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Intra and peridomiciliary comparison of density, sex ratio and gonotrophic stage of *Phlebotomus sergenti* in an active anthroponotic cutaneous leishmaniasis focus in Morocco

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ABSTRACT

Cutaneous leishmaniasis due to *Leishmania tropica* represents a major public health problem due to its ability to spread into non-endemic areas by means of its vectors, and the associated dramatic psychosocial impact. The objective of this work was to compare the intra and extradomiciliary density, sex ratio and gonotrophic stage of sand flies from a recent active focus in Morocco. This field study is based on the need to optimize the effectiveness of control programs. Two different capture methods, CDC light traps and sticky traps, were used at two different times of the year, corresponding with the peaks of sand fly abundance. 7,815 sand flies were captured and classified into 13 species belonging to genera *Sergentomyia* (50.8%) and *Phlebotomus* (49.2%). *Phlebotomus sergenti* was the most abundant and frequent species of the genus *Phlebotomus* both inside (49.3%) and outside houses (52.1%) and it showed the highest density in extradomiciliary captures in June. The proportion of blood-fed females was similar indoors and outdoors (21.5% and 26.3%, respectively). Females in the three gonotrophic stages were found in 26% houses and this was significantly associated with some factors related to housing conditions. Therefore, *P. sergenti* seems well adapted to both indoors and outdoors biotopes where these females coexist with males. These findings suggest that the adoption of additional measures could benefit the strategy of the Moroccan health authorities, currently consisting of indoor insecticide spraying, given that transmission may also occur outdoors.

1. Introduction

Leishmaniasis remains one of the world's most devastating neglected tropical diseases. Approximately 12 million people are currently infected showing three main clinical forms, visceral, cutaneous and mucosal leishmaniasis. Cutaneous leishmaniasis (CL) is a disfiguring and stigmatizing parasitic disease that is caused by a variety of *Leishmania* species. It is endemic in more than 70 countries with an incidence of 1.2 million cases per year. Morocco is considered as one of the 12 "high-burden countries" for CL. In this country, 54,838 leishmaniasis cases were reported from 2008 to 2017 (Moroccan Ministry of Health, 2018). Cutaneous leishmaniasis (CL) represents the main clinical form (> 95% of cases), followed by visceral leishmaniasis (VL), whereas only a few cases of mucosal leishmaniasis have been described (Iguermia et al., 2011; Bennis et al., 2017). CL represents a major public health problem due to its wide geographic distribution and its psychological impact (Aoun and Bouratbine, 2014; Bennis et al., 2017; Bailey et al., 2019).

Three *Leishmania* species can be found in Morocco: *Leishmania major* (zoonotic CL, ZCL), *Leishmania tropica* (anthroponotic CL, ACL) and *Leishmania infantum* (causative agent of zoonotic VL and CL) (Mouttaki et al., 2014; Merino-Espinosa et al., 2018). *Leishmania tropica* has the widest geographic distribution and has shown a highly emerging nature,

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being able to expand rapidly into non-endemic areas (Ajaoud et al., 2013; Es-Sette et al., 2014; Baghad et al., 2020). ACL lesions seem to be more insidious than those caused by *L. major* as some can last up to a year and relapses and treatment failure are common (Aoun and Bouratbine, 2014).

The first CL case in Settat province (central Morocco) was detected in El Borouj locality in 2006, preceding an epidemic outbreak due to *L. tropica* (Amarir et al., 2015; Gijón-Robles et al., 2018) in which hundreds of cases were reported between 2007 and 2012. Currently, El Borouj is the only active ACL focus in the province of Settat, and it is considered endemic; the number of cases has decreased considerably and only 25 cases were officially reported in 2017 (Moroccan Ministry of Health, 2018; personal communication from the Délégation du Ministère de la Santé, province de Settat, Morocco).

The presence of sand fly vectors is considered the main risk for the emergence of leishmaniasis and updated data on species abundance is needed for control strategies, including a good understanding of intra and peridomiciliary diversity and population density. It is generally considered that resting site collections usually provide more representative samples of the sand fly fauna whereas those involving attractive stimuli preferentially sample host-seeking females, usually with a bias for certain species (Alexander, 2000; Alten et al., 2015) so the combination of both types of traps may show a more realistic result.

Hence, the aim of this work was to evaluate the density, relative abundance, sex ratio and gonotrophic stage of sand flies from a recent active ACL focus in Morocco focusing on the differences between intra- and extradomiciliary sand fly fauna.

2. Material and methods

2.1. Study area

The present study was carried out in El Borouj, a semi-rural town in Settat (Central Morocco, coordinates 07°36′W-32°29′N), at 410 m above sea level. It has an arid climate characterised by annual average temperature of 23°C, annual average precipitation of 125 mm and 70% humidity. The population estimate in 2014 was 19,235 and its average growth rate is close to 2%. The economic activity is mainly based on agriculture and animal breeding. El Borouj is organised in different zones according to their urbanistic evolution. Differences in the systems of liquid and solid waste management, and absence of sanitation network in some areas have been noticed.

A large proportion of dwellings in El Borouj are unfinished homes and have unsuitable cement wall with numerous holes. Many households have domestic animals (livestock or poultry) inside the house and often accumulate manure in their periphery.

2.2. Sand fly collection and morphological identification

Sand flies were captured using CDC light traps and sticky traps (21 × 29.5 cm paper sheets covered in castor oil) spread across the different town districts. Captures were performed during the last week of September and the first week of October in 2014 and 2015. In addition, some sites were sampled during the last week of June and the first week of July 2015.

A total of 1,617 sticky traps (total surface 200.4 m^2) were set in 99 sampling sites placed in different biotopes close to dwellings (usually holes in the dwellings' walls, abandoned buildings, stables and other domestic animals sheds) or further away from houses (in road drainage holes). These traps were left for 4 days (1.3-75.5 traps per sampling site, average 13.8) and then they were collected in a coded envelope. Individuals were taken with care from the surface of sticky papers using a brush soaked in 96°alcohol and subsequently stored in 70% alcohol. Later, the specimens were placed in Marc André solution and heated to boiling point, and finally mounted on slides under a coverslip using Berlese solution.

The intradomiciliary survey was carried out placing 179 CDC light traps in 97 houses (1-4 traps/house, average 2 traps/house) covering all districts. Trapping sites included houses where an ACL case was found (52 houses). In most cases, indoor rooms and outdoor spaces (patios and terraces) were sampled. Traps were placed in the evening and collected the following morning. Sand fly genitalia and head were dissected and mounted while the rest of the body was stored at -20 °C for subsequent molecular analysis.

Morphological identification was performed using taxonomic keys (Rioux and Golvan, 1969; Abonnenc, 1972; Rioux et al., 1978; Benabdennbi et al., 1999; Sáez et al., 2018) and based on the observation of male genitalia, female spermathecas, the pharyngeal armature and the length of some ascoids and antennal segments. The gonotrophic cycle of the female sand flies was categorised as blood-fed, unfed or gravid.

2.3. Data analysis

Density (number of specimens per trap and night when using CDC light traps; number of specimens per square meter of trap for sticky traps), relative abundance (percentage of specimens of a given species over the total number of capture sand flies) and frequency (percentage of stations where a particular species was found) were calculated for all species identified. Mann-Whitney's U test was used to check significant density differences between capture periods, biotopes and sex ratio at a 95% confidence level.

To assess the association between presence of all three stages of female *P. sergenti* (fed, unfed and gravid) and factors related to housing conditions (type of house –low brick; plants and terrace-, condition of house walls –poor with hole; good-, dwellers, vegetation inside the house –No, Yes-, vegetation outside the house –No, Yes-, neighbourhoods, presence of water well –No, Yes-, accumulation of organic matter –No, Yes-, frequency of garbage collection –daily, every 15 days, others- and presence of animals –livestock, dogs, cats poultry, rabbits, sheep, goats-) we recorded the presence of three stages into a binary variable ("Yes" if all three stages were present in the same household during the same trapping night, otherwise "No") and fitted a logistic regression model. A two-step procedure was used to select the most parsimonious model. First, all explanatory variables were assessed in a univariate model; variables significant at the $p= 0.20$ level were included in the final model. We then used a stepwise backward elimination procedure; variables with a p-value of greater than 0.05 were removed one at a time. Software package IBM SPSS Statistics versión 21.0 was used for the statistical analysis.

3. Results

Overall 7,815 sand flies were captured of which 4,430 were male specimens (56.7%) and 3,385 females (43.3%). Most sand flies were captured using sticky traps (5,588; 71.5%) and the rest using CDC light traps (2,227; 28.5%). Captured sand flies belonged to 13 species classified into 2 genera, *Sergentomyia* 3,971 (50.8%) and *Phlebotomus* 3,844 (49.2%). *Sergentomyia minuta* was the most abundant species (2,310; 29.6%), followed by *Phlebotomus sergenti* (1,944; 24.9%). Table 1 shows the species distribution and relative abundance, both intradomiciliary and extradomiciliary.

3.1. Association between sand fly fauna density, capture method and trap setting

Phlebotomus spp. were the predominant sand flies in intradomiciliary settings using CDC light traps (2,140/2,227; 96.1%) while only 87 *Sergentomyia* specimens were captured (3.9%). In contrast, *Sergentomyia* spp. were the most abundant in captures carried out using sticky traps in extradomiciliary settings (3,884/5,588; 69.5%) whilst 30.5% sand flies were identified as *Phlebotomus* sp.

Phlebotomus sergenti was the most abundant *Phlebotomus* species,

Table 1
Sand flies captured intradomiciliary (CDC light traps) and extradomiciliary (adhesive traps).

Genus	Subgenus	Species	Intradomiciliary captures					Extradomiciliary captures					T	A
			♂	♀	T	D (179 CDC traps)	A	♂	♀	T	D (200.39 m^2)	A		
Phlebotomus	*Paraphlebotomus*	*P. sergenti*	461	595	1056	5.90	47.42	713	175	888	4.43	38.34	1944	24.88
		P. alexandri	0	1	1	0.01	0.04	0	0	0	0.00	0.00	1	0.01
		P. chabaudi	0	1	1	0.01	0.04	0	0	0	0.00	0.00	1	0.01
	Larroussius	*P. longicuspis*	461	311	772	4.31	34.67	103	14	117	0.58	5.05	889	11.38
		P. perniciosus	121	38	159	0.89	7.14	231	28	259	1.29	11.18	418	5.35
		P. langeroni	6	0	6	0.03	0.27	1	0	1	0.00	0.04	7	0.09
	Phlebotomus	*P. papatasi*	60	76	136	0.76	6.11	350	77	427	2.13	18.44	563	7.20
		P. bergeroti	0	8	8	0.04	0.36	7	5	12	0.06	0.52	20	0.26
		P. duboscqi	1	0	1	0.01	0.04	0	0	0	0.00	0.00	1	0.01
Sergentomyia	*Sergentomyia*	*S. minuta*	12	11	23	0.13	1.03	1060	1227	2287	11.41	98.75	2310	29.56
		S. fallax	36	22	58	0.32	2.60	802	790	1592	7.94	68.74	1650	21.11
		S. antennata	0	0	0	0.00	0.00	5	0	5	0.00	0.22	5	0.06
	Grassomyia	*S. dreyfussi*	0	6	6	0.03	0.27	0	0	0	0.00	0.00	6	0.08
Total *Phlebotomus* spp.			1110	1030	2140	11.96	96.09	1405	299	1704	8.50	73.58	3844	49.19
Total *Sergentomyia* spp.			48	39	87	0.49	3.91	1867	2017	3884	19.38	167.70	3971	50.81
TOTAL			1158	1069	2227	12.44	100	3272	2316	5588	27.89	241.28	7815	100

D = Density (sand flies per CDC trap and night or sand flies per square meter sticky trap), A =Abundance, T =total number of sand flies captured.

both inside and outside dwellings. In intradomiciliary captures, 49.3% sand flies captured were identified as *P. sergenti* (1,056/2,140) followed by *Phlebotomus longicuspis* (36.1%; 772/2,140) and *Phlebotomus perniciosus* (7.4%; 159/2,140). Similarly, *P. sergenti* summed 52.1% extradomiciliary captures (888/1,704), followed by *Phlebotomus papatasi* (25.1%; 427/1,704), *P. perniciosus* (15.2%; 259/1,704) and *P. longicuspis* (6.9%; 117/1,704).

Several *Phlebotomus* species were absent from captures with sticky traps (*Phlebotomus duboscqi, Phlebotumus chabaudi, Phlebotomus alexandri* and *Sergentomyia dreyfussi*) whereas *Sergentomyia antennata* was absent from CDC captures.

3.2. Association between sand fly fauna density and biotope characteristics

Most sand flies caught using sticky traps were collected in traps set around dwellings (peridomiciliary; 61.4%; 3,428/5,588) while the remaining sand flies were captured in traps set in walls in the outskirts of El Borouj (38.7%; 2,160/5,588). *Phlebotomus langeroni* and *S. antennata* were only captured in walls in the outskirts and *P. longicuspis* density was significantly higher in the outskirts (0.86 specimens/m^2, p =0.05) than in the peridomestic biotope (0.37 specimens/m^2). *P. sergenti* density was similar in these biotopes (p =0.846).

Sand fly density inside dwellings (indoor rooms) or outdoors (patios and terraces) was similar for all species when using CDC traps, including *P. sergenti* (p =0.763) (Table 1, disaggregated data not shown).

3.3. Association between sand fly fauna and captured period

Table 2 shows the comparison between the sand flies captured in the two periods (June and October) at 7 domiciliary and 18 extradomiciliary capture sites. Although the absolute figures are higher in June (834) than in September (488) in intradomiciliary captures, and higher in September (1296) than in June (1019) in extradomiciliary captures, there are no statistically significant differences. Overall, sand fly captures were similar at both times in the intradomiciliary (p = 0.49) and in the extradomiciliary (p = 0.40) biotopes. However, extradomiciliary captures were significantly different at the two capture times for two sand fly species: *P. sergenti* density was higher in June (p = 0.034) and *S. fallax* density was higher in October (p = 0.035).

3.4. Sand fly gender distribution and female gonotrophic cycle

Female sand fly capture rate was 48% in the intradomiciliary biotope (1,069 female specimens, 5.97 female sand flies/trap/night). The female/male ratio was close to 1 in October (4.40 and 4.04 specimens/trap/night) whereas this ratio decreased to 0.7 in June, capturing 24.5 females and 35.07 males/trap/night. In the whole of the two periods, significant differences between the densities of both genders were found in some species such as *P. sergenti* (p =0.045), *P. papatasi* (p ≤0.001), *Phlebotomus bergeroti* (p =0.004), and *S. dreyfussi* (p =0.024) with a predominance of females, and *P. perniciosus* (p ≤0.001) and *Sergentomyia fallax* (p =0.015) with a predominance of males.

Male sand flies were predominant in the extradomiciliary captures regardless of the period, where females counted 41.5% captures (2,316: 11.6 sand flies/m^2). Female sand fly density was 6.3 and 15.5 sand flies/m^2 in June and October respectively. In the whole of the two periods, significant gender density differences were found in some species: *P. sergenti* (p ≤0.001), *P. perniciosus* (p ≤0.001), *P. longicuspis* (p =0.001) and *P. papatasi* (p ≤0.001) with a predominance of males.

Table 3 shows the gonotrophic physiological abdomen conditions of the female sand flies captured. In intradomiciliary captures, 28.4% females were gravid (304/1,069), 19.1% blood engorged (204) and 52.4% unfed (561). In contrast, unfed females in extradomiciliary captures counted 87.6% (2,029/ 2,316) while gravid were only 6.0% (140) and 6.4% blood-engorged (147). Figure 1 shows a comparison between the abundance of the different gonotrophic stages in captured *P. sergenti*.

The presence of three gonotrophic physiological abdomen conditions of female *P. sergenti* in the same household at the same time was evaluated. *P. sergenti* females were captured inside 77.3% dwellings (75/97); in 23 of these houses (26%) females in the three stages of the gonotrophic cycle were found. Only 3 factors related to the conditions of the houses (neighbourhoods, presence of water well and accumulation of organic fertilizers) were statistically associated with the dependent variable represented by the simultaneous presence of the 3 stages, both in the univariate and multivariate models (supplementary material).

4. Discussion

Anthroponotic cutaneous leishmaniasis foci originate as a consequence of ecological and demographic changes usually driven by urbanisation of highly populated settlements, particularly when the sanitation and hygiene conditions are poor (Rhajaoui et al., 2012), which increases vector growth. Sand fly diversity is very high in Morocco since 23 species have been found (Es-Sette et al., 2014) and only in El Borouj, 13 species were identified in the present work, counting more than half species reported in the country. Recently, Mhaidi et al., 2021 also found 13 species in a CL focus from the Azilal province confirming the previous result of Ajaoud et al., 2015. This diversity is the highest reported to date in Morocco, since 5 to 11 species

Table 2
Sand flies captured intradomiciliary (CDC light traps) and extradomiciliary (sticky traps) in sites sampled throughout the two sampling periods (June-July and September-October) and comparison of catches in both periods.

		Intradomiciliary captures September-October	June-July	Extradomiciliary captures September-October	June-July	p-value	
	N stations	7	7	18	18	I	P
Species	N traps	14	14	318.5 (S= 39.46 m^2)	396.25 (S=49.09 m$^{2)}$		
P. sergenti	N (♂/♀)	142 (52/90)	352 (189/163)	75 (57/18)	248 (184/64)	0.608	0.034
	D	10.14	25.14	1.90	5.05		
	A	29.10	42.21	5.79	24.34		
	F	1.00	0.86	0.83	0.89		
P. alexandri	N (♂/♀)	0	1 (0/1)	0	0	0.317	1.000
	D	0	0.07	0	0		
	A	0	0.12	0	0		
	F	0	0.14	0	0		
P. longicuspis	N (♂/♀)	224 (147/77)	415 (257/158)	13 (11/2)	34 (31/3)	0.520	0.871
	D	16	29.64	0.33	0.69		
	A	45.90	49.76	1.00	3.34		
	F	1	0.71	0.39	0.39		
P. perniciosus	N (♂/♀)	77 (61/16)	43 (12/31)	60 (56/4)	60 (56/4)	0.974	0.730
	D	5.50	3.07	1.52	1.22		
	A	15.78	5.16	4.63	5.89		
	F	0.57	0.57	0.56	0.61		
P. langeroni	N (♂/♀)	1 (1/0)	4 (4/0)	1 (1/0)	0	0.476	0.317
	D	0.07	0.29	0.03	0		
	A	0.20	0.48	0.08	0		
	F	0.14	0.29	0.06	0		
P. papatasi	N (♂/♀)	24 (8/16)	17 (10/7)	54 (48/6)	97 (72/25)	0.478	0.625
	D	1.71	1.21	1.37	1.98		
	A	4.92	2.04	4.17	9.52		
	F	0.86	0.71	0.67	0.67		
P. bergeroti	N (♂/♀)	0	0	2 (2/0)	2 (1/1)	1.000	0.954
	D	0	0	0.05	0.04		
	A	0	0	0.15	0.20		
	F	0	0	0.11	0.11		
S. minuta	N (♂/♀)	2 (0/2)	1 (0/1)	635 (295/340)	416 (230/186)	0.530	0.082
	D	0.14	0.07	16.09	8.47		
	A	0.00	0.12	49.00	40.82		
	F	0.29	0.14	1.00	1.00		
S. fallax	N (♂/♀)	16 (9/7)	1 (0/1)	456 (189/267)	160 (103/57)	0.085	0.035
	D	1.14	0.07	11.56	3.26		
	A	0.03	0.12	35.19	15.70		
	F	0.57	0.14	0.89	0.94		
S. antennata	N (♂/♀)	0	0	0	2 (2/0)	1.000	0.317
	D	0	0	0	0.04		
	A	0	0	0	0.20		
	F	0	0	0	0.06		
S. dreyfussi	N (♂/♀)	2 (0/2)	0	0	0	0.317	1.000
	D	0.14	0	0	0		
	A	0.41	0	0	0		
	F	0.14	0	0	0		
TOTAL	N (♂/♀)	488 (278/210)	834 (491/343)	1296(659/637)	1019 (679/349)	0.749	0.402
	D	34.86	59.57	32.84	20.76		
	A	100	100	100	100		
	F	1	0.86	1	1		

F =Frequency, D =Density (sand flies per CDC trap and night or sand flies per square meter sticky trap), A =Abundance. The column p-value indicated the p-value when comparing the two different periods for intradomiciliary (I) and peridomiciliary (P) captures.

have been reported in other areas of the country (Boussaa et al., 2005, 2016; Es-Sette et al., 2014). The present study found that *Phlebotomus* species were the most abundant in intradomiciliary captures performed with CDC traps while *Sergentomyia* species where the most abundant in the extradomiciliary areas mainly surveyed with sticky traps. These differences may well be due to the different biotope characteristics: the herpetophilic *S. minuta* prefers holes within rocks or walls because these biotopes allow sympatry with reptiles (Alten et al., 2015) what explains the fact that this genus only comprised 3.9% captures in our intradomiciliary surveys. Species abundance may be associated with the type of trap as well: CDC light traps are more effective at capturing species that exhibit positive phototropism such as *Phlebotomus* species, while *Sergentomyia* species are not attracted to light (Hilmy et al, 1989; Alten et al., 2015). When using sticky traps, species of *Sergentomyia* genus were the most abundant sand fly species in some entomological studies carried out in Morocco and other countries (Barón et al., 2013; Alcover et al., 2014; Boussaa et al., 2016) while other studies showed a greater presence of *Phlebotomus* species (Boussaa et al., 2005; Ouanaimi et al., 2015; González et al., 2017). However, in entomological studies carried out with CDC traps, species of the genus *Phlebotomus* are predominant both in studies carried out in Morocco and other countries (Es-Sette et al., 2014; Ajaoud et al., 2015; Sáez et al., 2018; Díaz-Sáez et al., 2021).

When using the same type of traps, we did not find significant difference between intradomiciliary captures of *P. sergenti* performed indoors and captures in patios or terraces and this constitutes an interesting finding since *P. sergenti* is usually considered endophilic. No significant differences were found between peridomiciliary captures and other carried out in the outskirts of the city, except for a higher *P. longicuspis* density in the outskirts.

Only a few *P. langeroni* and *S. antennata* specimens were captured,

Table 3
Female sand flies captured intradomiciliary (CDC light traps) and extradomiciliary (adhesive traps) throughout the two sampling periods (June-July and September-October) and classified according to their gonotrophic cycle stage (unfed, blood-fed and gravid).

Number of stations/ Number and type of traps		Intradomiciliary captures September-October 97/ 165 CDC			June-July 7/ 14 CDC			Extradomiciliary captures September-October 82/ 924.1 ST (114.5 m^2)			June-July 35/ 693.3 ST (85.9 m^2)		
Species	Parameter	Unfed	Blood-fed	Gravid	Unfed	Blood-fed	Gravid	Unfed	Blood-fed	Gravid	Unfed	Blood-fed	Gravid
P. sergenti	Number	138	103	191	71	25	67	42	28	13	50	18	24
	Density	0.84	0.62	1.16	5.07	1.79	4.79	0.37	0.24	0.11	0.58	0.21	0.28
	Abundance	19.01	14.19	26.31	20.70	7.29	19.53	2.36	1.58	0.73	9.28	3.34	4.45
P. chabaudi	Number	0	0	1	0	0	0	0	0	0	0	0	0
	Density	0	0	0.01	0	0	0	0	0	0	0	0	0
	Abundance	0	0	0.14	0	0	0	0	0	0	0	0	0
P. alexandri	Number	0	0	0	1	0	0	0	0	0	0	0	0
	Density	0	0	0	0.07	0	0	0	0	0	0	0	0
	Abundance	0	0	0	0.29	0	0	0	0	0	0	0	0
P. longicuspis	Number	115	25	13	141	13	4	6	1	1	2	3	1
	Density	0.70	0.15	0.08	10.07	0.93	0.29	0.05	0.01	0.01	0.02	0.03	0.01
	Abundance	15.84	3.44	1.79	41.11	3.79	1.17	0.34	0.06	0.06	0.37	0.56	0.19
P. perniciosus	Number	15	6	5	7	3	2	13	5	4	4	1	1
	Density	0.09	0.04	0.03	0.50	0.21	0.14	0.11	0.04	0.03	0.05	0.01	0.01
	Abundance	2.07	0.83	0.69	2.04	0.87	0.58	0.73	0.28	0.23	0.74	0.19	0.19
P. papatasi	Number	31	24	14	0	2	5	26	14	8	18	6	5
	Density	0.19	0.15	0.08	0	0.14	0.36	0.23	0.12	0.07	0.21	0.07	0.06
	Abundance	4.27	3.31	1.93	0	0.58	1.46	1.46	0.79	0.45	3.34	1.11	0.93
P. bergeroti	Number	6	1	2	0	0	0	4	0	0	0	0	1
	Density	0.04	0.01	0.01	0	0	0	0.03	0	0	0	0	0.01
	Abundance	0.83	0.14	0.28	0	0	0	0.23	0	0	0	0	0.00
S. minuta	Number	8	1	0	1	0	0	855	29	30	280	11	22
	Density	0.05	0.01	0	0.07	0	0	7.47	0.25	0.26	3.26	0.13	0.26
	Abundance	1.10	0.14	0	0.29	0	0	48.11	1.63	1.69	51.95	2.04	4.08
S. fallax	Number	20	1	0	1	0	0	643	30	25	86	1	5
	Density	0.12	0.01	0	0.07	0	0	5.62	0.26	0.22	1.00	0.01	0.06
	Abundance	2.75	0.14	0	0.29	0	0	36.18	1.69	1.41	15.96	0.19	0.93
S. dreyfussi	Number	6	0	0	0	0	0	0	0	0	0	0	0
	Density	0.04	0	0	0	0	0	0	0	0	0	0	0
	Abundance	0.83	0	0	0	0	0	0	0	0	0	0	0
TOTAL	Number	339	161	226	222	43	78	1589	107	81	440	40	59
	Density	2.05	0.98	1.37	15.86	3.07	5.57	13.88	0.93	0.71	5.12	0.47	0.69
	Abundance	46.69	22.18	31.13	64.72	12.54	22.74	89.42	6.02	4.56	81.63	7.42	10.95
TOTAL FEMALES	Number		726			343			1777			539	
	Density		4.4			24.5			15.52			6.3	
	Abundance		100			100			100			100	

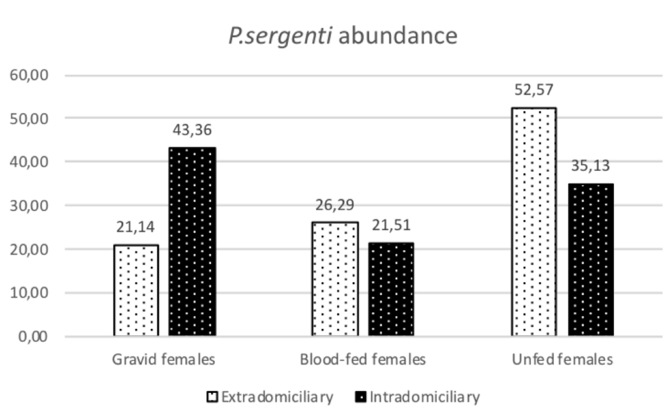

Fig. 1. Abundance of *Phlebotomus sergenti* female sand flies at different gonotrophic stages (gravid, blood-fed and unfed) in intradomiciliary and extradomiciliary captures.

similar to other entomological surveys in the country (Es-Sette et al., 2014; Ajaoud et al., 2015).

The capture method can influence the sex ratio: CDC traps usually attract host-seeking females whereas males are more abundant in sticky traps (Alexander, 2000; Alten et al., 2015). Male sand flies were more abundant in the present study using both methods, but this imbalance was more evident when using sticky traps. In other entomological studies carried out in Morocco and other countries, it seems widespread that more males are captured with sticky traps (Boussaa et al., 2005; Barón et al., 2013; Alcover et al., 2014; Ouanaimi et al., 2015) however, with CDC traps both males and females can predominate (Es-Sette et al., 2014; Ajaoud et al., 2015; Díaz-Sáez et al., 2021).

Phlebotomus sergenti was the most abundant *Phlebotomus* species captured in the present study (24.9%) followed by *P.longicuspis* (11.4%) and *P. papatasi* (7.2%), similar to other emergent ACL foci in Morocco (Ajaoud et al., 2013, 2015). *Phlebotomus sergenti* is a highly tolerant species that has adapted to different rural and urban areas in Morocco. Although this vector has even been capture at 1,400 m above sea level, the highest densities have been reported at 800-999 m a.s.l. (Es-Sette et al., 2014). El Borouj is an arid zone where *P. sergenti* is abundant both inside and outside dwellings, constituting 50.6% of captures (1,944/3, 844) within *Phlebotomus* genus. This vector is also the most frequent and dense *Phlebotomus* sand fly species and presumably the only *L. tropica* vector in this focus.

Population dynamics studies are a highly valuable tool that ideally requires periodic captures throughout the year in a given study area. This was not possible in the present paper and a limited capture schedule was planned according to the results obtained by other authors. According to Talbi et al., 2015, the population dynamics of *P. sergenti* in central Morocco follows a bimodal trend with two peaks in June and October. In the present study, June corresponds to the highest peak (Table 2) in agreement with previous studies of sand fly abundance in Morocco (Boussaa et al., 2016) and this was also the case for female sand flies. However, *L. tropica* infected *P. sergenti* frequency increases throughout the warm weather season and therefore the risk of infection is at its highest at the beginning of autumn (Guilvard et al., 1991). In El Borouj, *L. tropica* was found in 2.5% female *P. sergenti* in October 2014 (Gijón-Robles et al., 2018), 1% in October 2015 but 0% in June 2015 (unpublished data). The rate of blood-fed females captured inside and outside dwellings was found similar (21.5 and 26.3%, respectively; Fig. 1), suggesting that *P. sergenti* may be able to transmit the parasite both inside and outside dwellings. This hypothesis is supported by the local custom of having an evening walk or meeting the neighbours in community areas where sand fly capture was frequent (Gijón-Robles et al., 2018). Sand fly blood-feeding behaviour can determine the efficacies of indoor residual spraying, topical insecticides and bed nets (Courtenay et al., 2017). The sub analysis examining whether or not all three feeding conditions of female *P. sergenti* were present in the same household at the same time showed that this was the case in 25 of 75 households (26.0%). This finding may indicate that these houses are likely to be diurnal resting or breeding sites (Alexander, 2000; Malaviya et al., 2014). Presence of all three conditions was significantly associated with some factors related to housing conditions. The odds of finding all three conditions in the same house during the same trapping night were highest for households in Ouled Bouchair and Ouled Youssef neighbourhoods, settlements that evolved from the "douar" status (little rural settlement gathering families) and are characterised by the presence of water well, and near daily garbage collection. Daily collection may imply that garbage remains indoors until it is taken out for collection in the evening, as opposed to neighbourhoods where garbage is collected once every fortnight, where people would usually keep litter bins outdoors.

The existence of damp sources that provide moisture supply has been identified as an important risk factor for *P. sergenti* presence (Barón et al., 2013).

The presence of males inside dwellings was remarkable supporting the hypothesis that houses may be breeding sites. According to Alexander (2000) male/female aggregations in microhabitats represent mating leks, where males intercept females descending from higher up to lay eggs in the organic litter. Similarly in the present study, 17.2% walls sampled with sticky traps where *P. sergenti* females were found, also contained specimens in the all three gonotrophic conditions (data not shown).

It is also important to highlight the abundance of subgenus *Larroussius* specimens (16.8% total captures and 34.2% of *Phlebotomus*). *P. longicuspis* was the most abundant intradomiciliary species and *P. perniciosus* was the most abundant extradomiciliary (Table 1). In the light of these results, leishmaniasis due to *L. infantum* (visceral, cutaneous and mucosal forms) could be possible in the area given the vectorial role of these species (Benabdennbi et al., 1999; Martín-Sánchez et al., 2000; Es-Sette et al., 2014).

5. Conclusions

Phlebotomus sergenti is the most abundant, densest and frequent sand fly species of the genus *Phlebotomus* in the ACL active focus in El Borouj, in both intradomiciliary and extradomiciliary surveys. Although this species showed the highest level of endophilia among the 13 sand fly species found, it appears to be a well adapted species to both indoors and outdoors environments (such as the holes found in the exterior walls of dwellings in poor condition, and in the outskirts) where males and females in all gonotrophic states coexist. As the best choice of a vector-control strategy is dictated by sand fly behaviour, the results obtained in this study seem to explain the partial success of indoor residual spraying of insecticides, given the probably existence of ACL transmission inside and outside the home. This study provides a framework for future monitoring on sand fly population dynamics in a recent active focus of ACL.

Author contribution

JMS, VDS and MR conceived research

PGR, NA, NEK, FMM and VDS conducted captures and morphological identification

PGR, FMM, VCL, MR, JMS and VDS analysed data and conducted statistical analyses

PGR, VDS, VCL, MR and JMS wrote the manuscript

All authors read and approved the manuscript

Declaration of Competing Interest

We declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2021.106005.

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