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RESEARCH ARTICLE

A preliminary investigation on *Phlebotomus longicusps* Nitzulescu, 1930 (Diptera: Psychodidae), the suspected vector of visceral leishmaniasis in the northeastern region of Libya

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Abstract

This study was carried out in two endemic areas of visceral leishmaniasis (Batta and Mirrad Massoud) which are located on the plateau of Al-Jabel Akhdar, Libya. Sandflies were collected using sticky traps over a period of eight months from April till November 2008. A total number of 1584 sandflies were collected consisting *Phlebotomus longicuspis* (1552= 97.98%) and *Sergentomyia* spp (32= 2.02%). The seasonal abundance of *P. longicuspis* showed one peak in August and it was the only *Phlebotomus* spp found in these areas. This enhances the evidence of its role as the main vector of visceral leishmaniasis in these foci. Further detailed studies are indicated to investigate the bionomics of this species in relation to visceral leishmaniasis transmission in the region.

Introduction

There are about 700 species of sandflies in 6 genera of which some have been proven or suspected vectors of Leishmania (Alexander, 2000; Maroli et al., 2008). There are many types of *Leishmania* species are transmitted by tens of phlebotomine sandflies (Poché et al., 2011). Leishmaniasis is one of the most important vector borne diseases of humans, infecting people in 88 countries. Visceral leishmaniasis (VL) is a severe disease, usually fatal, if untreated (Alvar et al., 2006); about 500,000 new cases each year are estimated, and causing 59,000 deaths worldwide (Desjeux, 2004; Poché et al., 2011). Visceral leishmaniasis (VL), caused either by *L. infantum* or *L. donovani* (WHO, 2000).

There are about 21 species of phlebotomine recorded in Libya: *Phlebotomus papatasi*, *P. perniciosus*, *P. longicuspis*, *P. sergenti*, *P. chabaudi*, *P. langeroni*, *P. alexandri*, *P. bergeroti*, *P. tobbi*, *P. (Larroussius)* sp, *Sergentomyia minuta*, *S. fallax*, *S. antennata*, *S. clydei*, *S. christopehrsi*, *S. dreyfussi*, *S. schwetzi*, *S. bedfordi*, *S. cincta*, *S. palestinensis*, *S. Adleri* (El-Buni et al., 1993; Annajar, 1999).

The infantile visceral leishmaniasis (IVL) is known to occur in North Africa (Aoun et al., 2013); in northern-Algeria (Belazzoug et al., 1985), in southern Algeria (Biskra) (Belazzoug, 1986), in Egypt (Alagamy) (Schnur et al., 1985), in Tunisia (Bouratbine et al., 1998; Aoun et al., 2002). In Libya, there are two endemic foci of (IVL), the causative pathogen of this type is *Leishmania infantum*, MON-1. (Gramiccia, 2003). IVL infects children under 5 years, in Al-Jabel Al-khdar (northeastern region) and Ubary (Southern region) (Dar et al., 1987; Jain et al., 1990; Mehabresh and El-Mauhoub, 1992; Mehabresh, 1994). About 120 cases were recorded in the last two decades (NCDC, 2008).

The main reservoir hosts of VL in the Mediterranean is the domestic dogs (*Canis familiaris*), while sandflies genus *Phlebotomus* are the main vectors (WHO, 1990).

The biology and ecology of vector facets are essential for epidemiological investigations on leishmaniasis (Fish, 2008). Knowledge of the population dynamics of the phlebotomine vector of VL agent in the endemic areas in Libya is limited.

This study was to determine the temporal and spatial distribution of sandflies within villages that lie within the VL endemic area to figure out the species composition of sandflies.

Materials and Methods

Study area

This study was carried out in the villages of Batta (32°40'00'N, 21°05'00'E), and Mirrad Massoud (32°40'00'N, 21°15'00'E), from April to November 2008. Both villages locate in the area of Al-Jabel Akhdar, Libya (figure 1), north-west to Al Baida city (~15 Km) and east to Benghazi (~120 km), 120-400 meters above sea level. This area is characterized by chapparal biome, with an annual mean temperature of 18.0° C and monthly rainfall ranging from 0 to 200 mm. Climate is markedly seasonal, with a dry season from mid-April to mid-October and a rainy season for the rest of the year. The total population of villages Batta and Mirrad Massoud are 13,000 and 4,000 respectively. It is known as one of the VL foci in Libya (Dar et al., 1987; Jain et al., 1990; Mehabresh and El-Mauhoub, 1992; Mehabresh, 1994).

Sand fly collection

Each village divided into ten stations. Sticky traps were used to collect sandflies. Sticky trap is a castor-oiled paper (25 × 25 cm) (Annajar, 1999). In each site, a total of 48 traps/station/night were placed in domestic animal barns around farmer houses for three nights each month. All traps were put overnight then collected at the morning and treated to free sandflies, then sorted and preserved in 70% ethanol for identification. Heads and genetalia were used for species identification using identification keys (Annajar, 1999).

Data analysis

For each location, we summarized the species of sandflies, abundance, density and sex ratio. The density of phlebotomine sandflies that collected by sticky traps was calculated using the formula: Density= number of specimens/m^2 of oiled paper surface (one exposed side). The numbers of sandflies captured in sampling sites were tested for normality by the 1-Sample Kolmogorov - Smirnov Z test (K-S). Thereafter, the data were compared using the Mann Whitney rank sum test and Kruskal-Wallis Test, considering $P < 0.05$ as significant. All statistical analyses were carried out on SPSS 20.0 statistical software (SPSS, 2011).

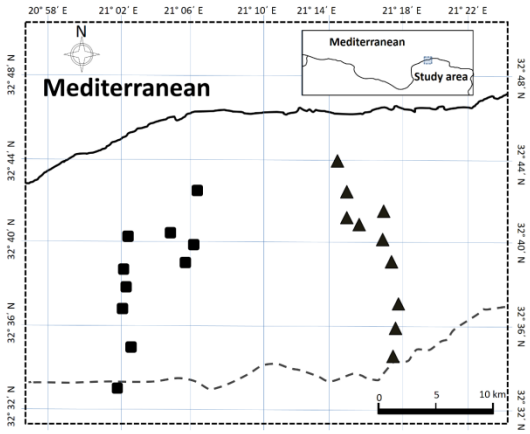

Figure 1. Map of the study area in northeastern Libya showing sampling sites: Batta (■) and Mirrad Massoud (▲).

Results

Sand fly collection

A total of 1,584 sandflies were collected over the 8-month study period (Tables 1 and 2), 936 sandflies from the village of Batta, and 648 sandflies from Mirrad Massoud. Sandflies species differed in abundance ($P<1.00 \times 10^{-9}$), these included *P. longicuspis* (97.98%), *Sergentomyia* spp (2.02%) (Table 1).

Table 1 Total sand fly captures from villages of Batta and Mirrad Massoud, Libya

	P. longicuspis		*Sergentomyia* spp	
	Batta	Mirrad Massoud	Batta	Mirrad Massoud
April	0	6	0	0
May	73	45	0	0
June	39	19	1	0
July	171	56	0	0
August	313	409	2	21
September	252	59	0	4
October	61	18	0	1
November	24	7	0	3
Total	933	619	3	29

The activity of the species extended from April to November. Total sand fly captures over the year reflected one peak in August (Figure 2). Among the villages, figure 3 shows a similar trend, with Batta having many more flies in September as well. There were significant differences among months either in Batta (Kruskal-Wallis test, $\chi^2= 37.6$, df= 7, $P= 3.7 \times 10^{-6}$) or in Mirrad Massoud (Kruskal-Wallis test, $\chi^2= 23.1$, df= 7, P= 0.002), which resulted from the significant differences in most pair-wise comparisons between months in both villages (Mann-Whitney Test). The density showed the same pattern (Table 2).

Sand fly numbers decreased in late October as the climate became cooler. No sandflies were trapped during the cooler months (December till March).

For the villages of Batta and Mirrad Massoud the total captured sandflies were 936 and 648, respectively. The total flies captured was higher in Batta than in Mirrad Massoud (χ^2, $P<4.6X10^{-13}$). The village of Batta was consistently high in sand fly numbers for all but one month during the sampling period, Batta had more sandflies captured (χ^2, $0.016>P<1.76 \times 10^{-26}$) (Figure. 3).

In both villages Batta and Mirrad Massoud the males formed the majority of population of sandflies 89.64% and 91.36% respectively (Table 2).

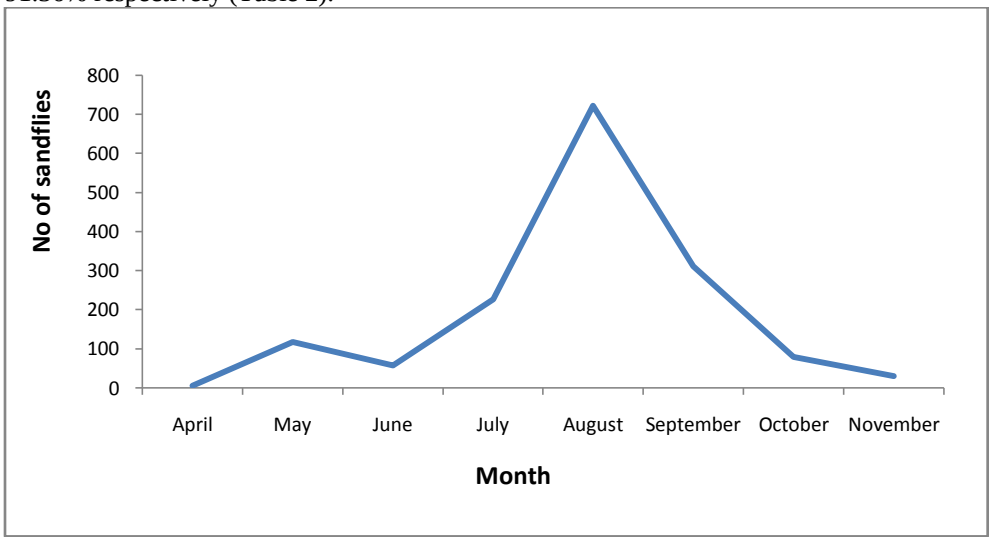

Figure2. The number of *P. longicuspis*s and flies trapped in both villages during the study period

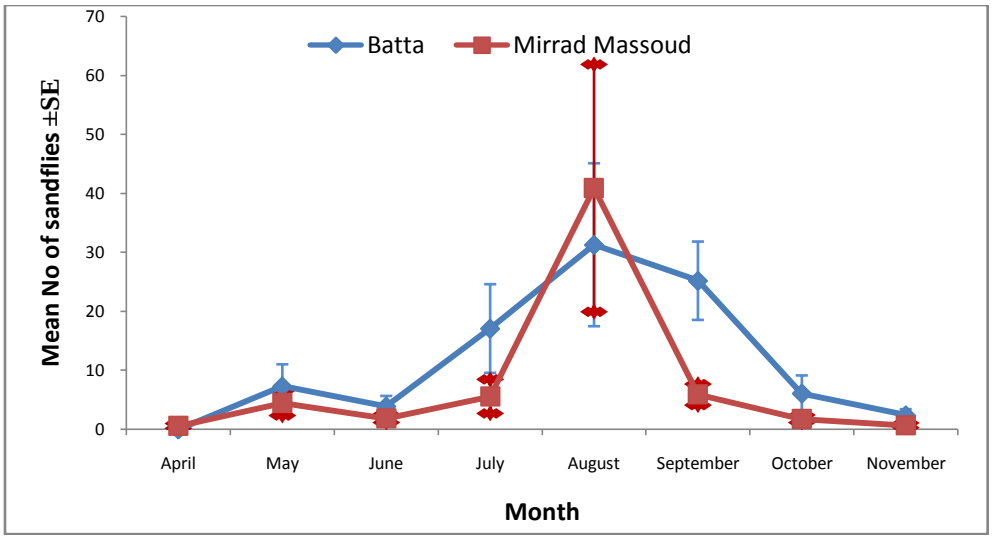

Figure. 3 The average number of *P. longicuspis* sandflies trapped in Batta and Mirrad Massoud during the study period

Table 2.No. of specimens, sex and density of captured sandflies

	No. of specimens		No. of Males		No. of Females		Density/m^2	
Month	Batta	Mirrad Massoud	Batta	Mirrad Massoud	Batta	Mirrad Massoud	Batta	Mirrad Massoud
April	0	6	0	5	0	1	0	0.3
May	73	45	70	34	3	11	3.65	2.25
June	40	19	23	15	17	4	2	0.95
July	171	57	166	49	5	8	8,55	2.85
August	315	430	300	409	15	21	15,75	21.5
September	252	63	231	58	21	5	12.6	3.15
October	61	19	44	15	17	4	3.05	0.95
November	24	10	5	8	19	2	1.2	0.5

Discussion

In this study, it was obvious that *Phlebotomus longicuspis* is dominant over *Sergentomyia* spp in the study area. The total number of *Phlebotomus longicuspis* forms 97.98%. This trend was seen in both villages, Batta and Mirrad Massoud. The presence of these species of sandflies in the northeastern region of Libya has been confirmed in previous studies (Ward, 1983) and in other areas (Ashford et al., 1977; El-Buni et al., 1993; Annajar, 1999). It has wide distribution in the Mediterranean (Lane, 1986). *S. minuta* (the possible species) less abundant in human settings (Maroli et al., 1994), it feeds on cold-blooded animals (Lewis, 1978).

Phlebotomus longicuspis has well-defined seasonal activity, in study area, peaking during August and September. These data are in accordance with previous findings in northwestern Libya (Dokhan, 2008).

Sex ratio was male biased (89.64%). Males remain near the animal host for longer time than females do (Fahmy et al., 2009). They also have lekking behavior which is believed to allow males to attract females by spreading high levels of sex pheromone (Morrison et al., 1995; Killick-Kendrick, 1999). This is consistent with other studies carried out on sandflies; males forms 83% in *Lutzomyia longipalpis* (Morrison et al., 1995), 85% in *P. papatasi* and 70% in

P. sergenti (Reza and Mansour, 2006), more than 60% in *P. argentipes* (Dinesh et al., 2008), 80% of the captured sandflies (Kasap et al., 2009).

Control of Leishmaniasis depends on epidemiological, biological and ecological data regarding the disease especially those of vector (Hazratian, 2011) such as Knowledge of spatial and temporal changes of vector population (Gálvez, 2010).

The species *P. longicuspis* considered as vector of VL in Biskra, Algeria (Belazzoug, 1986). *P. longicuspis* present in Biskra where *L. infantum* identified in human cases and reservoir dogs (Dedet et al., 1984). Killick-Kendrick mentioned that *L. infantum* is the responsible agent of kala-azar in El Agmy of Egypt and *P. langeroni* is the vector (Killick-Kendrick, 1985).

Dogs are thought to be the main 'reservoir' hosts in the Al-Jabel Akhdar and blood samples from 68 dogs have been investigated in both villages 40 from Batta and 28 from Mirrad Massoud and found 12.5% and 21.4% as positive cases, respectively (unpubl. data).

To emphasis that the presence of the only sand fly species *P. longicuspis* in the VL endemic areas in the NE region of the country enhances its role as the main suspected vector of VL in the studied foci. Therefore, It is recommended that further detailed investigation to confirm that *P. longicuspis* is the main vector has to be carried out.

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