HOSTED BY

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Document heading doi: 10.1016/S2222-1808(14)60787-8

Phlebotomine sand flies (Diptera: Psychodidae) of Morocco: results of an entomological survey along three transects from northern to southern country

Fouad Ouanaimi[1*], Samia Boussaa[1,2], Ali Boumezzough[1*]

[1]*Laboratory of Ecology and Environment L2E, (URAC 32, CNRST ERACNERS 06), Faculty of Sciences Semlalia, Cadi Ayyad University, BP 2390-40080 Marrakesh, Morocco*

[2]*Higher Institute of Nursing and Technical Occupations Health, Marrakesh, Morocco*

PEER REVIEW

Peer reviewer

Dr. Maribel Jiménez, PhD. Research Fellow, Unidad de Entomología Médica, Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Ctra. Majadahonda-Pozuelo s/n, 28220 Majadahonda, Madrid, Spain.
Tel: +34 91 822 3674
E-mail: mjimenez@isciii.es
Co-reviewer: Dr. Andre Antonio Cutolo, São Paulo, Brazil.

Comments

In this work authors conducted an entomological survey along three transects of Morocco where foci of cutaneous leishmaniasis occur. The data obtained gives practical information and suggests the need for a continuous entomological surveillance in this country.
Details on Page 304

ABSTRACT

Objective: To study the sandflies distribution within their climatic and ecological context in three transects of Morocco: Ouarzazat-Mhamid, Foum Zguid-Marrakesh and Erfoud-Nador.
Methods: In total, twenty-nine stations were prospected, through 1800 Km, including four zoonotic cutaneous leishmaniasis foci (Ouarzazat, Zagora, Tata and Errachidia). Sand flies were collected using sticky paper traps for one night.
Results: Overall, 7140 sandflies were collected along the three transects. In the combined collections, nine *Phlebotomus* species: *Phlebotomus papatasi* (27.6%), *Phlebotomus longicuspis* (19%), *Phlebotomus sergenti* (18.2%), *Phlebotomus perniciosus* (6.2%), *Phlebotomus bergeroti* (2.9%), *Phlebotomus alexandri* (1.4%), *Phlebotomus chadlii* (0.8%), *Phlebotomus chabaudi* (0.5%) and *Phlebotomus ariasi* (0.5%) and five *Sergentomyia* species: *Sergentomyia minuta* (10.4%), *Sergentomyia fallax* (8.1%), *Sergentomyia* dreyfussi (2.1%), *Sergentomyia christophersi* (1.7%) and *Sergentomyia africana* (0.5%) were detected.
Conclusions: We update the entomological data in zoonotic cutaneous leishmaniasis foci and discuss the possible effect of many ecological factors as bioclimate, biotopes and altitude on the diversity and distribution of caught species.

KEYWORDS

Sandflies, Entomological survey, Ecology, Bioclimate, Morocco

1. Introduction

Phlebotomine sandflies (Diptera: Psychodidae) are the vectors for leishmaniasis as well as for arboviruses and bartonellosis and responsible of sandfly fever, summer meningitis, vesicular stomatitis, Chandipura virus encephalitis and Carrion's disease[1].

*Corresponding author: Fouad Ouanaimi, Laboratory of Ecology and Environment L2E, (URAC 32, CNRST ERACNERS 06), Faculty of Sciences Semlalia, Cadi Ayyad University, BP 2390-40080 Marrakesh, Morocco.
Tel: 00212602567417
E-mail: fouad.ouanaimi@yahoo.fr
Ali Boumezzough, Laboratory of Ecology and Environment L2E, (URAC 32, CNRST ERACNERS 06), Faculty of Sciences Semlalia, Cadi Ayyad University, BP 2390-40080 Marrakesh, Morocco.
E-mail: aboumezzough@gmail.com
Foundation Project: Supported by the Laboratory of Ecology and Environment, (CNRST, URAC 32; ERA-CNERS 06) and the National Centre for Studies and Research on the Sahara, CNERS Project (Contract N. 06/ ERACNERS).

Article history:
Received 11 Sep 2014
Received in revised form 15 Oct, 2nd revised form 16 Oct, 3rd revised form 5 Nov 2014
Accepted 2 Feb 2015
Available online 4 Feb 2015

In Morocco, leishmaniases are endemic diseases posing a major threat to public health. In 2011, Moroccan Ministry of Health reported 4 319 cases of human cutaneous leishmaniases (CL) and 107 cases of visceral leishmaniases (VL)[2]. CL caused by *Leishmania major* (*L. major*)(Kinetoplastida: Trypanosomatidae) is the most dominant form in the country with more than 24 450 cases reported in the last decade[2].

Phlebotomus papatasi (*P. papatasi*) and *Phlebotomus sergenti* (*P. sergenti*) are known to be the common vectors spreading *L. major* and *Leishmania tropica* (*L. tropica*), respectively in Morocco.

In the Mediterranean countries, *Leishmania infantum* (*L. infantum*) is the etiologic agent of VL[3]. While the subgenus "*Larroussius*" species: *Phlebotomus perniciosus* (*P. perniciosus*), *Phlebotomus ariasi* (*P. ariasi*) and *Phlebotomus longicuspis* (*P. longicuspis*) are considered as *L. infantum* vectors[4].

Moreover, Asmae *et al*. (2014) have demonstrated the coexistence of *L. tropica* and *L. infantum* as causative agents of CL in the Sefrou province, northeast of the country[5]. Knowledge of the distribution of vectors is important in predicting the spatial variations in the risk of disease. Previous studies in Morocco[6-10] showed that the distribution of sandflies was due to the bioclimate in great part. Current findings in Morocco showed that altitude (through the gradient on temperature, pressure and precipitation) and aspect (through climate and vegetation) have an influence upon the spatial distribution and density of the sand fly fauna[11,12].

In Morocco, other ecological factors are studied in their relationship with sand fly abundance and distribution such urbanization, proximity of humans and domestic animals, organic matter in the soil, shelter and vegetation type[13,14]. In southern Morocco, little is known about species composition of sand fly fauna. Epidemiological situation of leishmaniases, especially in extreme south, needs to be updated. Actually, zoonotic cutaneous leishmaniases (ZCL) has spread endemically in the southern Ouarzazate region and in the northern oriental highlands. This form is caused by *L. major*, and transmitted by *P. papatasi*, with *Meriones shawi* as the main reservoir host[15].

Rioux *et al.* have led an entomological survey carried out in the littoral zone of the western Saharan region until Dakhla province[7]. Recently, entomological investigations were carried out on both the northern and southern slopes of the mountains until Ouarzazate province[16].

In the present work, we discuss the possible effect of many ecological factors on the diversity and distribution of Moroccan sandflies through three transects: Ouarzazate-M'Hamid, Foum Zguid-Marrakesh and Erfoud-Nador (Table 1 and Figure 1). We give a particular attention to ZCL foci (Ouarzazate, Zagora, Tata and Errachidia) with the aim to update their entomological data.

Table 1

Information about the study areas showing the 29 sandfly collection sites.

Site (code)	Co-ordinates	Elevation above sea level (m)
Ouarzazate (S1)	30°50'N 6°46'W	1 100
Tisserghate (S2)	30°40'N 6°21'W	920
Tinzouline (S3)	30°39'N 6°20'W	803
Beni zoli (S4)	30°25'N 5°91'W	760
Zagora (S5)	30°21'N 5°48'W	732
Tamegroute (S6)	30°13'N 5°39'W	676
Zaouia Sidi Moktar (S7)	30°11'N 5°34'W	371
Anagam (S8)	30°13'N 5°41'W	653
Tagounite (S9)	30°20'N 5°50'W	642
M'Hamid (S10)	29°49N 5°43'W	552
Foum Zguid (S11)	30°4'N 6°52'W	645
Tata (S12)	29°44'N 7°58'W	693
Issafn (S13)	29°51'N 8°31'W	1 141
Igherm (S14)	30°5'N 8°27'W	1 720
Ait aiaaza (S15)	30°30'N8° 47'W	282
Ouled Berhil (S16)	30°38'N 8°28'W	485
Tinmel (S17)	30°59'N 8°30'W	1 247
Ouirgane (S18)	31°10'N 8°4'W	931
Tahanaout (S19)	31°21'N 7°57'W	925
Marrakech (S20)	31°35'N 8°0'W	482
Erfoud (S21)	31°26'N 4°14'W	802
Errachidia (S22)	31°55'N 4°25'W	1 030
Midelt (S23)	32°40'N 4°43'W	1 470
Azrou (S24)	33°5'N 5°13'W	1 250
El Hajeb (S25)	33°41'N 5°22'W	985
My Yaacoub (S26)	34°5'N 5°10'W	260
Chefchaouen (S27)	35°10'N 5°16'W	593
Al Hoceima (S28)	35°14'N 3°56'W	76
Nador (S29)	35°10'N 2°55'W	61

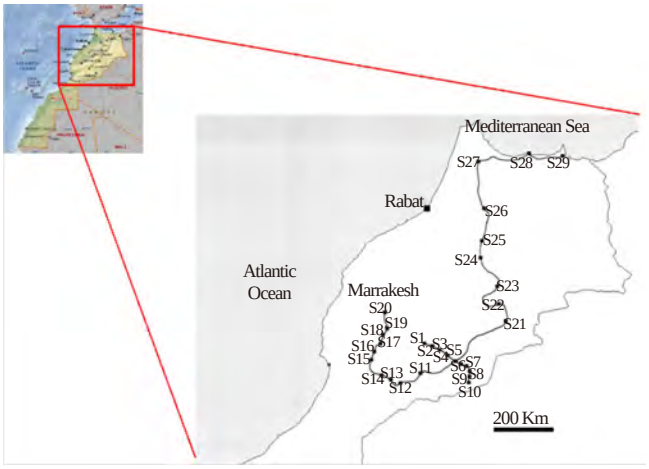

Figure 1. Study area showing the 29 sandfly collection sites.

2. Materials and methods

2.1. Transects

Three transects were studied. On June 2010, the first transect was 267 km long and linked the cities of Ouarzazate and M'Hamid. This transect went through eight collection sites, where altitude varies from 552 to 1 100 m, on the southern slopes of the High Atlas Mountains. The second transect was 537 km long from Foum Zguid to Marrakesh city and was studied on June 2011. This transect went through eight collection sites where altitude varies between 282 and 1 720 m. The third and last transect was 1 006 km

long and connected Erfoud city to the city of Nador. This transect went through seven collection sites with altitude ranges from 61 to 1 470 m and was studied on June 2012 (Figure 1).

2.2. Sandfly collection and identification

Sandflies were collected using sticky paper traps (each an A4 sheet of paper coated with castor oil) for one night. Specimens were preserved in 70% ethanol, cleared in 20% (w/v) KOH and Marc–André solution, and then mounted on microscope slides, in Hoyer's medium.

Most of the sandflies were then identified to species by the morphological examination of the genitalia (males) or the pharyngeal armature and spermathecae (females), with the help of the keys and descriptions published by Moroccan Ministry of Health[3,17].

For *P. perniciosus* complex, the females were identified by examining the dilatation of distal parts of spermathecal ducts and males by examining the shape of the copulatory valves and counting the number of coxite hairs[18,19].

2.3. Data analysis

Various ecological parameters were calculated to characterize the sand fly populations in the different sites and habitats:

$$\text{Relative frequency} = \frac{\text{Number of specimens of one species}}{\text{Total of specimens}} \times 100\%$$

Biodiversity index=(S-1)/logN

Where N is the number of individuals, S refers to the number of species in the sample.

Sorensen index (Sorensen's similarity coefficient) which can have values from 0 (no similarity) to 1.0 (complete similarity)= 2a/2a+b+c (a: number of species in both sample A and sample B, b: number of species in sample A but not in sample B and c: number of species in sample B but not in sample A).

3. Results

Overall, 7 140 sandflies were collected along the three transects. In the combined collections, nine *Phlebotomus* species: *P. papatasi* (27.6%), *P. longicuspis* (19%), *P. sergenti* (18.2%), *P. perniciosus* (6.2%), *Phlebotomus bergeroti* (*P. bergeroti*) (2.9%), *Phlebotomus alexandri* (*P. alexandri*) (1.4%), *Phlebotomus chadlii* (*P. chadlii*) (0.8%), *Phlebotomus chabaudi* (*P. chabaudi*) (0.5%) and *Phlebotomus ariasi* (*P. ariasi*) (0.5%) and five *Sergentomyia* species: *Sergentomyia minuta* (*S. minuta*) (10.4%), *Sergentomyia fallax* (*S. fallax*) (8.1%), *Sergentomyia dreyfussi* (*S. dreyfussi*) (2.1%), *Sergentomyia christophersi* (*S. christophersi*) (1.7%) and *Sergentomyia africana* (*S. africana*) (0.5%) were detected. The detail of sampling in each transect is shown respectively in Tables 2-4.

Among the 2 056 sandflies collected on the Ouarzazat-M'Hamid road, *P. longicuspis* was the most common species (25.5%), followed by *P. papatasi* (23.8%), *S. fallax* (12.6%), *P. sergenti* (16.6%), *S. minuta* (11%), *P. bergeroti* (5.4%), *S. dreyfussi* (1.8%), *S. christophersi* (1.7%) and *P. ariasi*, *P. perniciosus*, *P. alexandri*, *P. chabaudi* and *P. chadlii* with less than 1% each. The sex ratio was in favor of males for all species in all stations with the exception of *S. minuta* (M/F=13/14) in Tisserghate station and *P. chadlii* (0/1) in Ouarzazat city (Table 2).

On the Foum Zguid-Marrakesh road, *P. papatasi* was the most prevalent species (25.2%) of a total of 2 370 sandflies collected, followed by *P. longicuspis* (17.5%), *S. minuta* (16.2%), *P. perniciosus* (11.7%), *P. sergenti* (11.7%), *S. fallax* (7.1%), *S. dreyfussi* (3.1%), *S. christophersi* (2.2%), *P. bergeroti* (1.9%) and *P. alexandri* (1.1%). We collected *S. africana*, *P. chabaudi*, *P. ariasi* and *P. chadlii* with less than 1% each as well. The sex ratio was in favor of females for *P. ariasi* in Ait aiaaza, *S. dreyfussi* and *S. christophersi* in Tata city, while, it was in favor of males for all caught species in all other stations (Table 3).

Sandflies (2 714) were collected on the Erfoud-Nador road.

Table 2

Number of males (M) and females (F) sandflies collected along the Ouarzazate-M'Hamid transect.

Species		*P. papatasi*		*P. bergeroti*		*P. sergenti*		*P. alexandri*		*P. chabaudi*		*P. ariasi*		*P. perniciosus*		*P. longicuspis*		*P. chadlii*		*S. fallax*		*S. minuta*		*S. dreyfussi*		*S. christophersi*		Total
Site (code)		M	F	M	F	M	F	M	F	M	F	M	F	M (PNA)	F	M	F	M	F	M	F	M	F	M	F	M	F	
Ouarzazate (S1)		36	15	0	0	24	11	3	1	2	1	11	2	0	0	43	24	0	1	12	3	12	2	1	3	0	0	207
Tisserghate (S2)		106	13	0	0	144	25	0	0	0	0	2	0	5	1	224	20	0	0	18	15	13	14	0	0	0	0	600
Tinzouline (S3)		52	11	1	0	20	2	0	0	0	0	0	0	2	0	123	6	0	0	19	7	12	10	0	0	2	0	267
Beni zoli (S4)		30	12	14	5	13	11	0	0	0	0	0	0	0	0	10	2	0	0	23	8	19	3	0	0	3	1	154
Zagora (S5)		23	7	15	3	20	9	0	0	0	0	0	0	0	0	2	0	0	0	9	2	11	9	2	1	6	1	120
Tamegroute (S6)		28	10	30	10	6	0	0	0	0	0	0	0	0	0	12	2	0	0	12	4	21	2	0	0	9	0	146
Zaouia Sidi Moktar (S7)		33	18	11	1	4	2	0	0	0	0	0	0	0	0	3	0	0	0	15	7	16	7	7	0	5	3	132
Anagam (S8)		22	4	8	0	10	2	1	0	0	0	0	0	0	0	20	8	0	0	18	11	23	11	2	0	2	0	142
Tagounite (S9)		19	8	10	2	16	3	0	0	0	0	0	0	0	0	16	4	0	0	23	2	18	9	10	4	1	0	145
M'Hamid (S10)		30	12	1	0	15	4	0	0	0	0	0	0	0	0	5	0	0	0	33	18	12	2	7	1	3	0	143
Total	M/F	379/110		90/21		272/69		4/1		2/1		13/2		7/1		458/66		0/1		182/77		157/69		29/9		31/5		1 624/432
	All	489		111		341		5		3		15		8		524		1		259		226		38		36		2 056

Table 3

Number of males (M) and females (F) sandflies collected along the Foum Zguid-Marrakech transect.

Species	*P. papatasi*		*P. bergeroti*		*P. sergenti*		*P. alexandri*		*P. chabaudi*		*P. ariasi*		*P. perniciosus*		*P. longicuspis*		*P. chadlii*		*S. fallax*		*S. minuta*		*S. dreyfussia*		*S. africana*		*S. christophersi*		Total
Site (code)	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Foum Zguid (S11)	60	18	18	3	24	8	4	1	0	0	0	0	0	0	4	0	0	0	10	2	24	13	0	0	4	1	10	2	180
Tata (S12)	88	61	9	0	36	14	12	2	0	0	0	0	0	0	2	0	0	0	8	5	38	21	0	1	12	4	4	6	323
Issafn (S13)	24	16	3	0	23	16	3	1	11	5	3	1	14	3	16	8	4	2	0	0	17	5	13	4	0	0	0	0	182
Igherm (S14)	0	0	0	0	2	0	0	0	0	0	3	1	8	1	24	18	0	0	2	0	3	1	0	0	0	0	0	0	63
Ait aiaaza (S15)	18	6	4	0	4	1	2	0	0	0	0	1	0	0	2	0	0	0	13	3	27	5	3	1	0	0	18	11	119
Ouled Berhil (S16)	32	14	6	2	22	8	0	0	0	0	0	0	0	0	6	0	0	0	43	19	83	49	14	6	0	0	0	0	304
Tinmel (S17)	25	11	0	0	13	6	2	0	0	0	2	0	14	6	33	12	0	0	0	0	8	0	0	0	0	0	0	0	132
Ouirgane (S18)	16	10	0	0	8	2	0	0	0	0	0	0	33	18	43	11	0	0	7	2	20	12	5	0	0	0	0	0	187
Tahanaout (S19)	23	14	0	0	10	7	0	0	0	0	0	0	115	65	123	77	0	0	11	9	16	2	3	1	0	0	0	0	466
Marrakech (S20)	90	72	0	0	50	23	0	0	0	0	0	0	0	0	22	13	0	0	24	11	28	12	17	6	0	0	0	0	348
Total M/F	376/222		40/5		192/85		23/4		11/5		8/3		184/93		275/139		4/2		118/51		264/120		55/19		16/5		32/19		1558/746
All	598		45		277		27		16		11		277		414		6		169		384		74		21		51		2370

Table 4

Number of males (M) and females (F) sandflies collected along the Erfoud-Nador transect.

Species	*P. papatasi*		*P. bergeroti*		*P. sergenti*		*P. alexandri*		*P. chabaudi*		*P. ariasi*		*P. perniciosus*			*P. longicuspis*		*P. chadlii*		*S. fallax*		*S. minuta*		*S. dreyfussi*		*S. Africana*		*S. christophersi*		Total
Site (code)	M	F	M	F	M	F	M	F	M	F	M	F	M (PN)	M (PNA)	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Erfoud (S21)	22	13	14	6	1	0	4	1	0	0	0	0	0	0	0	0	0	0	0	17	11	0	0	3	1	7	1	12	4	117
Errachidia (S22)	56	34	8	2	12	8	40	17	2	1	0	0	0	0	0	4	1	1	0	11	4	5	2	0	2	4	0	0	0	214
Midelt (S23)	23	12	3	0	19	3	0	0	6	2	2	0	19	8	14	31	26	13	2	11	3	5	1	0	1	0	0	0	0	204
Azrou (S24)	88	53	0	0	76	42	3	1	4	1	6	2	12	5	10	56	29	11	6	2	0	11	7	1	1	0	0	0	0	427
El Hajeb (S25)	72	39	0	0	114	68	2	0	2	0	0	0	39	17	23	53	18	7	1	7	3	12	5	12	9	0	0	0	0	503
My Yaacoub (S26)	263	138	0	0	172	91	0	0	0	0	0	0	8	4	6	101	83	2	0	28	15	22	5	7	2	0	0	0	0	947
Chefchaouen (S27)	45	33	0	0	27	18	0	0	1	1	0	0	4	1	0	16	3	4	0	12	3	16	9	0	1	0	0	0	0	194
Al Hoceima (S28)	12	3	0	0	16	4	0	0	0	0	0	0	0	0	0	0	0	0	0	13	2	4	0	0	0	0	0	0	0	54
Nador (S29)	11	6	0	0	12	8	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	12	3	0	0	0	0	0	0	54
Total M/F	592/331		15/8		449/242		49/19		15/5		8/2		117/53			261/160		38/9		103/41		87/32		23/17		11/1		12/4		1790/924
All	923		33		691		68		20		10		170			421		47		144		119		40		12		16		2714

P. papatasi was the most prevalent species (34%), followed by *P. sergenti* (25.5%), *P. longicuspis* (15.5%), *P. perniciosus* (6.3%), *S. fallax* (5.3%), *S. minuta* (4.4%), *P. alexandri* (2.5%), *P. chadlii* (1.7%), *P. bergeroti* (1.2%) and *S. dreyfussi* (1.5%). *S. africana*, *S. christophersi*, *P. chabaudi* and *P. ariasi* with less than 1% each. Except for *S. dreyfussi* (in Errachadia, Midelt, Azrou and Chefchaouen) for each sandfly species, the males collected outnumbered the females in all stations (Table 4).

In the first and second transects, *P. perniciosus* males were collected only as atypical morphology (PNA), but in Erfoud-Nador road, both forms *P. perniciosus* typical (PN) and *P. perniciosus* atypical were collected in the same stations (Tables 2-4).

For ZCL foci, a total of 207 specimens were collected in Ouarzazat, 120 in Zagora, 323 in Tata and 214 in Errachidia city. *P. longicuspis* was the most abundant species in Ouarzazat with 32% while *P. papatasi* was the most prevalent once in Zagora, Tata and Errachidia with 25%, 46% and 42% respectively. Each of the 29 study sites was highly similar to the other sites with Sorensen similarity indexes varying from 0.74 to 1 (complete similarity).

Table 5 summarizes the numbers of sand fly species and individuals caught in each category of biotope and the corresponding sand fly biodiversity indices. If it is not null, the sand fly fauna biodiversity of peridomestic biotope of Issafn (S13) showed the greatest value while that in the Barbacanes of Al Houceima (S28) showed the least one.

Table 5

Sandflies caught in different biotopes along the 29 sites studied with the corresponding biodiversity index.

Code	Biotope	S	Individuals	Biodiversity index	Dominant species
S1	Ruin	4	27	0.91	*P. longicuspis*
	Peridomestic	10	170	1.75	*P. longicuspis*
S2	Peridomestic	7	307	1.05	*P. longicuspis*
	Intradomestic	7	280	1.06	*P. papatasi*
	Barbacanes	4	19	1.02	*P. longicuspis*
S3	Barbacanes	5	92	0.88	*P. papatasi*
	Peridomestic	8	175	1.36	*P. longicuspis*
S4	Intradomestic	6	30	1.47	*P. papatasi*
	Vegetation	5	44	1.06	*P. papatasi*
	Stable	6	80	1.14	*P. papatasi*
S5	Stable	7	66	1.43	*P. papatasi*
	Intradomestic	6	18	1.73	*P. papatasi*
	Barbacanes	4	16	1.08	*P. papatasi*
S6	Peridomestic	7	103	1.29	*P. papatasi*
	Ruin	6	43	1.33	*P. bergeroti*
S7	Peridomestic	7	83	1.36	*P. papatasi*
	Vegetation	5	39	1.09	*S. fallax*
S8	Wall	4	29	0.89	*P. papatasi*
	Peridomestic	8	81	1.59	*P. longicuspis*
	Rock crevices	5	32	1.15	*S. minuta*

Table 5, continued

Sandflies caught in different biotopes along the 29 sites studied with the corresponding biodiversity index.

Code	Biotope	S	Individuals	Biodiversity index	Dominant species
S9	Peridomestic	7	73	1.40	*S. fallax*
	Stable	6	45	1.31	*P. papatasi*
	Rock crevices	4	27	0.91	*S. minuta*
S10	Peridomestic	8	143	1.41	*S. fallax*
S11	peridomestic	8	102	1.51	*P. papatasi*
	Barbacanes	5	45	1.05	*P. sergenti*
	Wall	5	33	1.14	*S. minuta*
S12	Peridomestic	8	223	1.29	*P. sergenti*
	Barbacanes	7	76	1.39	*P. papatasi*
	Wall	6	24	1.57	*S. minuta*
S13	Peridomestic	11	182	1.92	*P. sergenti*
S14	Peridomestic	6	40	1.36	*P. longicuspis*
	Intradomestic	3	23	0.64	*P. longicuspis*
S15	Intradomestic	6	79	1.14	*P. papatasi*
	Peridomestic	8	40	1.90	*S. minuta*
	Intradomestic	7	183	1.15	*P. papatasi*
S16	Vegetation	6	40	1.36	*S. fallax*
	Barbacanes	6	81	1.14	*S. minuta*
S17	Peridomestic	7	52	1.52	*P. longicuspis*
	Wall	6	33	1.43	*P. longicuspis*
	Barbacanes	7	47	1.56	*P. longicuspis*
S18	Peridomestic	7	93	1.32	*P. papatasi*
	Barbacanes	6	74	1.16	*P. longicuspis*
	rock crevices	4	20	1.00	*P. longicuspis*
S19	Intradomestic	5	277	0.71	*P. perniciosus*
	Peridomestic	7	189	1.14	*P. longicuspis*
S20	Barbacanes	5	217	0.74	*P. papatasi*
	Peridomestic	5	101	0.87	*P. papatasi*
	Vegetation	4	30	0.88	*P. papatasi*
S21	Intradomestic	6	593	0.78	*P. papatasi*
	Peridomestic	8	231	1.29	*P. bergeroti*
	Vegetation	5	95	0.88	*S. fallax*
S22	Intadomestic	6	754	0.75	*P. papatasi*
	Barbacanes	8	179	1.35	*P. alexandri*
	Peridomestic	10	311	1.57	*P. papatasi*
S23	Peridomestic	11	1374	1.38	*P. perniciosus*
	Intradomestic	6	300	0.88	*P. longicuspis*
S24	Peridomestic	11	976	1.45	*P. longicuspis*
	Intradomestic	7	701	0.92	*P. perniciosus*
S25	intradomestic	6	373	0.84	*P. longicuspis*
	Vegetation	5	130	0.82	*P. sergenti*
	Peridomestic	10	985	1.31	*P. papatasi*
S26	Peridomestic	8	711	1.07	*P. longicuspis*
	Intradomestic	6	320	0.87	*P. sergenti*
	Barbacanes	8	176	1.35	*P. papatasi*
S27	Peridomestic	9	377	1.35	*P. sergenti*
	Intradomestic	5	261	0.72	*P. papatasi*
	Wall	6	149	1.00	*S. minuta*
S28	Peridomestic	4	76	0.69	*P. papatasi*
	Intradomestic	0	0	0.00	0
	Barbacanes	3	54	0.50	*P. sergenti*
S29	Intradomestic	0	0	0.00	0
	Peridomestic	4	74	0.70	*P. sergenti*

Some qualitative and quantitative differences between biotopes were noted. In terms of number and specific richness of sand fly fauna, we collected 11 species in peridomestic biotope of S13, S23 and S24 whereas only three species in intradomestic biotope and barbacanes of S14 and S28 respectively.

We noted also that most of the *Sergentomyia* species collected were from sites far from human residences like wall, rock crevices, vegetations and peridomestic habitats.

4. Discussion

In Morocco, twenty three species of phlebotomine sandflies have been reported; 14 species of *Phlebotomus* genus and 9 species of *Sergentomyia* genus[2] of which five species have been known to transmit the disease: *P. ariasi*, *P. perniciosus* and *P. longicuspis* are vectors of *L. infantum* and are spread mainly in northern regions[19], *P. papatasi* is vector of humid form of cutaneous leishmaniases caused by *L. major* seen in the south and southeast of the Atlas Mountains, while *P. sergenti* is the vector for the dry skin form of cutaneous leishmaniases caused by *L. tropica*, reported in the center of the country[20-22].

In this study, 14 sandfly species were identified, representing 60% of the Moroccan sand fly species. Two genera are identified; *Phlebotomus* (77.2%) and *Sergentomyia* (22.8%) and all vectors are well represented.

The five vectors - *P. papatasi*, *P. ariasi*, *P. perniciosus*, *P. longicuspis* and *P. sergenti* - made up 71% of the sandflies collected, while in 86% of study sites (25/29), three of them - *P. papatasi*, *P. sergenti* and *P. longicuspis* - coexist.

We suggest that leishmaniases in Morocco is more related to the parasite ecology rather than the vector distribution, even if the vector occurrence is very important. Role of different vectors are often determined by species-level co-evolution of susceptibility to *Leishmania* species, with selection being initiated and maintained by ecological contacts[23].

Vector-borne and zoonotic disease transmission risk is the result of interactions between different species in space and time[24]. But, many entomological investigations in Morocco show no correlation between these vectors distribution and disease distribution even if the hosts and reservoir playing a role in the transmission of Leishmania are present in both endemic and non-endemic area[11,24,25].

The distribution of *P. papatasi* extends from southern Europe and eastern regions to the Indian subcontinent and highly depends on environment factors particularly relative humidity and temperature. It thus largely exceeds those of *L. major*[11]. In Egypt for example, ZCL is primarily present in northern Sinai while the vector distribution is more extensive[26]. Until now, despite the wide distribution of both *P. papatasi* and *Meriones shawi* in Morocco, ZCL and *L. major* are restricted to the pre-Saharan areas.

In Iran, Yavar *et al.* (2013) found natural infection of *P. papatasi* by *L. infantum*, with the absence of human VL cases. This vector has the ability to transmit two species of Leishmania parasite: *L. major* and *L. infantum*[27].

P. sergenti has also an extensive geographical distribution, wider than that of the anthroponotic cutaneous leishmaniases[28,29]. In Portugal, *P. sergenti* showed a very short period of activity in

comparison with other sandflies[30].

It is suggested that the presence of *P. sergenti* in *L. tropica* free areas is related to the existence of some cryptic vector species with consequences in their capacity to readily transmit *Leishmania*[31] and/or mechanisms of transmission for *Leishmania* parasites[32]. In Spain, two *P. sergenti* lineages were identified, a typically Spanish mitochondrial lineage and another one that is common in Morocco[28,29].

Phlebotomus ariasi, *P. perniciosus* and *P. longicuspis* are considered as *L. infantum* vectors[4]. The vectorial role of *P. ariasi* has been described in northern Morocco[33] when, previous studies[18,19,34,35] showed the presence in Morocco of three phylogenetic species of *P. perniciosus* complex: *P. perniciosus* including PN and PNA-like morphs; *P. longicuspis* sensu stricto and a sibling species of *P. longicuspis* (LCx).The atypical morph of *P. perniciosus* was identified also in Tunisia[36] and in Algeria[37]. The vectorial capacity and competence of these *P. perniciosus* morphs as well as of *P. longicuspis* and its potential cryptic species should be confirmed.

L. infantum is widespread mainly in northern Morocco as causative agent of cutaneous and visceral leishmaniasis with sporadic cases of human visceral leishmaniasis in the south[20,38]. Boussaa *et al.* (2008) noted the same distribution of *P. perniciosus* forms[19]. In North of Morocco, typical morphs of *P. perniciosus* are the most abundant forms, while in the southern regions, are mainly an atypical form.

Our results confirm this distribution, atypical morphology of *P. perniciosus* males was collected in the first and second transects, but in Erfoud-Nador road, both forms were collected in the same stations. Monitoring the insects in natural ecosystem is one of the simplest ways to observe and provide early warning of changes to biodiversity and habitat structure[39].

In our study, species presence and diversity appeared to be affected by several factors. According to altitude, which acting through its relationship with climate, we noted relatively low biodiversity at the highest (1 720 m) and low-altitude (61 m) sites investigated. Jahanifard et al. (2014) did find similar results in Iran[40], showing *P. papatasi* to be found between 8 and 1 756 m[41-43]. In contrast, some species prefer high altitude such as *P. ariasi* collected only between 920 and 1720 m. Other species change their sites according to bioclimate. For example, *P. chabaudi* and *P. chadlii* were collected at high altitude in arid climate and low altitude with humid climate.

On the other hand, a high diversity level in Saharan areas has been found when the species are structured on bioclimate basis[44]. Our results are similar to the other authors that found a high diversity of sandflies in sites ranged from 900 to 1100 m[11,16,45]. Nador and Al Hoceima are two coastal cities and show a low specific richness level.

In conclusion, the wide distribution of vectors in different forms of leishmaniases in Morocco could increase the risk to spread of different species of *Leishmania* from many foci to nonendemic sites. This suggests the need for a continuously surveillance to control the situation in these foci and to prevent risk in nonendemic areas.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This article is supported by the Laboratory of Ecology and Environment, (CNRST, URAC 32; ERA-CNERS 06) and the National Centre for Studies and Research on the Sahara, CNERS Project (Contract No. 06/ERACNERS). The Authors would like to thank Pro. Pesson Bernard for his collaboration and support.

Comments

Background

Human CL is a serious public-health problem in Morocco with active foci from 1970s. Several faunistic studies related to the distribution of phlebotomine sandflies in these foci have provided important data by describing the species involved in CL. The study on populations of sandflies by entomological surveys and the climatic factors that could affect their distribution need to be studied in new foci of CL.

Research frontiers

This type of works contributes to the need for appropriate control programs for Leishmaniasis in endemic countries.

Related reports

There are many reports related to this research in other regions of Morocco. In overall, in the three transects studied, nine *Phlebotomus* species were found. *P. papatasi* was the most prevalent species responsible of *L. major* spreading and human CL.

Innovations & breakthroughs

This is the first contribution in which entomological studies are related with ecological factors from Northern to Southern of Morocco where twenty-nine sampling stations were studied along three transects with a high diversity of *Phlebotomus* species found.

Applications

Entomological surveys in areas where leishmaniasis foci occur are imperative for the control of the disease. This work contributes to a better understanding of the distribution of the different species of sandflies present along three transects in Morocco in relation to ecological factors to undertake better control programs in this country.

Peer review

In this work authors conducted an entomological survey along three transects of Morocco where foci of cutaneous leishmaniasis occur. The data obtained gives practical information and suggests the need for a continuous entomological surveillance in this country.

References

[1] Maroli M, Feliciangeli MD, Bichaud L, Charrel RN, Gradoni L. Phlebotomine sandflies and the spreading of leishmaniases and other diseases of public health concern. *Med Vet Entomol* 2013; **27**: 123-147.

[2] Moroccan Ministry of Health. [A report on progress of control programs against parasitic diseases]. Rabat: Directorate of Epidemiology and Disease Control, Ministry of Health; 2013. French.

[3] Ready PD. Epidemiology of visceral leishmaniasis. *Clin Epidemiol* 2014; **6**: 147-154.

[4] World Health Organization. Control of the leishmaniasis: report of a meeting of the WHO Expert Committee on the Control of Leishmaniases. Geneva: World Health Organization; 2010. [Online] Available from: http://whqlibdoc.who.int/trs/WHO_TRS_949_eng.pdf [Accessed on 15th July, 2014]

[5] Asmae H, Fatima A, Hajiba F, Mbarek K, Khadija B, Mohamed R, et al. Coexistence of *Leishmania tropica* and *Leishmania infantum* in Sefrou province, Morocco. *Acta Trop* 2014; **130C**: 94–99.

[6] Rioux JA, Rispail P, Lanotte G. Lepart J. [Sandflies-bioclimate relationships in leishmaniasis ecology. Moroccan Example]. *Bull Soc Bot Fr* 1984; **131**: 549-557. French.

[7] Rioux JA, Akalay O, Périères J, Dereure J, Mahjour J, Le Houérou HN, et al. [Eco-epidemiological leishmanian risk trends in the Moroccan Atlantic Sahara. Heuristic interest of bioclimates sandflies relationship]. *Ecol Mediterr* 1997; **23**: 73-92. French.

[8] Rispail P, Dereure J. Jarry D. Risk zones of human leishmaniases in the western Mediterranean basin: correlations between vector sand flies, bioclimatology and phytosociology. *Mem Inst Oswaldo Cruz* 2002; **97**: 477- 483.

[9] Rioux JA, de la Roque S. [Climates, leishmaniasis and trypanosomiasis]. In: Rodhain F, editor. [*Climate change, infectious and allergic diseases*]. Amsterdam: Éditions scientifiques et médicales, Elsevier; 2003, p. 41-62. French.

[10] Prudhomme J, Gunay F, Rahola N, Ouanaimi F, Guernaoui S, Boumezzough A, et al. Wing size and shape variation of *Phlebotomus papatasi* (Diptera: Psychodidae) populations from the south and north slopes of the Atlas Mountains in Morocco. *J Vector Ecol* 2012; **37**: 137-47.

[11] Guernaoui S, Boumezzough A, Laamrani A. Altitudinal structuring of sandflies (Diptera: Psychodidae) in the High-Atlas mountains (Morocco) and its relation to the risk of leishmaniasis transmission. *Acta Trop* 2006; **97**: 346–351.

[12] Boussaa S, Pesson B, Boumezzough A. Faunistic study of the sandflies (Diptera: Psychodidae) in an emerging focus of cutaneous leishmaniasis in Al Haouz province, Morocco. *Ann Trop Med Parasitol* 2009; **103**: 73-83.

[13] Boussaa S, Pesson B, Boumezzough A. Phlebotomine sandflies (Diptera: Psychodidae) of Marrakech city, Morocco. *Ann Trop Med Parasitol* 2007; **101**: 715-724.

[14] Guernaoui S, Boumezzough A. Habitat preferences of phlebotomine sand flies (Diptera: Psychodidae) in southwestern Morocco. *J Med Entomol* 2009; **46**: 1187-1194.

[15] Rioux JA, Petter F, Akalay O, Lanotte G, Ouazani A, Seguignes M, et al. [*Meriones shawi* (Duvernoy, 1842) (Rodentia, Gerbillidae), a reservoir of *Leishmania major*, Yakimoff and Schokhor, 1914 (Kinetoplastida, Trypanosomatidae) in south Morocco]. *C R Seances Acad Sci III* 1982; **294** : 515-517. French.

[16] Boussaa S, Neffa M, Pesson B, Boumezzough A. Phlebotomine sandflies (Diptera: Psychodidae) of southern Morocco: results of entomological surveys along the Marrakech-Ouarzazat and Marrakech-Azilal roads. *Ann Trop Med Parasitol* 2010; **104**: 163-170.

[17] DELM and Directorate of Epidemiology and Disease Control. *Fight against leishmaniasis. Activity guide. Parasitic Diseases*. Morocco: Department of Health; 1997.

[18] Benabdennbi I, Pesson B, Cadi-Soussi M, Morillas Marquez F. Morphological and isoenzymatic differentiation of sympatric populations of *Phlebotomus perniciosus* and *Phlebotomus longicuspis* (Diptera: Psychodidae) in northern Morocco. *J Med Entomol* 1999: **36**: 116-120.

[19] Boussaa S, Boumezzough A, Remy PE, Glasser N, Pesson B. Morphological and isoenzymatic differentiation of *Phlebotomus perniciosus* and *Phlebotomus longicuspis* (Diptera: Psychodidae) in Southern Morocco. *Acta Trop* 2008; **106**: 184-189.

[20] Rhajaoui M. [Human leishmaniases in Morocco: a nosogeographical diversity]. *Pathol Biol* (Paris) 2011; **59**: 226-229. French.

[21] Adler S, Ber M. The transmission of *Leishmania tropica* by the bite of

Phlebotomus papatasi. *Ind J Med Res* 1941; **29**: 803-809.

[22] Bounoua L, Kahime K, Houti L, Blakey T, Ebi KL, Zhang P, et al. Linking climate to incidence of zoonotic cutaneous leishmaniasis (*L. major*) in Pre-Saharan North Africa. *Int J Environ Res Public Health* 2013; **10**: 3172-3191.

[23] Ready P. Should sand fly taxonomy predict vectorial and ecological traits? *J Vector Ecol* 2011; **36**(Suppl 1): S17-S22.

[24] Boussaa S, Guernaoui S, Pesson B, Boumezzough A. Seasonal fluctuations of phlebotomine sand fly populations (Diptera: Psychodidae) in the urban area of Marrakech, Morocco. *Acta Trop* 2005; **95**: 86–91.

[25] Hanafi HA, el Sawaf BM, Fryauff DJ, Beavers GM, Tetreault GE. Susceptibility to *Leishmania major* of different populations of *Phlebotomus papatasi* (Diptera: Psychodidae) from endemic and non-endemic regions of Egypt. *Ann Trop Med Parasitol* 1998; **92**: 57-64.

[26] Samy AM, Campbell LP, Peterson AT. Leishmaniasis transmission: distribution and coarse-resolution ecology of two vectors and two parasites in Egypt. *Rev Soc Bras Med Trop* 2014; **47**: 57-62.

[27] Yavar R, Hadi K, Reza AM, M M, Hasan B, Ali OM, et al. First detection of *Leishmania infantum* DNA in wild caught *Phlebotomus papatasi* in endemic focus of cutaneous leishmaniasis, South of Iran. *Asian Pac J Trop Biomed* 2013; **3**: 825-829.

[28] Barón S, Martín-Sánchez J, Gállego M, Morales-Yuste M, Boussaa S, Morillas-Márquez F. Intraspecific variability (rDNA ITS and mtDNA Cyt b) of *Phlebotomus sergenti* in Spain and Morocco. *Acta Trop* 2008; **107**: 259-267.

[29] Ozbel Y, Balcio lu IC, Olgen MK, Simsek FM, Töz SÖ, Ertabaklar H, et al. Spatial distribution of phlebotomine sand flies in the Aydin Mountains and Surroundings: the main focus of cutaneous leishmaniasis in western Turkey. *J Vect Ecol* 2011; **36**(Suppl 1): S99-S105.

[30] Branco S, Alves-Pires C, Maia C, Cortes S, Cristovão JM, Gonçalves L, et al. Entomological and ecological studies in a new potential zoonotic leishmaniasis focus in Torres Novas municipality, Central Region, Portugal. *Acta Trop* 2013; **125**: 339-348.

[31] Depaquit J, Ferté H, Léger N, Lefranc F, Alves-Pires C, Hanafi H, et al. ITS2 sequences heterogeneity in *Phlebotomus sergenti* and *Phlebotomus* similis (Diptera, Psychodidae): possible consequences in their ability to transmit *Leishmania tropica*. *Int J Parasitol* 2002; **32**: 1123-1131.

[32] Yahia H, Ready PD, Hamdani A, Testa JM, Guessous-Idrissi N. Regional genetic differentiation of *Phlebotomus sergenti* in three Moroccan foci of cutaneous leishmaniasis caused by *Leishmania tropica*. *Parasite* 2004; **11**: 189-199.

[33] Hamdani A. The study of sandflies fauna in three leishmaniases foci in North Morocco: species, abundance, seasonality and vectors [dissertation]. Marrakesh: Cadi Ayyad University, Faculty of Sciences, 1999.

[34] Pesson B, Ready JS, Benabdennbi I, Martín-Sánchez J, Esseghir S, Cadi-Soussi M, et al. Sandflies of the *Phlebotomus perniciosus* complex: mitochondrial introgression and a new sibling species of *P. longicuspis* in the Moroccan Rif. *Med Vet Entomol* 2004; **18**: 25-37.

[35] Guernaoui S, Pesson B, Boumezzough A, Pichon G. Distribution of phlebotomine sandflies, of the subgenus *Larroussius*, in Morocco. *Med Vet Entomol* 2005; **19**: 111-115.

[36] Boudabous R, Jaouadi K, Bounamous A, Babba H. Morphological and molecular investigations of population structure of *Phlebotomus perniciosus* and *Phlebotomus longicuspis* (Diptera: Psychodidae) in Tunisia. *J Med Entomol* 2012; **49**(4): 787-793.

[37] Berchi S, Bounamous A, Louadi K, Pesson B. [Morphological differenciation of two sympatric species: *Phlebotomus perniciosus* Newstead 1911 and *P. longicuspis* Nitzulescu 1930 (Diptera: Psychodidae)]. *Ann Soc Entomol Fr* 2007; **43**: 201-203. French.

[38] Kahime K, Boussaa S, Bounoua L, Fouad O, Messouli M, Boumezzough A. Leishmaniasis in Morocco: diseases and vectors. *Asian Pac J Trop Dis* 2014; **4**(Suppl 2): S530-S534.

[39] Uribe-M N, Wolff M, de Carvalho CJB. Synanthropy and ecological aspects of Muscidae (Diptera) in a tropical dry forest ecosystem in Colombia. *Rev Bras Entomol* 2010; doi: 10.1590/S0085-56262010000300018.

[40] Jahanifard E, Yaghoobi-Ershadi MR, Akhavan AA, Akbarzadeh K, Hanafi-Bojd AA, Rassi Y, et al. Diversity of sand flies (Diptera, Psychodidae) in southwest Iran with emphasis on synanthropy of *Phlebotomus papatasi* and *Phlebotomus alexandri*. *Acta Trop* 2014; **140**: 173-180.

[41] Kassiri H, Javadian E. Composition of the sand fly fauna in Khash County, Southeast Iran. *J Insect Sci* 2012; **12**: 132.

[42] Yaghoobi-Ershadi M. Phlebotomine sand flies (Diptera: Psychodidae) in Iran and their role on *Leishmania* transmission. *J Arthropod Borne Dis* 2012; **6** :1-17.

[43] Kavarizadeh, F, Vazirianzadeh B, Rassi Y, Jalali Glusang A, Moravvej SA. A faunistic study of sand flies of Musian District, Southwestern of Iran. *Pakistan J Zool* 2013; **45**: 549-554.

[44] Rioux JA, Carron S, Dereure J, Périères J, Zeraia L, Franquet E, et al. Ecology of leishmaniasis in the South of France. 22. Reliability and representativeness of 12 *Phlebotomus ariasi*, *P. perniciosus* and *Sergentomyia minuta* (Diptera: Psychodidae) sampling stations in Vallespir (eastern French Pyrenees region). *Parasite* 2013; **20**: 34.

[45] Simsek FM, Alten B, Caglar SS, Ozbel Y, Aytekin AM, Kaynas S, et al. Distribution and altitudinal structuring of phlebotomine sand flies (Diptera: Psychodidae) in southern Anatolia, Turkey: their relation to human cutaneous leishmaniasis. *J Vect Ecol* 2007; **32**: 269–279.