Article available at http://www.parasite-journal.org or http://dx.doi.org/10.1051/parasite/2006131023

PHLEBOTOMINAE (DIPTERA: PSYCHODIDAE) OF HUMAN LEISHMANIOSIS SITES IN TUNISIA

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Summary:

In order to identify the phlebotomine sandfly populations in Tunisian leishmaniosis foci, an entomological survey was carried out through three entomological seasons (2002-2003-2004) in 19 visceral and cutaneous leishmaniosis areas, located in six bioclimatic zones. Sandfly collections were based on light and sticky traps placed around human leishmaniosis cases. 8,722 phlebotomine sandflies belonging to 12 species were collected. The dominance of subgenus *Larroussius* species in northern foci, *Phlebotomus papatasi* in south-western foci and their co-dominance in the centre of the country is in accordance with the distribution of *Leishmania infantum* and *L. major* in Tunisia. The low density found in the historical zoonotic cutaneous leishmaniosis focus of Metlaoui in the south-west may indicate the high competence of the local populations. Studied phlebotomine settlements have showed a low specific diversity in most of the studied sites. In *L. infantum* areas, the dominant species were respectively: *P. perfiliewi* in the cutaneous leishmaniosis site of the humid bioclimatic stage, *P. perniciosus* in the cutaneous and visceral leishmaniosis foci of semi-arid and arid bioclimatic stages and *P. longicuspis* in the visceral leishmaniosis focus of saharan bioclimate. In the zoonotic cutaneous leishmaniosis foci, *P. papatasi* was a dominant species. In the well-known south-eastern foci of cutaneous leishmaniosis due to *L. killicki*, *P. sergenti* was a dominant species with *P. perniciosus*. In the central emerging foci of *L. killicki*, *P. perniciosus* was a dominant species in some sites whereas it was very rare in others. In these sites, the subgenus *Paraphlebotomus* was always present with a higher abundance of *P. alexandri* than *P. sergenti*.

KEY WORDS : Phlebotominae, visceral leishmaniosis, cutaneous leishmaniosis, *Leishmania infantum*, *Leishmania major*, *Leishmania killicki*, epidemiology, entomology, Tunisia.

Résumé : PHLEBOTOMINAE (DIPTERA : PSYCHODIDAE) DES RÉGIONS DE LEISHMANIOSE HUMAINE EN TUNISIE

Afin de caractériser les populations phlebotomiennes des foyers de leishmaniose humaine en Tunisie, une enquête entomologique a été effectuée durant trois saisons entomologiques (2002-2003-2004) dans 19 foyers de leishmanioses viscérale et cutanée, situés dans six sous-étages bioclimatiques. Les collectes de phlébotomes ont été effectuées par des pièges lumineux et adhésifs placés autour des cas humains de leishmaniose. 8722 phlébotomes ont été capturés appartenant à 12 espèces. La dominance des espèces de Larroussius *dans les foyers du nord, de* P. papatasi *dans ceux du sud-ouest et leur codominance au centre du pays est en accord avec la répartition de* Leishmania infantum *et* L. major *en Tunisie. La faible densité des phlébotomes trouvée dans le foyer historique de leishmaniose cutanée zoonotique de Métlaoui suggère la haute compétence des populations phlébotomiennes locales. Les peuplements phlébotomiens étudiés ont montré une faible diversité spécifique dans la plupart des sites étudiés. Dans les foyers de* L. infantum, *les espèces dominantes étaient respectivement* P. perfiliewi *dans le foyer de leishmaniose cutanée de l'étage bio-climatique humide,* P. perniciosus *dans les foyers de leishmanioses cutanée et viscérale des étages bio-climatiques semi-aride et aride et* P. longicuspis *dans le foyer de leishmaniose viscérale de l'étage saharien. Dans les foyers de leishmaniose cutanée zoonotique,* P. papatasi *était une espèce dominante. Dans les foyers connus de leishmaniose cutanée à* L. killicki *du sud-est,* P. sergenti *était l'espèce dominante associée à* P. perniciosus. *Dans les foyers émergents du centre, si* P. perniciosus *était l'espèce dominante dans certains sites, elle était rarement trouvée dans d'autres. Le sous genre* Paraphlebotomus *était toujours présent avec une prédominance de* P. alexandri *par rapport à* P. sergenti.

MOTS CLÉS : *Phlebotominae, leishmaniose viscérale, leishmaniose cutanée,* Leishmania infantum, Leishmania major, Leishmania killicki, *épidémiologie, entomologie, Tunisie.*

INTRODUCTION

In Tunisia three species of *Leishmania* coexist. They occur in particular ecological features and cause human visceral and cutaneous leishmaniosis (Ben Ismail & Ben Rachid, 1989). *L. infantum* occurs in the north and in the centre of the country and causes two forms: visceral leishmaniosis (VLI) and cutaneous leishmaniosis (CLI) (Bouratbine *et al.*, 1998; Aoun *et al.*, 2000). Although *Phlebotomus (Larroussius) perniciosus* Newstead, 1911 seems to be the main vector, there is no available information concerning the possible role of other species of *Larroussius* in the transmission of VLI and CLI (Killick-Kendrick, 1990). *L. major* causes zoonotic cutaneous leishmaniosis (CLM), the most extensive and prevalent form with thousands of cases reported each year since the start of a large epidemic in 1982 (Ben Ismail & Ben Rachid, 1989; DSSB, 2001). This form is endemic in the centre and the south of

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Tunisia and is transmitted from wild rodents by *P. (Phlebotomus) papatasi* Scopoli, 1786 (Ben Ismail *et al.*, 1987). *L. killicki* is the agent of a chronic cutaneous leishmaniosis form (CLK). It was known to occur as scarce cases in micro foci located in the south-east (Rioux *et al.*, 1986d). Recently, cases due to *L. killicki* have been reported in the centre and the south-western Tunisia, in foci of CLM (Bouratbine *et al.*, 2005). The reservoir and the vector of this specie are unknown. The reservoir seems zoonotic, mainly because of the sporadic occurrence of cases and the rural distribution of disease (Ben Rachid & Ben Ismail, 1989). The vector could belong to the subgenera *Paraphlebotomus* or *Larroussius* as described elsewhere for *L. tropica,* a closely related species to *L. killicki* (Rioux *et al.*, 1986d; Lawyer *et al.*, 1991; Rioux *et al.*, 1990; Jacobson, 2003; Gebre-Michael *et al.*, 2004).
Since 1980's there has been an increase of case numbers, a geographic range extension and an occurrence of epidemics, which are related to all forms of leishmaniosis recorded in the country. This new epidemiological situation may be connected to changes in land use (urbanisation and especially the mobilisation of water resources) that was carried out in Tunisia in the past few years. These ecological modifications have probably induced an increase in reservoir and vector population densities and changes in their geographical distribution (Ben Ismaïl & Ben Rachid, 1989; Rioux & De la Roque, 2003; Aoun *et al.*, 2004; Baldi *et al.*, 2004). The aim of the present study is to describe the sandflies occurring in different human leishmaniosis sites in Tunisia and to discuss vector role in relation to species distribution and abundance.

MATERIAL AND METHODS

STUDY AREAS

• VL sites

The study was carried out in six VL sites, situated in five bioclimatic zones (Fig. 1). Five VL sites are located in different parts of the range of *L. infantum*. They were from north to south: Ghezala (north, sub-humid), Marthouma (north, lower semi-arid) and Aïn Jloula (centre, upper arid) which are active sites regularly generating sporadic cases ; Garet Ennam and Sidi Salem (centre, upper arid) which are located near the southern border of the range of *L. infantum* and where the last cases has been notified respectively in 1988 and 2001 (DSSB, 2001). The 6th site Mahassen (south-west, upper saharan) is the only known focus in the Saharan bioclimatic stage so far. It is an isolated site located outside of the continuous *L. infantum* range (Anderson, 1934 ; Bouratbine *et al.*, 1998).

• CL sites

15 CL sites were studied. Regarding the leishmaniosis form they were grouped as follows (Fig. 2):
- three CLI sites: Aïn Somra (north, sub-humid) and Jmila (north, lower humid) are active foci of CLI (Aoun *et al.*, 2000). Nchem (north, middle semi-arid) is an emerging epidemic focus located in the southern border of CLI area (Belhadj *et al.*, 2003);
- six CLM active sites located in different parts of the area of *L. major* (Ben Ismail & Ben Rachid, 1989): Zemla and Sidi Salem (centre, upper arid); Aouabdia (south-west, upper arid); Mahassen, Ras Dhraa and Boulifa (south-west, upper saharan);
- six CLK sites: two well known sites (Rioux *et al.,* 1986c), Nsefri and Rogba, located in the south-east, in Lower arid bioclimate and four new sites, which are confirmed or probable mixed foci of CLK and CLM (Bouratbine *et al.*, 2005). Ghanzour and Ennajeh (centre, upper arid) are located between the northern CLI area and the septentrional border of CLM area. In these emergent foci, *L. killicki* was isolated but the presence of *L. major* and *L. infantum* is also suspected. Gouleb (Centre, Upper arid) and Metlaoui (South-west, Upper arid) are confirmed mixed foci.

SAMPLE COLLECTIONS

Captures of phlebotomine sandflies were carried out through three entomological seasons: between July and October 2002 mainly in the north, from May to November 2003 in the south and from June to November 2004 mainly in the centre of the country.
All captures were done in anthroponized biotopes, around confirmed leishmaniosis cases. Target stations were houses with yards (used as resting places) and adjacent animal sheds (sheep, cows, dogs, chickens). Collections were based on CDC light-traps and sticky papers (Rioux *et al.*, 1982) placed indoors, outdoors and in animal sheds. Traps were placed before sunset and taken in the following morning. Some sticky-trap were left five nights according to weather conditions and to CDC output. Repeated samples were carried out in the entomological season in all *L. major* and/or *L. killicki* sites. *L. infantum* sites were sampled once.
Collected sandflies were identified according to morphological characters described by Croset *et al.* (1978) and by Léger *et al.* (1983) for *Larroussius* female's.

ANALYSIS

To characterize phlebotominae settlements of different human leishmaniosis sites the following ecological parameters and indexes were calculated separately for captures using sticky traps and those obtained with CDC traps:

- Relative abundance (A_r) = $\frac{\text{Number of specimens of taxon i}}{\text{Total number of phlebotomines in the sample}} \times 100$

- Degree of presence (D)* = $\frac{\text{Number of foci containing the species i}}{\text{Total number of foci}} \times 100$

*D was the only parameter calculated from mixed CDC and sticky traps captures

- Density (d): number of phlebotomines per m^2 of sticky paper per night (ph/m^2/night)

- Specific richness (S): number of species in the sample (Spellerberg & Fedor, 2003).

Besides specific richness, specific diversity was also measured by indexes diversity that expresses simultaneously relation between number of species and number of individuals (Spellerberg & Fedor, 2003).

- Index of Simpson (I_s) = $\frac{1}{\sum p_i^2}$

p_i = Ar/100

I_s vary between 1 and S

I_s = 1: dominance of one species

I_s = S: species having equal abundance

- Equitability (E) = $\frac{I_s - 1}{S - 1}$

E vary between 0 and 1

E = 0: dominance of one species

I_s = 1: species having equal abundance

Statistical analyses were performed with Epi-info and SPSS Software packages. χ^2 test and Kruskal-Wallis test

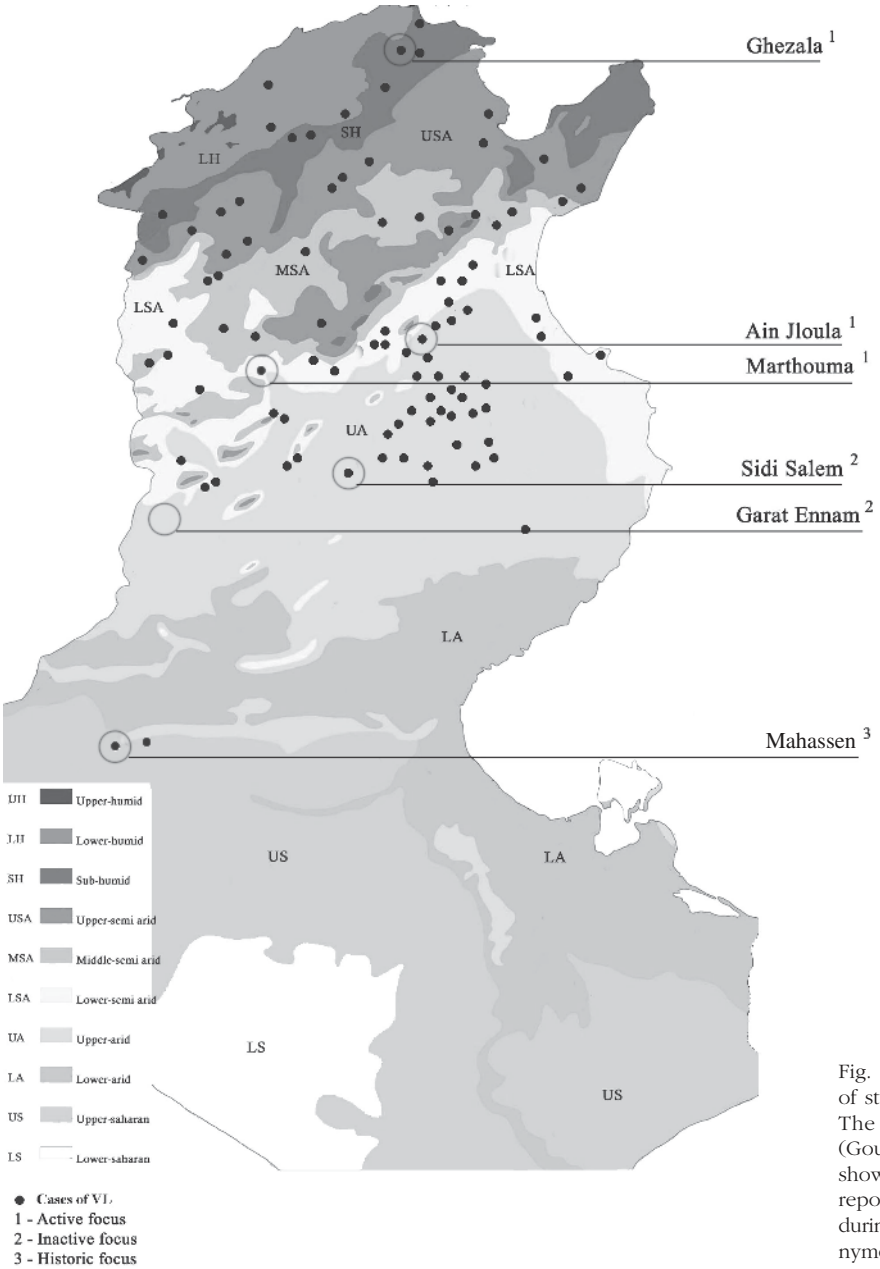

Fig. 1. – Geographical and bioclimatic situations of studied VL foci.
The map reproduces the bioclimatic stages (Gounot & Le Houerou, 1967 in Nabli, 1989) and shows the geographical distribution of VL cases reported by the Directory of Primary Health Care during the season of transmission 2001-2002 (Anonymous, 2003).

were used to compare percentages and averages respectively.

RESULTS

Global results

A total of 8,722 phlebotomine sandflies were collected and morphologically identified as follows: *P.* (*L.*) *perniciosus* Newstead, 1911: 3,395 (38.9 %); *P.* (*L.*) *perfiliewi* Parrot, 1939: 2,256 (25.9 %); *P.* (*L.*) *longicuspis* Nitzulescu, 1930: 266 (3 %); *P.* (*L.*) *langeroni* Nitzulescu, 1930: 46 (0.5 %); *P.* (*Paraphlebotomus*) *alexandri* Sinton, 1928: 194 (2.2 %); *P.* (*Pa.*) *sergenti* Parrot, 1917: 126 (1.4 %); *P.* (*Pa.*) *chabaudi* Croset, Abonnenc et Rioux, 1970: 3 (0.03 %); *P.* (*Ph.*) *papatasi* (Scopoli, 1786): 991 (11.4 %) *Sergentomyia* (*Sergentomyia*) *fallax* Parrot, 1921: 816 (9.4 %); *S.* (*S.*) *minuta parroti* Adler et Theodor, 1927: 540 (6.2 %); *S.* (*S.*) *antennata* (Newstead, 1912): 60 (0.7 %) and *S.* (*Grassomyia*) *dreyfussi* Parrot, 1933: 29 (0.3 %) (Tables Ia and Ib).

The mean density was significantly different according to the regions. It was high in the north (in humid, subhumid and semi-arid bioclimatic stages) (mean density = 29.3 ± 25.8 ph/m^2/night) still important in arid foci of the centre (mean density = 13.9 ± 17.7 ph/m^2/night)

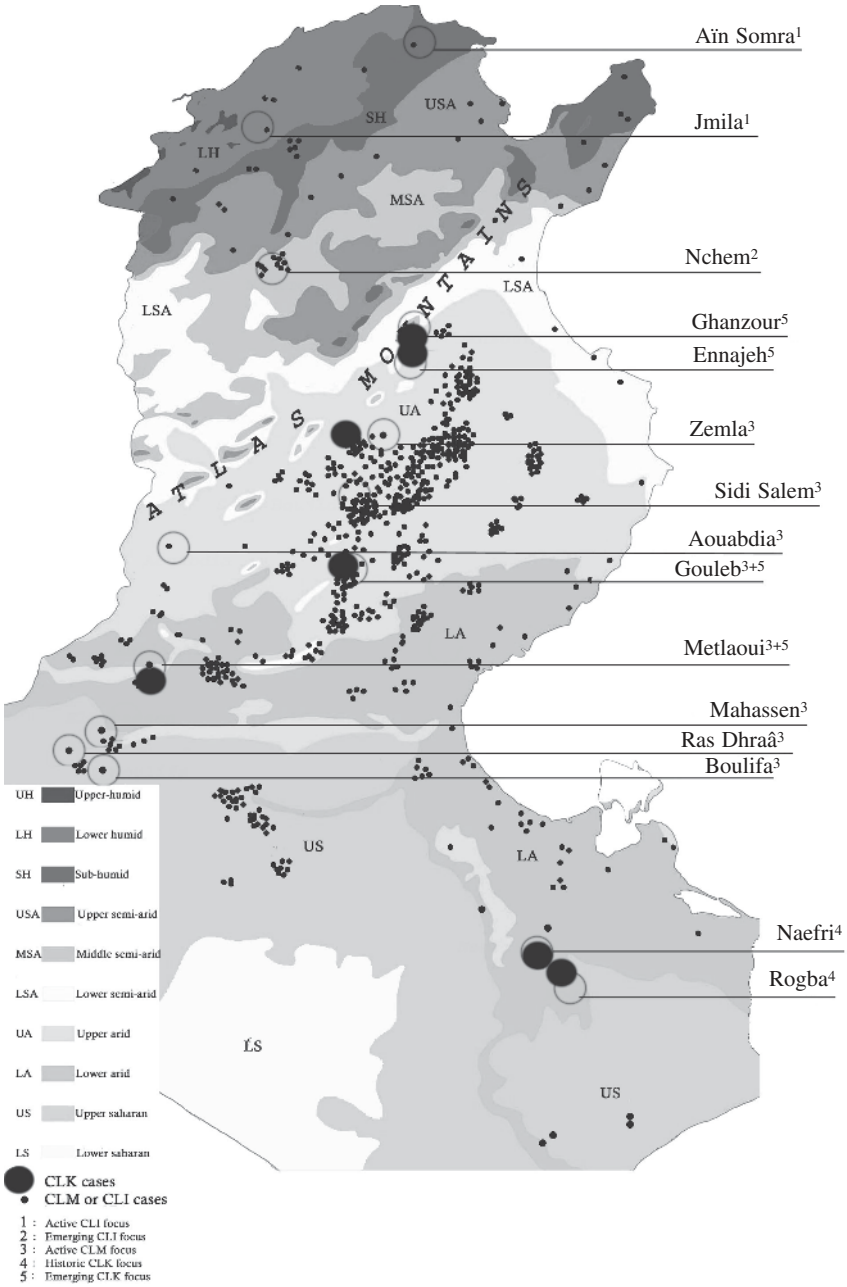

Fig. 2. – Geographical and bioclimatic situations of studied CL foci.
The map reproduces the bioclimatic stages (Gounot & Le Houerou, 1967 in Nabli, 1989) and shows the geographical distribution of CL cases reported by the Directory of Primary Health Care during the season of transmission 2001-2002 (Anonymous, 2003).

and decreased in southern foci (mean density = 0.56 ± 1.07 ph/m^2/night) (Fig. 3) ($p < 0.01$ %).

P. papatasi and *S. minuta parroti* were common species and showed the largest distribution as indicated by the high degree of presence (D). They were collected from 18 foci out of the 19 prospected (D = 94.7 %).

P. perniciosus was also a common species with a large distribution (D = 84 %). *P. langeroni* was collected only from Nchem (D = 5.3 %). It was the first report of this female specie in Tunisia (Ghrab *et al.*, 2005). *P. chabaudi* also a rare species, was collected from two localities in the southeast (D = 10.5 %) (Tables Ia and Ib).

	CDC traps		**Species**											
Foci	nb	nights	*perf.*	*pern.*	*long.*	*lang.*	*se.r*	*ale.*	*chab.*	*Pap.*	*fal.*	*min.*	*ant.*	*dre.*	**Total**
Jmila	6	1	1,986	243	6	–	–	–	–	3	2	33	–	–	2,273
Ghezala	3	1	4	38	1	–	–	–	–	13	–	103	–	–	159
A. Somra	3	1	–	7	1	–	–	–	–	3	–	3	–	–	14
Nchem	6	1	34	1,130	68	41	–	–	–	6	1	10	–	–	1,290
Marthouma	6	1	11	96	12	–	1	1	–	1	4	1	–	–	127
Ghanzour	12	3	4	321	58	–	9	83	–	244	213	30	4	16	982
Ennajeh	15	3	3	526	51	–	10	37	–	112	63	7	4	2	815
A. Jloula	1	1	–	56	–	–	–	5	–	52	29	–	–	1	143
G. Ennam	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
Zemla	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
S. Salem	9	3	4	53	13	–	–	2	–	53	5	7	–	–	137
Gouleb	9	3	–	4	–	–	–	23	–	24	232	88	–	2	373
Metlaoui	38	10	–	–	–	–	–	3	–	9	1	3	1		17
Aouabdia	19	6	–	–	–	–	–	–	–	20	–	–	2	2	24
Nsefri	25	10	–	58	–	–	60	6	2	40	21	3	9	–	199
Rogba	19	7	–	19	–	–	34	1	1	89	34	2	6	3	189
Mahassen	19	6	–	4	12	–	–	–	–	31	1	–	2	–	50
R Dhraâ	15	6	–	–	–	–	–	–	–	7	1	10	5	1	24
Boulifa	15	5	–	–	5	–	–	–	–	3	1	7	–	–	16
Total	220	68	2,046	2,555	227	41	114	161	3	710	608	307	33	27	6,832

perf.: *P. perfiliewi*; *pern.*: *P. perniciosus*; *long.*: *P. longicuspis*; *lang.*: *P. langeroni*; *ser.*: *P. sergenti*; *ale.*: *P. alexandri*; *chab.*: *P. chabaudi*; *pap.*: *P. papatasi*; *fal.*: *S. fallax*; *min.*: *S. minuta parroti*; *ant.*: *S. antennata*; *dre.*: *S. dreyfussi*.

Table Ia. – Distribution of phlebotomine species caught by CDC traps in the studied leishmaniosis sites.

	Stiky traps		**Species**											
Foci	M^2	nights	*perf.*	*pern.*	*long.*	*lang.*	*se.r*	*ale.*	*chab.*	*Pap.*	*fal.*	*min.*	*ant.*	*dre.*	**Total**
Jmila	5	1	44	24	–	–	–	–	–	2	–	11	–	–	81
Ghezala	3	5	2	28	1	–	–	–	–	38	–	96	–	–	165
A. Somra	4	5	1	30	2	–	–	–	–	3	–	63	–	–	99
Nchem	5	1	5	252	6	5	–	–	–	–	–	–	–	–	268
Marthouma	4	1	158	73	2	–	–	1	–	6	3	–	–	–	243
Ghanzour	6,3	2	–	151	6	–	5	25	–	84	120	6	6	2	405
Ennajeh	4	2	–	165	6	–	–	2	–	1	5	–	–	–	179
A. Jloula	1.3	1	–	41	–	–	1	2	–	9	–	–	–	–	53
G. Ennam	19.5	20	–	–	–	–	–	–	–	–	–	5	–	–	5
Zemla	11.7	15	–	37	4	–	1	–	–	53	3	16	10	–	124
S. Salem	9	15	–	33	2	–	–	–	–	23	–	6	1	–	65
Gouleb	9.8	15	–	–	–	–	–	2	–	4	65	19	–	–	90
Metlaoui	19.5	10	–	1	–	–	2	–	–	9	–	2	1	–	15
Aouabdia	20	6	–	–	2	–	–	–	–	22	1	5	4	–	34
Nsefri	17	6	–	4	–	–	3	1	–	14	7	–	1	–	30
Rogba	1	1	–	–	–	–	–	–	–	2	1	–	–	–	3
Mahassen	34	11	–	–	4	–	–	–	–	3	3	2	–	–	12
R Dhraâ	14.1	6	–	–	–	–	–	–	–	6	–	2	3	–	11
Boulifa	14.9	5	–	1	4	–	–	–	–	2	–	–	1	–	8
Total	203	128	210	840	39	5	12	33	0	281	208	233	27	2	1,890

perf.: *P. perfiliewi*; *pern.*: *P. perniciosus*; *long.*: *P. longicuspis*; *lang.*: *P. langeroni*; *ser.*: *P. sergenti*; *ale.*: *P. alexandri*; *chab.*: *P. chabaudi*; *pap.*: *P. papatasi*; *fal.*: *S. fallax*; *min.*: *S. minuta parroti*; *ant.*: *S. antennata*; *dre.*: *S. dreyfussi*.

Table Ib. – Distribution of phlebotomine species caught by sticky traps in the studied leishmaniosis sites.

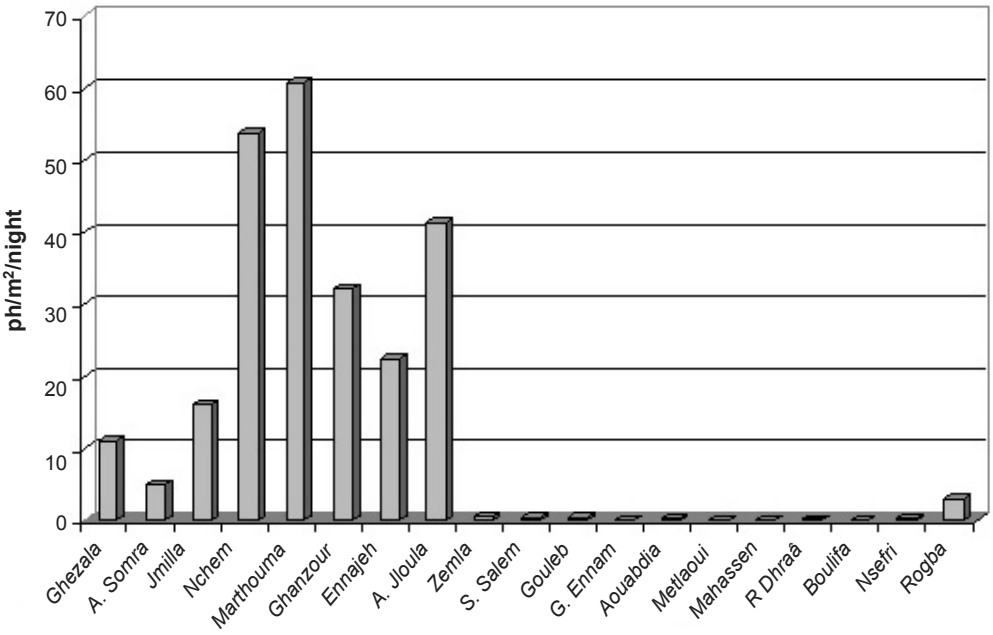

Fig. 3. – Density of phlebotomine sandflies per focus calculated on collections of sticky traps.

Specific richness (S) was higher in CDC captures than in sticky ones (Tables IIa and IIb). Sampled with CDC traps, most of the sites (14 out of 19) have got a specific richness equal or superior to five, with a maxima in central foci situated on the southern side of the Atlas Mountains (S = 10). However, Simpson index (I_s) showed a low diversity in all sites. This result is confirmed by the equitability index (E) showing values near to zero. Thus using this kind of traps, we could group the sites as follows: those with an Equitability $E \leq 0.2$ in which only one species dominates; those with $0.2 < E \leq 0.4$ in which two species co-dominate and finally sites with $0.4 < E \leq 0.6$ in which three species co-dominate (Table IIa). Using sticky traps, Simpson index and equitability showed a higher diversity in some sites so a fourth group where all the species have an equal dominance ($0.6 < E < 1$) was added (Table IIb).

Two foci of the south-east (Nsefri and Rogba) presented specimens of *P. perniciosus* with curved distal portion of aedeagi which can be confused with *P. longicuspis* (Fig. 4). However, the number of coxite hairs varied from 10 to 17 (mean = 14) whereas this number varied in *P. longicuspis sensu stricto* from 21 to 27 (mean = 24.8). In this area only one specimen of *P. perniciosus* displayed the typical bifid aedeagus as the males collected in all other prospected sites.

Fig. 4. – Aedeagi of *P. perniciosus* collected in south-eastern foci.

Phlebotominae of VL sites

Among the 955 phlebotomine sandflies identified from captures carried out between July and September in each of the six VL foci, 706 (73.9 %) belonged to the genus *Phlebotomus* including 561 (79.5 %) *Larroussius* (Table III). 144 (25.7 %) were females shared as follows: 109 (75.7 %) in animal sheds, 29 (20 %) indoors and six (4.3 %) outdoors. No statistical significance was found according to the site of capture.

The subgenus *Larroussius* was absent in Garet Ennam, a site located on the border of the area of *L. infantum*. In Ghezala, Aïn Jloula and Sidi Salem the specific composition showed a dominance of *P. perniciosus*. In Marthouma, *P. perfiliewi* was the dominant species in sticky traps whereas *P. perniciosus* was dominant in CDC traps. In the south-western focus of Mahassen *P. longicuspis* predominated (Tables IIa, IIb and III).

Phlebotominae of CL sites

Among the 7,827 phlebotomine sandflies collected in CL sites, 4,025 (51.5 %) were caught in the northern CLI sites, 505 (6.4 %) in the central and south-eastern CLM sites, 421 (5.4 %) in the classic south-eastern sites of CLK and finally 2,876 (36.7 %) in the emergent sites of CLK.

Foci	S*	Is**	E***	Dominant species (relative abundance)
Jmila	6	1.29	0.06	*P. perfiliewi* (87.4 %)
Ghezala	5	2.07	0.27	*S. m. parroti* (64.8 %); *P. perniciosus* (23.9 %)
A. Somra	4	2.88	0.63	*P. perniciosus* (50 %); *P. papatasi* (21.4 %); *S. m. parroti* (21.4 %)
Nchem	7	1.3	0.05	*P. perniciosus* (87.6 %)
Marthouma	8	1.7	0.1	*P. perniciosus* (75.6 %)
Ghanzour	10	4.39	0.38	*P. perniciosus* (32.7 %); *P. papatasi* (24.8 %)
Ennajeh	10	2.23	0.14	*P. perniciosus* (64.5 %); *P. papatasi* (13.7 %)
A. Jloula	5	3.05	0.51	*P. perniciosus* (39.2 %); *P. papatasi* (36.4 %); *S. fallax* (20.3 %)
G. Ennam	–	–	–	–
Zemla	–	–	–	–
S. Salem	7	3.19	0.37	*P. perniciosus* (38.7 %); *P. papatasi* (38.7 %)
Gouleb	6	2.22	0.24	*S. fallax* (62.2 %); *S. m. parroti* (23,6 %)
Metlaoui	5	2.86	0.47	*P. papatasi* (52.9 %); *P. alexandri* (17.6 %); *S. m. parroti* (17.6 %)
Aouabdia	3	1.41	0.21	*P.papatasi* (83.3 %)
Nsefri	8	4.34	0.48	*P. sergenti* (30.2 %); *P. perniciosus* (29.1 %); *P. papatasi* (20.1 %)
Rogba	9	3.36	0.3	*P. papatasi* (47.1 %); *P. sergenti* (18 %)
Mahassen	5	2.22	0.31	*P. papatasi* (62 %); *P. longicuspis* (24 %)
R Dhraâ	5	3.27	0.57	*S. m. parroti* (41.7 %); *P. papatasi* (29.2 %); *S. antennata* (20.8 %)
Boulifa	4	3.05	0.68	*S. m. parroti* (43.8 %); *P. longicuspis* (31.3 %); *P. papatasi* (18.8 %)

* S: specific richness; ** I_s: index of Simpson; and *** E: equitability.

Table IIa. – Diversity and dominant species of phlebotomine caught on CDC traps in leishmaniosis sites.

Foci	S*	Is**	E***	Dominant species (relative abundance)
Jmila	4	2.49	0.5	*P. perfiliewi* (54.3 %); *P. perniciosus* (29.6 %); *S. m. parroti* (13.6 %)
Ghezala	5	2.38	0.35	*S. m. parroti* (58.2 %); *P. papatasi* (23 %); *P. perniciosus* (17 %)
A. Somra	5	2.01	0.25	*S. m. parroti* (63.3 %); *P. perniciosus* (30.3 %)
Nchem	4	1.13	0.04	*P. perniciosus* (94 %)
Marthouma	6	1.95	0.19	*P. perfiliewi* (65 %)
Ghanzour	9	3.64	0.33	*P. perniciosus* (37.3 %); *S. fallax* (29.6 %); *P. papatasi* (20.7 %)
Ennajeh	5	1.17	0.04	*P. perniciosus* (92.2 %)
A. Jloula	4	1.59	0.2	*P. perniciosus* (77.4 %)
G. Ennam	1	1	–	*S. m. parroti* (100 %)
Zemla	7	3.37	0.4	*P. papatasi* (42.7 %); *P. perniciosus* (29.8 %); *S. m. parroti* (12.9 %)
S. Salem	5	2.55	0.39	*P. perniciosus* (50.8 %); *P. papatasi* (35.4 %); *S. m. parroti* (19.2 %)
Gouleb	4	1.76	0.25	*S. fallax* (72.2 %); *S. m. parroti* (21.1 %)
Metlaoui	5	2.47	0.37	*P. papatasi* (60 %); *P. sergenti* (13.3 %); *S. m. parroti* (13.3 %)
Aouabdia	5	2.18	0.3	*P. papatasi* (64.7 %); *S. m. parroti* (14.7 %); *S. antennata* (11.8 %)
Nsefri	6	3.31	0.46	*P. papatasi* (46.7 %); *S. fallax* (23.3 %); *P. perniciosus* (13.3 %)
Rogba	2	1.8	0.80	*P. papatasi* (66.7 %); *S. fallax* (33.3 %)
Mahassen	4	3.79	0.93	*P. longicuspis* (33.3 %); *P. papatasi* (25 %); *S. fallax* (25 %); *S. m. parroti* (16.7 %)
R Dhraâ	3	2.47	0.74	*P. papatasi* (54.5 %); *S. antennata* (27.3 %); *S. m. parroti* (18.2 %)
Boulifa	4	2.91	0.64	*P. longicuspis* (50 %); *P. papatasi* (25 %); *S. antennata* (12.5 %); *P. perniciosus* (12.5 %)

* S: specific richness; ** I_s: index of Simpson; and *** E: equitability.

Table IIb. – Diversity and dominant species of phlebotomine caught on sticky traps in leishmaniosis sites.

					Number (% among *Larroussius*)			
Form of leishmaniasis	**Foci**	**Date of catch**	**Total sandflies**	**Total *Larroussius***	***P. perfiliewi***	***P. perniciosus***	***P. longiscuspis***	***P. langeroni***
VL	Ghezala	22/09/2004	324	74	6 (8,1)	66 (89,2)	2 (2,7)	–
	Aïn Jloula	18/07/2002	196	97	–	97 (100)	–	–
	Sidi Salem	21/09/2004	49	33	–	29 (78,9)	4 (12,1)	–
	Marthouma	11/09/2002	370	352	169 (48)	169 (48)	14 (4)	–
	Gt Ennam	21/08/2004	5	0	–	–	–	–
	Mahassen	30/072003	11	5	–	1 (20)	4 (80)	–
SCL	Jmila	07/09/2002	2,354	2,303	2,030 (88,1)	267 (11,6)	6 (0,3)	–
	Aïn Somra	22/09/2004	113	41	1 (2,4)	37 (90,3)	3 (7,3)	–
	Nchem	28/08/2002	1,558	1,541	39 (2,5)	1,382 (89,7)	74 (4,8)	46 (3)

Table III. – Specific composition of subgenus *Larroussius* caught by CDC and sticky traps in the studied *L. infantum* foci.

Among the 4,025 phlebotomine sandflies identified from captures carried out on August and September in the three CLI sites, 3,902 (96.9 %) belonged to the genus *Phlebotomus,* of which 3,885 (99.6 %) belonged to the subgenus *Larroussius* (Table III). 2,039 females of this subgenus were collected mainly in animal sheds (87 %, $p < 0.001$). In the focus of Jmila situated in the humid stage, *P. perfiliewi* was the dominant species with a relative abundance (A_r) varying from 54,3 % (Sticky traps) to 87,4 % (CDC traps) whereas in Aïn Somra (sub-humid) and Nchem, the southern limit of CLI area in semi-arid zone, *P. perniciosus* predominated (Tables IIa, IIb and III).

Among the 505 sandflies caught monthly between May and October in the central and southern sites of *L. major*, 404 (80 %) belonged to the genus *Phlebotomus* of which 223 (55.2 %) were *P. papatasi* (Tables Ia and Ib). It was a dominant species in all sites, associated with *Larroussius* species in Zemla, Sidi Salem, Mahassen and Boulifa and with *Sergentomyia* species specially in fauna sampled with sticky traps (Table IIa and IIb). 69 females of *P. papatasi* were caught. A significantly higher proportion of these females (56.5 %) was collected in animal sheds ($p < 0.01$). However a non negligible proportion (24.6 %) was caught indoors.

Among the 3,297 phlebotomine sandflies regularly caught between April and October in CLK sites, 2,318 (70.3 %) belonged to the genus *Phlebotomus* (Tables Ia and Ib).

In Nsefri, the best known south-east focus of CLK; *P. perniciosus* and *P. sergenti* were the co-dominant species with *P. papatasi* (Table IIa). In Rogba, the second best known focus, *P. papatasi* and *P. sergenti* were the dominant species (Table IIa). 37 females of *P. sergenti* were caught in these two foci and 65 % of them were indoors. 36 females of *P. perniciosus* were collected, of which 55 % were in animal sheds. However no statistical significance was found according to the site of capture for both species.

In Ghanzour and Ennajeh, the emergent foci of the Centre, *P. perniciosus* was a dominant species with a significantly higher proportion of females caught in animal sheds ($p < 0.01$). *P. alexandri* represented the highest relative abundance among subgenus *Paraphlebotomus* (85.9 %) with 55 % of female specimens present indoors (Tables Ia and Ib). In Gouleb, *Sergentomyia* was the dominant genus (Tables IIa and IIb). *P. perniciosus* was rare (0.8 %) and the subgenus *Paraphlebotomus* was represented exclusively by *P. alexandri* (Tables Ia and Ib).

In the mixed focus of Metlaoui (south-west), only some specimens were collected, although the captures had been repeated twice a month. They were three *P. alexandri*, two *P. sergenti*, one *P. perniciosus*, 18 *P. papatasi* and eight specimens of subgenus *Sergentomyia* (Tables Ia and Ib).

DISCUSSION

In order to identify the phlebotomine sandfly populations in Tunisian leishmaniosis sites, an entomological survey was carried out through three entomological seasons (2002-2003-2004) in 19 visceral and cutaneous leishmaniosis areas, located in six bioclimatic zones. Two complementary kind of traps were used: CDC light traps acting by attraction and sticky ones sampling by interception.

In the studied Tunisian leishmaniosis sites, we found 12 species of Phlebotominae out of the 16 recorded in Tunisia (Croset *et al.*, 1978; Depaquit *et al.*, 1998). *P. (L.) ariasi*, *P. (L.) chadlii*, *P. (Pa.) riouxi* and *S. (S.) christophersi* were not found. Our failure to find them is probably due to their rarity and their presence in natural biotopes that were not sampled in our study (Croset *et al.*, 1978). Eight out of the 12 collected species belonged to the genus *Phlebotomus*, proven vectors of human leishmaniosis in the Old World (Killick-Kendrick, 1985; Killick-Kendrick, 1990). Among these species, four belong to the subgenus *Larroussius*, the proven vector of *L. infantum* in the Mediterranean basin, and three were *Paraphlebotomus,* among which is found *P. sergenti* the proven vector of *L. tropica* in the Old Word and the last was *P. papatasi* the proven vector of *L. major* (Killick-Kendrick, 1990).

The dominance of *Larroussius* species in northern sites, *P. papatasi* in the south-western ones and their co-dominance in the centre is in accordance with the distribution of *L. infantum* and *L. major* in Tunisia (Tables IIa and IIb, Figs 1 and 2). The low density found in the historical CLM focus of Metlaoui in the south-west may indicate the high competence of the local populations. It can also suggest that CL could be transmitted out of the village. Sites located on the southern side of the Atlas Mountains are transitional ones. They have been submitted to northern Mediterranean and southern Ethiopian influences and therefore exhibit a mixture of mediterranean species as *P. perniciosus* and *P. perfiliewi* and peritethysian ones as *P. papatasi, P. sergenti* and *P. alexandri* (Croset *et al.*, 1978). In spite of the high proportion of *P. perniciosus* in south-eastern sites, no human leishmaniosis cases due to *L. infantum* were reported. It's important to underline that the east-southern population of *P. perniciosus* is morphologically different from populations caught in *L. infantum* foci. The male specimens does not present the typical bifid aedeagi but exhibit curved ones. It can be confused with *P. longicuspis*, but has been differenciated by the number of coxite hairs which is higher in *P. longicuspis sensu stricto* as described on specimens from Morocco and Spain. (Benabdennbi *et al.*, 1999; Martin-Sanchez *et al.*, 2000; Pesson *et al.*, 2004; Guernaoui *et al.*, 2005).

In five out six prospected VL sites, the subgenus *Larroussius* predominated. It was absent in Garet Ennam, a site located near the south-western limit of the area of VL (Fig. 1), where the absence of recent human cases can be explained by a rigorous climate limiting the occurrence and the abundance of the vector. In the other VL sites, the succession from north to south, of different *Larroussius* species explains the large geographical distribution of VL in Tunisia. In fact, *L. infantum* is not restricted to a particular *Larroussius* species and can be encountered in different bioclimates (Killick-Kendrick, 1985; Rioux, 2001). *P. (L.) perniciosus* has got the largest distribution (D = 84 %) (Tables Ia and Ib). It was the most abundant *Larroussius* species in most studied VL sites (Ghezala, Aïn Jloula and Sidi Salem) and probably represents the main vector of VL in northern and central Tunisia as reported in other Mediterranean countries (Table IIa and IIb) (Alves-Pires, 1984; Bettini *et al.*, 1986; Rioux *et al.*, 1986b; Maroli *et al.*, 1988; Izri *et al.*, 1990; Izri *et al.*, 1992). However, in Marthouma, the dominance of *P. perniciosus* in CDC traps and *P. perfiliewi* in sticky traps suggests that more than one species could be incriminated in the transmission (Table IIa and IIb) (Dancesco *et al*, 1970; Alves-Pires, 1984; Rioux *et al.*, 1986b; Izri *et al.*, 1992). In Mahassen, the southern focus situated in a oasis in the saharan bioclimatic zone, *P. perniciosus* was very rare. *P. longicuspis*, the dominant *Larroussius* species, could be the main vector in this area as reported in other Saharan foci (Tables IIa and IIb) (Rioux, 2001).

In CLI sites, specimens of subgenus *Larroussius* represented almost the totality of sandflies caught (95 %). In Jmila, a focus located in the humid bioclimatic zone, *P. perfiliewi* the proven vector of CLI in Algeria and Italy, was the dominant species (Tables IIa and IIb) (Izri & Belazzoug, 1993; Maroli *et al.*, 1988). However, in Aïn Somra and Nchem located in the subhumid and the semi-arid bioclimatic stages *P. perfiliewi* was rare. *P. perniciosus* was the dominant specie, and probably can be the vector of CLI in these areas.

Larroussius females predominated in animal sheds as demonstrated in previous studies (Dansesco *et al.,* 1969). A non negligible proportion of females was caught indoors in VL sites and outdoors in CLI sites. Therefore, a transmission of these two forms of leishmaniosis could occur in different sites.

In the arid CLM sites of the centre and the south-west, *P. papatasi* the proven vector of *L. major* in Tunisia was a dominant species with a significantly higher proportion of females in animal sheds and a non negligible proportion indoors (Tables IIa and IIb) (Ben Ismail *et al.*, 1987). In fact, although this species is known to be endophilic, it was also already collected with a high abundance outdoor (Rioux *et al.*, 1986a; Maroli *et al.,* 1994). In particular sites located on the side of stony hills, as Gouleb, species of *Paraphlebotomus* were abundantly collected as reported in similar biotopes of Saudi Arabia (Büttiker & Lewis, 1983). The presence of this subgenus could explain the co-existence, with *L. major,* of *L. killicki* a closely related complex to *L. tropica* (Rioux *et al.*, 1986d).

In Tunisia, the vector of *L. killicki* still unknown. It could belong, as reported for the closely related complexes, *L. tropica* and *L. infantum,* to the subgenus *Paraphlebotomus* or *Larroussius* (Serre & Roux, 1986; Killick-Kendrick, 1990; Lawyer *et al.*, 1991). In the well known foci of the south-east, the dominante species, *P. (Pa.) sergenti,* proven vector of *L. tropica* in the Old World is probably the vector of this form (Rioux *et al*, 1986c; Killick-Kendrick, 1990). However, interestingly *P. (L) perniciosus* was also abundant in the historic foci of Nsefri (Tables I and IIa) and seems to be endophilic in its behaviour. In the emerging foci of *L. killicki* of the Centre, *P. perniciosus* was a dominant species in some sites whereas it was very rare in others (Tables IIa and IIb). In these sites, the subgenus *Parasphlebotomus* was always present. Among this subgenus, only some specimens of *P. sergenti* were found whereas *P. alexandri* was relatively abundant with a non negligible proportion of females indoors which suggests its possible role in the transmission (Tables Ia and Ib).

CONCLUSION

This study is a contribution to the knowledge of phlebotomine sandflies of human leishmaniosis sites in Tunisia and its possible consequences in the epidemiology of leishmaniosis. It would gain in being compleeted by the isolation and identification of the parasite in potential vector species particulary those present in *Leishmania killicki* foci.

ACKNOWLEDGEMENTS

This study was carried out with the financial support of the Research Unit "Parasitoses emergentes" 02/UR/08-05 Faculté de Médecine de Tunis. We thank the Regional Directors and heads of Services of Public Health: Dr A. Garraoui (Bizerte), N. Kouzana (Beja), Dr M. Hamdi (Siliana), Dr H. Hadhri (Kairouan), Dr MZ Ahmadi (Sidi Bouzid), Dr H. Khmaïs and Dr B. Mahmoudi (Kasserine), Dr S. Tlijani (Gafsa), Dr S. Doukali and Dr R. Dani (Tozeur), Dr Kodia and Dr M. Raouene (Tataouine), all of whom facilitated the field work together with Miss Karima Hmida and the health agents who contributed to the achievement of this survey.

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Reçu le 18 août 2005
Accepté le 5 décembre 2005