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Preliminary field investigations on Phlebotomine sandflies (Diptera: Psychodidae) from a recent cutaneous leishmaniasis focus in Northern-Sinai, Egypt

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ABSTRACT

Species composition, sex ratio and nocturnal activity of sandflies were studied in El Barth, a recent focus of cutaneous leishmaniasis (CL) in North Sinai, Egypt. CDC light traps and sticky paper traps were used for insect collection. Two species of sandflies were identified; *Phlebotomus papatasi* and *P. sergenti*. *P. papatasi* constituted more than 82 % of the collected sandflies in the surveyed sites. Number of caught *P. papatasi* females was almost double that of males, however, it was slightly male biased in case of P. *sergenti.* Both sandfly species exhibited nocturnal activity and peaked at 24:00-2:00. This study might be a step towards a better understanding of vector population dynamics in this recent focus of CL. Using the results of this study, health workers in this area can better manage control and prevention tactics of cutaneous leishmaniasis.

Keywords: Northern Sinai, *P. papatasi*, *P. sergenti*, sex ratio, nocturnal activity

INTRODUCTION

Sandflies are important vectors of leishmaniasis, a vector-born zoonotic disease which is endemic in 88 countries throughout Africa, Asia, Europe, and North and South America. Twelve million cases worldwide, with 1.5 to 2 million new cases each year are estimated (Desjeux, 2001 and Dedet *et al*, 2003). Sandflies are widely distributed across the Middle East, from Morocco to Egypt, and the Arabian Peninsula to Jordan, Turkey and Iran (Lane *et al*. 1988; Yuval 1991; Sawalha *et al*., 2003; Ibrahim *et al.* 2005; and Sahin and Nurdan 2007). Cutaneous leishmaniasis (CL) is an important health problem in many parts of the world, especially Mediterranean and Middle East countries (WHO 2006).

Two forms of leishmaniasis have been recorded in Egypt, cutaneous leishmaniasis (CL) in Sinai (Kamal *et al.*, 2003) and visceral leishmaniasis (VL) in the northern costal margins (Youssef *et al*., 1989).

Phlebotomus papatasi has been reported to comprise more than 94% of the sandfly population in North Sinai, Egypt (Hanafi, *et al*., 2007). *P. papatasi* is the proven vector of cutaneous leishmaniasis and the *Leishmania major* is the etiological agent associated (Wahba *et al*., 1990).

In September, 2006, physicians from a hospital in El Barth village, Rafah, Northern-Sinai, Egypt, collected tissue samples from ten patients with suspected CL. *Leishmania major* was the responsible etiological parasite for clinically diagnosed CL cases among Bedouins inhabiting El-Barth village (Shehata *et al*., 2008) (unpublished data).

Preliminary field observations including species composition, sex ratio and nocturnal activity rhythm of sandflies were studied in El Barth village to help in the implementation of a strategic vector control measures and disease prevention tactics of cutaneous leishmaniasis in this recent focus of the disease in North Sinai, Egypt.

MATERIALS AND METHODS

Study area:

El Barth village is 35 Km southeast of Rafah, North Sinai, on the Egyptian border with Palestine (31° 01´N, 34° 12´ E to 30° 8´N, 34° 17´E). Rafah is a city in the far North East of Sinai, some 400 kilometers far from Cairo, on the Egyptian border with Palestine and is inhabited principally by Bedouins. El Barth is located on the El-Ghoora road and has approximately 5000 inhabitants. It is divided into four sectors namely Kilo 25, Kilo 30, Kilo 33 and Kilo 36 located on the Egyptian Palestinian borders (Fig. 1).

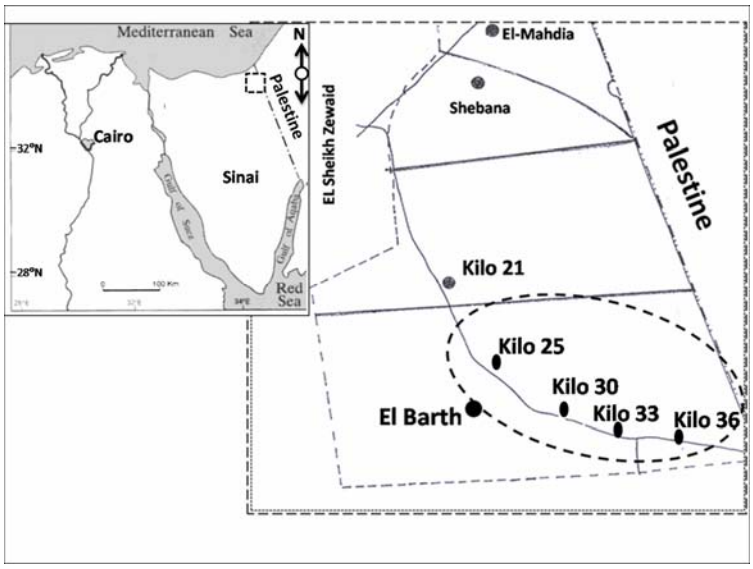

Fig. 1: Regional and local map of the study site in North-Sinai, Egypt.

Climatic conditions and microhabitat of study area:

The average temperature in the study area during this investigation was 17°C min. and 30°C max. Relative humidity (R.H.) ranged from 70% - 100%. Average wind velocity during the study period was 5.3 Km. /hour. The land is mainly dunes, with small scattered cultivations of citrus, olives and almonds. Houses are primitive with straw sheep barns attached.

Sandfly collection and processing:

Sandfly collection was carried out for ten consecutive days in May 2007. Sticky traps and CDC light traps were used for collection. After trap recovery, dead flies were kept in 70% alcohol and transported to a temporary laboratory for identification. Gravid live females were left till oviposition in order to initiate sand fly colonies (for future studies) and after oviposition they were dissected for identification; while the remaining live flies were dissected under sterile conditions. Heads and genetalia were used for species identification using the morphological key of Lane (1986).

a.Sticky trap collection:

Sticky traps were made of 20 × 20 cm, castor-oiled paper. A total of 200 sticky traps was distributed along the four sectors under investigation. Two collection sites were chosen in each sector and 25 traps were set at each collection site. Collection sites were chosen close to the suspected rodent burrows surrounding houses where leishmaniasis cases were diagnosed. Recovered traps were placed in labeled plastic bags and transported to a temporary field laboratory located in El-Barth hospital. Sandflies adhering to the oiled papers were collected with a fine paintbrush and prepared for identification and sexing.

b. CDC-light trap collection:

Sandflies were also collected using CDC-light traps (John W. Hock Company, Gainesville, FL, USA). Two – three traps were set in each collection site. They were set inside the houses or in domestic animal barns where CL cases were detected. Captured flies were collected with an aspirator, cleared and mounted for species identification and sexing.

Nocturnal activity rhythm:

Sticky traps were used for sandfly collection for ten consecutive nights during May 2007 in the same four sectors of El Barth village. Traps were set and replaced at two hours intervals starting before sunset till after sunrise (18:00 - 6:00) i.e. six collections / night to clarify the characteristics of nocturnal activity rhythm. Collected paper traps were then placed in labeled plastic bags indicating the date, place of collection, time of collection and number of traps used. The removed traps were replaced by another set of traps for the next two hours.

RESULTS

Sandfly species composition:

An overall total of 2049 sandflies was collected during May 2007. Two species of sandflies were identified; *P. papatasi* and *P. sergenti*. *P. papatasi* was the most predominant species found; being recorded in all sites monitored and comprised more than 82 % of the total collected sandflies (Table 1).

Table 1: Sandfly species collected from different sectors of El Barth village.

Study sector	Sticky paper traps				CDC light traps				Total
	P. papatasi		*P. sergenti*		*P. papatasi*		*P. sergenti*		
	♂	♀	♂	♀	♂	♀	♂	♀	
Kilo 25	107	53	3	0	51	293	9	21	537
Kilo 30	73	46	51	32	47	209	84	49	591
Kilo 33	112	82	39	26	34	276	0	7	576
Kilo 36	83	72	17	23	29	107	3	11	345
Grand total	375	253	110	81	161	885	96	88	2049

Sex ratio:

The number of female *P. papatasi* captured by CDC light traps (set indoors) was more than five times that of males i.e. 885 and 161, respectively. On the other hand, the number of female *P. sergenti* caught by the same traps was slightly male-biased (96 female and 88 male) (Table 1). However, it was observed that sticky traps,

which were set outdoors at the rodent burrows entrances, collected more males than females of both sandfly species detected in the study area.

Nocturnal activity rhythm:

Both sandfly species, *P. papatasi and P. sergenti*, exhibited nocturnal activity and peaked after midnight (24:00 – 2:00 a.m). The weighted average of caught sandflies is plotted against the collection time (Fig. 2). A gradual increase in the numbers of captured sandflies was recognized starting from 18:00 till the peak at midnight then a gradual decrease took place till dawn 4:00 – 6:00. Just after sunrise, (during the month of May sun rises a few minutes past 6:00) sandflies were absent from sticky paper traps recovered.

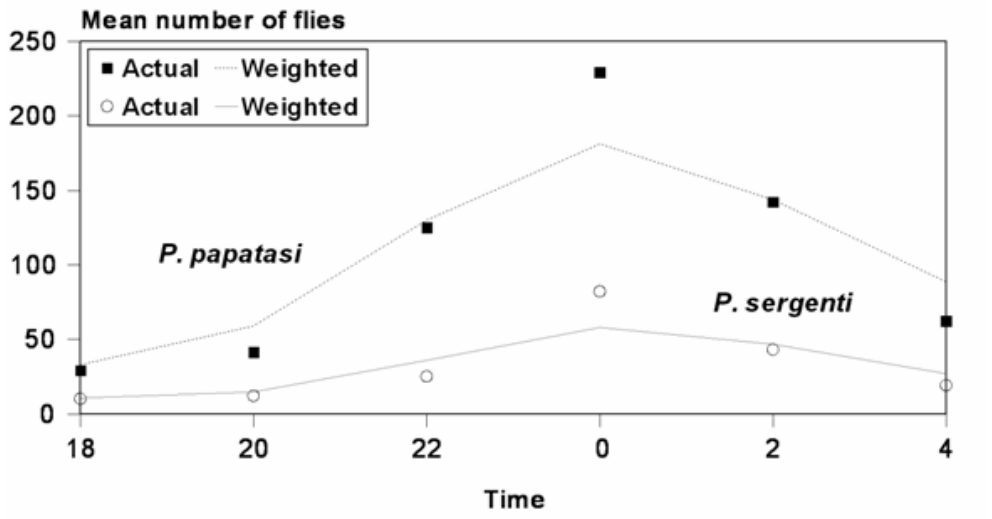

Fig. 2. Mean number of sandflies *P. papatasi* and *P. sergenti* collected from the study area each two hours throughout ten consecutive nights.

DISCUSSION

In this study, the dominance of *P. papatasi* over *P. sergenti* in the four collection sectors was very obvious in the study area. The total number of *P. papatasi* sandflies was almost five times higher than that of *P. sergenti*, (82% of the total capture). The presence of these two species of sandflies and the dominance of *P. papatasi* in north Sinai has also been confirmed in previous surveys. Hassan *et al*. (1999) and El Hossary *et al*. (2000) identified the same two species in a study at Nekhel, Northern Sinai; an area that has a similar habitat characteristics to the area investigated in this study. Recently, Hamadto *et al*. (2007) have also reported the same observations at four study areas in North Sinai, El Hassanah, Nekhel, El Arish and Bir El-Abd. Similar observations have also been reported in surveys in South Sinai. (El Sawaf *et al*., 1987).

It was observed that CDC light traps captured more than 60% of the total capture of sandflies. Sahin and Nurdan (2007) in Turkey reported that among the trapping methods used, light traps showed the highest capture efficiency, above aspirators and sticky papers. The same observations were also recorded by Dinesh *et al*., (2008) in India and by Hilmy *et al*., (1989) in Egypt. It might be concluded that light traps alone can be sufficient to determine the sand fly fauna in any study area. However, and in spite of the above reports, it was observed that sticky traps in this study collected more males than females of both sandfly species. Sticky traps were set

close to and at the level of rodent burrows. It was reported that sandfly males attracted by the odor and Co_2 emitted by the rodent host in addition to high R.H. (70 – 100%) in the study area, these males attract the females by emitting pheromones and wing vibrations and compete for mating privileges (Ward et al 1988, 1990 and 1993). This might explain why sex ratio was male biased near rodent burrows. Males seem to remain near the animal host for longer time than females which reproductive strategy seems to be taking blood meal, mating then flying off to seek a shelter elsewhere.

The number of female *Phlebotomus papatasi* captured Indoors using CDC light traps was significantly higher than that of males (F/M ratio = 5: 1) whereas, the number caught was slightly male-biased in case of *P. sergenti*, (F/M = 0.82 : 1). This might suggest that female *Phlebotomus papatasi* is more endophagic compared to *Phlebotomus sergenti* in the study area. Deviations in sex ratios from the natural 1 : 1 ratio in this study is largely attributed to behavioral variations since it was reported by Ford (1961) that sex is a case of balanced polymorphism and that selective forces will oppose departure from the optimum proportion of males and females which is generally near equality.

Both sandfly species, *P. papatasi and P. sergenti*, exhibited nocturnal activity and peaked after midnight (24:00 – 2:00 a.m.) rather than crepuscular-nocturnal as reported by Lewis (1971). When phlebotomine sandflies are active, they fly to disperse, to feed, to find mates and to search for oviposition sites. This peak of sandflies in this area is probably due to the fact that this study was performed during the month of May where temperature remains as high as 30^{o}C to 35^{o}C almost till midnight when a gradual decline takes place and sandflies are enhanced for host finding. The current results coincide with that of Guernaoui *et al*. (2006) who reported that variations of the nocturnal activity rhythm could be related to the variations in temperature and relative humidity. Also, Shehata et al. (1995) reported that in a Cl focus in Wadi Feiran area of Sinai Peninsula, Egypt, man-biting activity of sandflies *P. bergeroti* and *P. sergenti* displayed a sharp peak around 22.00-23.00 hrs and 24.00-01.00 hrs, respectively.

Data obtained in this study which constituted population activity and resting behavioral patterns might be helpful in the implementation of successful tactics for vector control and disease preventive measures. Further knowledge on the seasonal abundance and biting preferences of Cl vectors in this new focus are of great importance to be investigated in the future.

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ARABIC SUMMERY

دراسات حقلية أولية على ذباب الرمل الفليبوتومينى (ثنائيات الأجنحة: سايكوديدى) من بؤرة حديثة لمرض الليشمانيا الجلدية فى شمال سيناء – جمهورية مصر العربية

عادل رمزى فهمى[1] – عبد اللة سامى[1] – سعيد ضحا[2] – مجدى جبريل شحاتة[1]

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ذبابة الرمل هي العامل الناقل لداء الليشمانيا الجلدي حيث تتواجد في الأماكن الرطبة الدافئة وهي تنشط ليلا" فقط أما نهارا" فتختبئ في شقوق الجدران القديمة والمهجورة والزوايا المظلمة والرطبة. و يعتبر داء الليشمانيا الجلدية من المشاكل الصحية الهامة. وهو من الآمراض الجلدية الشائعة في الشرق الأوسط و حوض البحر الأبيض المتوسط وخاصة في المنطقة الشمالية والساحلية، أما عالميا" فهو ينتشر في المناطق الحارة والدافئة بشكل رئيسي ويتواجد فيها بشكل مستوطن.

فى بؤرة تم اكتشافها حديثا لهذا المرض فى قرية "البرث" الواقعة فى أقصى الشمال الشرقى لمصر و على الحدود مع فلسطين تمت هذة الدراسة لتحديد انواع ذبابة الرمل الموجود، النسبة الجنسية (الذكور للأناث) و نمط النشاط الليلى لهذة الحشرات.

استخدمت المصائد الضوئية (سى دى سى) و المصائد الورقية اللاصقة لتجميع الحشرات و حصلنا على النتائج الأتية:

1- تم رصد و تعريف نوعين من ذبابة الرمل (بالتشريح و باستخدام المفاتيح التصنيفية) و هما *فليبوتوماس باباتاسى* و *فليبوتوماس سرجنتى*. النوع الأول مثل ٨٢ % من العدد الكلى الذى تم تجميعة.

2- عدد الأناث كان خمس اضعاف عدد الذكور تقريبا فى نوع *فليبوتوماس باباتاسى* عند التجميع داخل البيوت بأستخدام المصائد الضوئية ، و لكن كان عدد الذكور كان اكثر فى النوعين عند التجميع بواسطة المصائد اللاصقة قرب جحور القوارض.

3- كلا النوعين كان نشاطهما ليليا و ظهرت قمة النشاط عند الفترة من منتصف الليل و حتى الثانية صباحا (2:00 – 24:00).

ستساعد هذة الدراسة على فهم أفضل لطبيعة هذة الحشرة الناقلة للمرض و نشاطها و بيئتها الخاصة مما قد يساعد المختصين على وضع استراتيجية ناجحة فى مقاومة الحشرة و منع أو السيطرة على انتشار المرض.