species, suggesting that factor(s) other than a change in the dynamics of sand fly populations precipitated the decline of the human leishmaniasis epidemic in Sanliurfa.

KEY WORDS *Leishmania tropica*, sand fly, cutaneous leishmaniasis

LEISHMANIASIS IS CAUSED by a wide range of parasites in many countries and is transmitted by different species of sand fly vectors as a crucial part of the life cycle for both cutaneous and visceral forms of the disease. In Turkey, cutaneous leishmaniasis is highly endemic in the south and southeast, whereas visceral leishmaniasis and canine leishmaniasis have been sporadically observed mainly along the Aegean and Mediterranean regions (Ozcel et al.1999). Previous studies of sand flies in Turkey revealed 18 *Phlebotomus* species (or subspecies raised recently to species) belonging to subgenera *Adlerius*, *Larroussius*, *Paraphlebotomus*, and *Phlebotomus* (Houin et al. 1971, Yasarol 1980, Daldal et al. 1989, Budak et al. 1991, Yagci et al. 1998, Alptekin et al. 1999). Nine of them are proven or probable vectors of the Old World leishmaniases (Killick-Kendrick 1999).

Self-healing anthroponotic cutaneous leishmaniasis (ACL) has persisted in and around Sanliurfa (formerly Urfa)—the provincial capital in the southeast of the country near the Syrian border. The agent was isoenzyme-typed as *Leishmania tropica* zymodeme MON-53 (Gramiccia et al. 1984) and confirmed by gene sequence analysis of isolates from a recent epidemic (Akman et al. 2000). Clinical records compiled by the Municipal Department of Health showed that all confirmed cases of ACL were restricted to the old districts of the city where the yearly incidence peaked in 1993, with 2,980 cases (≈1% of the city population), which declined gradually to 2,369–2,780 in 1994–1996, and then abruptly dropped to ≈800 between 1997 and the present. Alptekin et al. (1999) concluded from their survey of sand flies conducted at the height of the epidemic in 1994–1996 that "*P. sergenti* and *P. papatasi* were the probable vectors of cutaneous leishmaniasis during this outbreak and control of these sand flies may eliminate transmission." Investigation of sand fly fauna in that area becomes all the more important with the completion of the Southeast Anatolia Water and Land Resources Development Project (GAP), which is expected to bring about drastic climatic changes, thereby affecting vector and parasite populations in the region.

Here, we report the species of sand flies collected after the decline of the epidemic in Sanliurfa from 1997 to 1999. We described several minor species previously not reported from the area.

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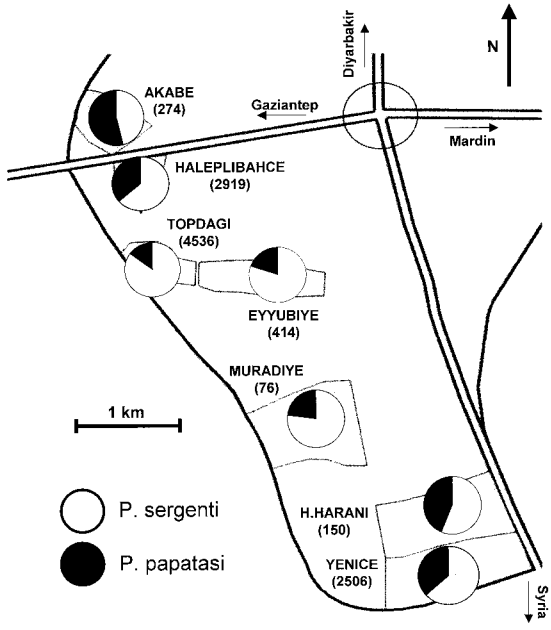

Fig. 1. Districts of Sanliurfa studied. Ratios of the two dominant species and their total numbers (*n*) collected in individual districts indicated.

Materials and Methods

Sampling was performed in seven districts in the old part of Sanliurfa city (Fig. 1) where >95% of the ACL cases were reported. The old part of the city is a mixture of urban and rural sites. Housing varies from two-story cement block enclosures to simple brick, stone, or cement houses with basements, cellars, caves, or barns for keeping poultry or livestock animals. The careful clinical management of leishmaniases including active search for patients and antimony therapy (usually Glucantime) has been applied in Sanliurfa since 1994. All patients were injected three times a week (at least 10 doses in total) either intralesionally or intramuscularly when more lesions were present. However, there was no spatial spraying of insecticides in study area since 1996.

Study was performed in 1997–1999, between May and September, which is the hot and dry season. Sand flies were collected by sticky paper traps made from sheets of paper (20 by 20 cm) coated with castor oil and with CDC miniature light traps. Traps were placed in and near houses of active or past ACL patients. In September 1999, both trapping methods were applied in the same houses. On each catching night, 15–20 sticky papers and three to four light traps were set in each of the selected houses.

Flies caught by sticky papers were immersed first in 96% ethanol to remove the oil, transferred to 70% ethanol, and mounted in chloralhydrate medium for later identification. Live female flies caught by light traps in August 1998 and September 1999 were examined for parasite infection. Females were immobilized on ice, washed in 70% ethanol, dissected in sterile saline and their guts were microscopically examined for the presence of promastigotes.

Species identification was done using the keys and descriptions presented by Perfiliew (1968), Lewis (1982), Artemiev (1980), and Killick-Kendrick et al. (1991). Most specimens were placed in the collection of Department of Parasitology, Charles University in Prague. Some *Adlerius* samples were sent to R. Killick-Kendrick (Imperial College, Ascot, UK) and J. Depaquit (U.E.R. de Pharmacie, Reims, France) for further study.

Results and Discussion

In total, 10,937 sand flies were collected throughout this study from seven districts of Sanliurfa (Fig. 1). Altogether, 10 species were identified, consisting of eight *Phlebotomus* spp. and two *Sergentomyia* spp. (Table 1). In most districts, *P. (Paraphlebotomus) sergenti* Parrot was the dominant species, whereas *P. (Phlebotomus) papatasi* (Scopoli) was the second most dominant one. These two abundant species consti-

Table 1. Sand fly species collected in Sanliurfa and their relative abundance

Species	Sex	Year 1997	1998	1999	Total	%
P. sergenti	M	1,203	2,064	1,769	5,036	72.25
	F	570	1,188	1,108	2,866	
P. papatasi	M	295	586	817	1,698	27.18
	F	206	430	639	1,275	
P. brevis	M	0	7	6	13	0.20
	F	0	6	3	9	
P. neglectus	M	4	1	3	8	0.13
	F	5	0	1	6	
P. perfiliewi	M	2	0	1	3	0.05
	F	0	1	1	2	
P. mascitti	M	0	0	1	1	0.01
P. halepensis	M	0	0	1	1	0.01
P. alexandri	M	0	0	1	1	0.01
S. theodori	M	2	5	0	7	0.15
	F	2	5	2	9	
S. adleri	F	0	0	2	2	0.02
Total		2,289	4,293	4,355	10,937	100

Table 2. Comparison of CDC light traps and sticky paper traps for two dominant species

	P. sergenti				*P. papatasi*				P.s./P.p. ratio	
	Male	Female	Total	%	Male	Female	Total	%		
Light trap	792	594	1,386	65	359	382	741	35	1.87	
Sticky paper	152	39	191		64	80	28	108	36	1.76

tuted 99.43% of the total flies collected throughout the study (Table 1). The ratio of *P. sergenti* /*P. papatasi* varied from 0.85:1 to 5.80:1 in different sites, the average being 2.65:1 (Fig. 1; Table 2).

For *Phlebotomus* species, more males were collected than females from all sites. The average of male/female ratio was 1.63:1. In September 1999, the male/female ratio as well as the ratio of *P. sergenti* /*P. papatasi* were found to be similar in individual houses, irrespective of the methods of collection using light traps or sticky papers (Table 2). This indicates that the local *P. sergenti* and *P. papatasi* populations are equally attracted to the light. Thus, the results from CDC light traps are suitable for determining the ratio of these two abundant species.

The remaining species identified were far less abundant but two of them deserved mentioning in more detail.

One was tentatively identified as *P. (Adlerius) brevis* Theodor & Mesghali. This species was captured repeatedly (13 males and nine females) in the cellar and basement of houses in Top Dagi and Eyyubiye, two districts located in the western part of the old city (Fig. 1). The same species was first reported from Turkey by Yasarol (1980) and from Sanliurfa recently by Alptekin et al. (1999).

Females in subgenus *Adlerius* cannot be readily distinguished by conventional morphological criteria; and despite the comprehensive work by Artemiev (1980), the status of several *Adlerius* species has remained confusing. Therefore, we present some measurements of specimens collected in 1998 as follows: males ($n = 7$): third antennal segment (A3) was 332 (306–354) μm long, labrum 295 (276–330) μm long, ratio A3/labrum: 1.13 (1.07–1.22). Antennal ascoid formulae 2/3–5, 1/6–15 for all specimens. Coxite 359 (348–372) μm long with 27 (23–31) hairs positioned partly on distal half (position 0.56–0.60). Aedeagus with subterminal tooth 14 (12–15) μm from the tip. Females ($n = 6$) A3: 295 (282–318) μm, labrum: 377 (354–402) μm, A3/labrum: 0.78 (0.76–0.81).

Our samples were consistent with following data reported by Artemiev (1980) for *P. brevis:* the measurements of A3/labrum for both sexes, the shape of male coxite, the position of coxite hairs, and the morphology of pharyngeal armature of females. Inconsistent with his data were the lengths of A3, labrum, and coxite, which are larger in the specimens collected in Sanliurfa than those of *P. brevis* reported so far. Differences were also found in the antennal ascoid formulae in males (for *P. brevis* 2/3–8, eventually 2/3–6), but this character may not be an absolute parameter for species-identification because of its light intraspecific variation (N. Leger, U.E.R. de Pharmacie, Reims, personal communication). The true identity of the specimen collected in Sanliurfa awaits further investigation.

Phlebotomu (Larroussius) neglectus Leger & Pesson was captured in low numbers (eight males and six females) from the Topdagi district (Fig. 1). Previous studies from Sanliurfa (Alptekin et al. 1999, Akkafa and Tasci 1999) reported either *P. syriacus* or *P. major.* At present, *P. neglectus* and *P. syriacus,* former subspecies of *P. major,* are well-accepted species living generally in allopatry. Differentiation of the two is based on the size of male claspers (style and coxite), morphology of the base of the spermathecal ducts (Leger et al. 1983), and the uppermost segment of the spermatheca (Killick-Kendrick et al. 1991). Claspers of the males collected in Sanliurfa were consistent in size with those of *P. neglectus;* their coxites were 0.335–0.355 μm and styles 0.155–0.180 μm long. *P. neglectus* specimens of similar features were also found by our group around Izmir at Aegean coast in West Turkey. All these flies differ considerably from *P. syriacus* collected in north Syria only ≈50 km southeast of Sanliurfa.

The remaining five species of flies [*P. (Adlerius) halepensis* Theodor, *P. (Larroussius) perfiliewi* Parrot, *P. (Paraphlebotomus) alexandri* Sinton, *P. (Transphlebotomus) mascitti* Grassi, *S. theodori* Parrot and *S. adleri* Theodor] were caught only occasionally from various districts of Sanliurfa. Three of them, *P. mascitti* and both *Sergentomyia* species, were reported for the first time from this site. In *P. perfiliewi* males, the transparent part of aedeagus was narrow, corresponding to the description given by Perfiliew (1968) for subspecies *transcaucasicus.*

No promastigotes were found in any of the 1,139 live females (859 *P. sergenti,* 278 *P. papatasi,* and two *P. brevis*) dissected for microscopic examinations in August 1998 and September 1999. This was not unexpected in view of the rapid decline in the number of patients from several thousands at the peak of the epidemic to several hundreds during the period of this investigation.

Phlebotomus sergenti was the most dominant species in Sanliurfa. It is a proven vector of *L. tropica* in most endemic sites elsewhere, e.g., Morocco (Guilvard et al. 1991) and Saudi Arabia (Al-Zahrani et al. 1988). This peridomestic and anthropophilic sand fly is highly susceptible to *L. tropica* (Killick-Kendrick et al. 1995). In contrast, *P. papatasi* is susceptible to *L. major,* but not to *L. tropica* (Killick-Kendrick et al. 1994). These data support the consideration of *P. sergenti* as the vector of ACL in Sanliurfa.

The relative abundance of *P. sergenti* versus the other species remained as high as that recorded earlier in the peak epidemic years (Alptekin et al. 1999). The decline of ACL from ≈2,500 to ≈800 cases since 1997,

thus, appeared to be independent of dynamic changes in the population of this sand fly. Moreover, isoenzyme typing of the *L. tropica* strain isolated in June 1999 revealed again zymodeme MON-53 (Y.O., unpublished data), exactly as reported from the same site >16 yr ago by Gramiccia et al. (1984). These data further suggest that factor(s) other than a change in vector population or parasite strains precipitated the decline of epidemic human leishmaniasis in Sanliurfa. We hypothesize that effective clinical management or development of immunity in the resident population may be the precipitating factor(s). Taken together, the cyclic emergence and decline of epidemic leishmaniasis in this area appears to deviate from the usual patterns proposed, i.e., introduction of new parasites into a susceptible population, migration of susceptible population into an endemic area or fluctuation of reservoir populations (Ashford 1999).

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