RESEARCH NOTE

SEASONAL OCCURRENCE OF PHLEBOTOMINAE SAND FLIES (PHLEBOTOMINAE: DIPTERA) AND IT'S CORRELATION WITH KALA-AZAR IN EASTERN UTTAR PRADESH, INDIA

NS Singh and Doris Phillips Singh

Department of Zoology, Lucknow Christian College, Golaganj, Lucknow (U.P.), India

Abstract. In this investigation, the species composition of sand flies, and their seasonality, nocturnal activity, sex ratio, and resting site, for implementation of future control measures, were surveyed in eastern (Gonda and Basti) Uttar Pradesh, India. Adult sand flies (2.893) were collected from internal and external sites by sticky and light traps. The sand flies were captured using light traps hung at different heights in trees and in peridomiciliary and extradomiciliary areas of a forest during both dry and rainy months. The traps were kept out between sunset and sunrise of the following day. In the extradomiciliary environment, the traps were installed at 1, 3 and 5 m above the ground. In this investigation, a total of 5 species were obtained: Phlebotomus papatasi, P. sergenti, Sergentomyia sintoni, S. punjabensis and S. dentata. The number of sand flies peaked in September and declined by December. The maximum and minimum numbers were found at 8:00 PM and 5:00 to 6:00 AM, respectively. The female to male ratio of the phlebotominae sand flies varied from a high in October to a low in June. The number of sand flies in the external regions was significantly more (p < 0.05) than the internal regions in all months except May, June, December and January. No flagellate infections were observed in any other species of sand flies. Using the results of this investigation, health workers in this area may be better able to control and prevent leishmaniasis.

INTRODUCTION

Sand flies (Psychodidae and Phlebotominae) belonging to genera *Phlebotomus* and *Lutzomyia*, are the vectors of the leishmaniasis, in the old and new worlds, respectively. About 30 species of sand flies are proven vectors of at least 20 species of *Leishmania*. Leishmaniasis is a vector-borne zoonotic disease with three distinct climate manifestations: cutaneous, mucocutaneous, and visceral. It is endemic in approximately 90 countries in tropical and subtropical regions of the world (Desjeux, 1996). One study identified the sandfly species and their behavior in forest and anthropic environments in Paraná State, Brazil (Silva *et al*, 2008). Habitats of these flies can be in different areas, including rain forests, desert, rural, urban, sylvatic and domestic areas in Asia, Africa, Europe and South America (Magill *et al*, 1993; Herwaldt, 1999). Singh

Correspondence: NS Singh, Department of Zoology, Lucknow Christian College, Golaganj, Lucknow-226018 (UP), India. E-mail: naveendoris@rediffmail.com

and Ipe (2006) identified the distributional patterns of sand flies in India. The objectives of this investigation were to give basic information about the seasonal abundance, nocturnal activity, resting sites, sex ratios and species composition of sand flies in the study area, so better control measures can be developed. There have been no studies of vectors of visceral leishmaniasis in this region and the present study provides the basic information.

MATERIALS AND METHODS

Description of the study area

The investigations were carried out from April 2006 to February 2008, in Gonda and Basti districts of eastern Uttar Pratesh, India.

Gonda District (27.13º N 81.93º E) is the headquarters of Devi Patan Division. In this region the year is divided into four seasons. The winter from mid-November to February is followed by the summer season lasting until about the middle of June. The period from mid-June to the end of September constitutes the south-west monsoon season. October to mid-November is the post monsoon or transition period. The average annual rainfall in the district is 1,166 mm. During the winter the mean minimum temperature is about 9°C and the mean maximum temperature is 23°C while in the summer the mean minimum temperature is about 25°C and the mean maximum temperature is about 44°C. The relative humidity is high during the south-west mansoon and the post-mansoon seasons, being above 70%. The humidity decreases and in the summer the air is very dry.

Basti (27.15° N 83°00 E) lies between Sant Kabir Nagar District to the east and Gonda District to the west. In this district the period from mid-June to the end of September is the southwest monsoon season, and October to mid-November is the postmonsoon or transition period. During the monsoon season, and for brief spells of a day or two in winter, heavily clouded or overcast skies prevail. During the rest of the year the skies are mostly clear or lightly clouded. The average annual rainfall of the district is 1,166 mm. During the winter the mean minimum temperature is about 9°C and the mean maximum temperature is about 23°C. During the summer, the mean minimum temperature is about 25°C and the mean maximum temperature is about 44°C. In the southwest monsoon and post-monsoon seasons the relative humidity is above 70%.

Sand fly collection and examination

Sticky traps and aspirators were used to collect sand flies at selected indoor and outdoor sites, on the basis of previous investigations about the habitat of sand flies, which are cracks in walls, caves, on riverbanks, and in private homes. Sand flies were collected biweekly using sticky paper traps, CDC light traps and aspirators from outdoors (cow shelters, dog shelters and holes in rocks and caves) as well as indoors (stable and living rooms). Dinesh et al (2008) found indoor CDC light traps for collecting the sand fly P. argentipes, vector of Leishmania donovani was the most efficient method for monitoring *P. argentipes* populations in the Indian subcontinent. Sticky paper traps (15 x 20 cm) installed after sunset were collected before sunrise in 20 catching sites. Collection by aspirators was made within houses from 6:00-8:00 AM. To keep safe from wind damage, light-traps were placed in confined locations, and were set out at 7:00 PM and put away 5:00 AM the next day. Collected specimens were preserved dry at 4°C. For identification of species and sex, specimens were slide mounted in Faure's medium after a clearing process in lactophenol for three days (Maroli and Fausto, 1986). Female identification was based on the

Species	Males	Females	Total	Relative abundance (%)
Phlebotomus papatasi	389	132	521	18
Phlebotomus sergenti	827	215	1,042	36
Sergentomyia punjabensis	278	213	491	17
Sergentomyia sintoni	312	122	434	15
Sergentomyia dentata	265	140	405	14

Table 1 The number of different species of sand fly collected from Gonda and Basti (districts of eastern Uttar Pradesh) and their relative abundance.

shape of spermathecae and the disposition of the teeth in the pharyngeal armature, whereas males were identified based on their genitalia (hypopygium), in particular the parameres and aedeagus, the shape, insertion, disposition, and length of the hairs in the coxite, and the spines in the style (Corradetti *et al*, 1961; Rioux and Golvan, 1967; Dolmatova and Demina; 1971, Killick-Kendrick *et al*, 1991; Maroli *et al*, 1994; Singh *et al*, 2007). Air temperature and relative humidity were recorded hourly from 6:00 PM to 6:00 AM, to report nocturnal activity. The taxonomic keys of Lewis (1978) were used for identification of the sand fly species.

RESULTS

A total of 2,893 adult sand flies were collected from April, 2006 to February, 2008, comprising five species of two genera (*Phlebotomus* and *Sergentomyia*) were identified. *P. sergenti* was the most abundant species, followed by *P. papatasi*, *S. punjabensis*, *S. sintoni*, and *S. dentata*. One thousand two hundred seventeen (42%) sand flies from indoors and 1,676 (58%) from outdoors were captured (Table 1).

Seasonal abundance

A total of 2,893 adult sand flies comprised of *P. sergenti* (1,042) *P. papatasi* (521) *S. punjabensis* (491), *S. sintoni* (434), and *S. dentata* (405) were collected from January 2006 to December 2008. According to Fig 1, sand flies were not active in December and January. February and September were the months of peak activity. The population of species increased to a maximum during September and then decreased to a minimum during November. In February, the month when activity began, the mean temperature was 16.5°C and the relative humidity was 22.5%. In September, the peak-activity month, the mean temperature was 28.5°C and the relative humidity was 31.5%.

Nocturnal activity

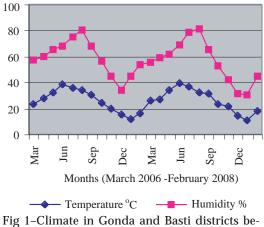
The peak activity of adult sand flies was at 8:00 PM and the minimum for all species, but especially mainly *Sergentomyia* species was at 6:00 AM in September, the month of peak activity (Fig 2).

Sex ratio

Significantly more adult male than female sand flies were collected in sticky traps each month. The sex ratio (female:male) in adults varied from a high in March and September, to a low in December and January. The overall percentage of male flies was 83% and female flies was 17% (Fig 3).

Resting site

Sand flies were more abundant out-



tween March 2006 and February 2008.

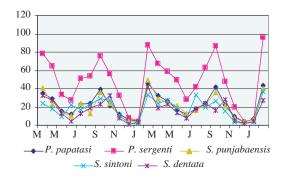


Fig 2-Seasonal prevalence of *P. papatasi, P. sergenti, S. punjabensis, S. sintoni*, and *S. dentata* in Gonda and Basti districts from March 2006 to February 2008.

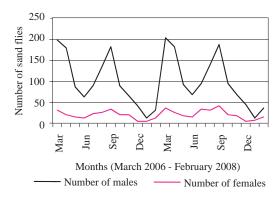


Fig 3–Monthly sex ratio of phlebotominae sand flies collected from Gonda and Basti districts of Uttar Pradesh, India from January 2006 to December 2008.

doors than indoors during all months, while the monthly abundance of *S. punjabensis* outdoors was almost equal to that indoors. The nocturnal activity of both *S. sintoni* and *S. dentata* outdoors was less than indoors, especially from 7:00 -11:00 PM.

DISCUSSION

The distribution of phlebotomine sand flies varies highly within its range, depending on local environmental factors, such as precipitation and temperature, physical factors, such as geographical barriers and habitat availability, and biotic factors, such as the distribution and abundance of vertebrate hosts (Cross et al, 1996; Ghosh et al, 1999). Although altitude is not a selective factor, biotic and abiotic properties of the environment are highly correlated with altitudinal gradients, the most obvious of which is climate (Karan et al. 2000). Future research in this area will be to make a detailed investigation regarding the biology and ecology of P. sergenti, P. papatasi, S. punjabensis, S. sintoni, and S. dentata.

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REFERENCES

- Corradetti A, Neri I, Verolini F, Palmieri G, Proietti AM. Procedimento tecnico per lo studio del faringe dei flebotomi e descrizione dei faringi dei flebotomi italiani. *Parassitologia* 1961; 3: 101-3.
- Cross ER, Newcomb WW, Tucker CJ. Use of weather and remote sensing to predict the geographic and seasonal distribution of *Phlebotomus papatasi* in Southwest Asia. Am

J Trop Med Hyg 1996; 54: 530-6.

- Desjeux P. Leishmaniasis, public health aspects and control. *Clin Dermatol* 1996; 14: 417-23.
- Dinesh DS, Das P, Picado A, *et al.* The efficacy of indoor CDC light traps for collecting the sandfly *Phlebotomus argentipes*, vector of *Leishmania donovani. Med Vet Entomol* 2008; 22: 120-3.
- Dolmatova AV, Demina NA. Les phlébotomes (*Phlebotominae*) et les maladies qu'ils transmettent. *ORSTOM Bull Liaison* 1971; 18: 168.
- Ghosh KN, Mukhopadhyay JM, Guzman H, Tesh RB, Munstermann LE. Interspecific hybridization of genetic variability of *Phlebotomus* sand flies. *Med Vet Entomol* 1999; 13: 78-88.
- Herwaldt Bl. Leishmaniasis. Lancet 1999; 354: 1191-9.
- Karan D, Dubey S, Moreteau B, Parkash R, David JR. Geographical clines for quantitative traits in natural populations of a tropical drosophilid: *Zaprionus indanus. Genetica* 2000; 108: 91-100.
- Killick-Kendrick R, Tang Y, Killick-Kendrick M, et al. The identification of female sandflies of the subgenus *Larroussius* by the morphology of the spermathecal ducts. *Parassitologia* 1991; 33: 335-47.
- Lewis DJ. The phlebotomine sandflies (Diptera:

Psychodidae) of the Oriental Region. *Bull Br Mus Nat Hist Ent* 1978; 37: 217-343.

- Magill AJ, Grogl M, Gasser RA, Sun W, Oster CN. Visceral infection caused by *Leishmania tropica* in veterans of Operation Desert Storm. *N Engl J Med* 1993; 328: 1383-7.
- Maroli M, Fausto AM. Metodi di campionamento e montaggio dei flebotomi (Diptera; Psychodidae). *Rapporti Istisan*, 1986: 74 pp.
- Maroli M, Bigliocchi F, Khoury C. I flebotomi in Italia: osservazioni sulla distribuzione e sui metodi di campionamento. *Parassitologia* 1994; 36: 251-64.
- Rioux JA, Golvan XJ. Écologie des leishmanioses dans le sud de la France. – I. Le phlábotomes, échantillonnage – éthologie. *Ann Parasitol* 1967; 42: 561- 603.
- Silva AM, Camargo NJ, Santos DR, *et al.* Diversity, distribution and abundance of sand flies (Diptera: Psychodidae) in Parana State, Southern Brazil. *Neotrop Entomol* 2008; 37: 209-25.
- Singh NS, Ipe M. A study on distributional pattern of Phlebotomidae sand flies (Diptera: Phlebotomidae) from India. *Indian J Entomol* 2006; 68: 178-81.
- Singh NS, Phillips-Singh D, Ipe IM. A Review on the collection, preservation and systematic techniques in Phlebotominae. *Pak J Biol Sci* 2007; 10: 3983-91.