J Vector Borne Dis 46, June 2009, pp. 136–140

The sandflies of the Satluj river valley, Himachal Pradesh (India): some possible vectors of the parasite causing human cutaneous and visceral leishmaniases in this endemic focus

Nand Lal Sharma,Vikram K. Mahajan, Nitin Ranjan, Ghanshyam K. Verma, Ajit K. Negi & Karan Inder S. Mehta

Department of Dermatology, Venereology & Leprosy, Indira Gandhi Medical College, Shimla, India

Abstract

Background & objectives: The recently recognized endemic focus of leishmaniasis in Satluj river valley in Himachal Pradesh (India) lies in north-western Himalayas (30°N, 70°E). This endemic focus of leishmaniasis appears peculiar where localized cutaneous leishmaniasis (LCL) co-exists with visceral leishmaniasis (VL), and *Leishmania donovani* is predominant pathogen for LCL whereas only a few cases have been due to *Leishmania tropica*. This study was carried out to collect sandflies, identify and delineate their habitat and role in transmission of human leishmaniasis in this endemic focus.

Methods: During June 2003 to September 2007, 142 (M–22, F–120) sandflies were collected with aspirators from 10 endemic villages of Kinnaur and Shimla districts.

Results & conclusion: Sixty-two of the identified sandflies caught belonged to the genus *Phlebotomus* species, including some species that are known to act as vectors of the parasites causing human leishmaniasis. The *Phlebotomus* (*Adlerius*) *chinensis longiductus* (Parrot), 1928 (28 sandflies), *P. major* (8 sandflies), *P.* (*Larroussius*) *kandelakii burneyi* (Lewis), 1967 (8 sandflies) were identified. The identification of the main species of vector sandfly in the region is complicated because it is still uncertain which *Leishmania* species cause(s) the local human leishmaniasis. Circumstantially it seems likely, however, that *Phlebotomus* (*Adlerius*) *chinensis longiductus* is the main vector. Other species found, such as *P. major* and *P.* (*Larroussius*) *kandelakii burneyi*, may also be responsible for some cases. A more elaborate study is recommended.

Key words Cutaneous leishmaniasis – Himachal Pradesh – *Phlebotomus longiductus* – sandflies vector – visceral leishmaniasis

Introduction

More than 30 species of *Leishmania* have been incriminated to cause human leishmaniasis that affects at least two million people every year worldwide. While visceral leishmaniasis is an important cause of mortality, the cutaneous disease too is responsible for significant morbidity. The vector, for both visceral and cutaneous leishmaniasis, is a small (2–5 mm), hairy hematophagous female sandfly belonging to the genus *Phlebotomus* in the Old World, and *Lutzomiya* and *Psychodopygus* in the New World. The males of the species are not hematophagous and can be differentiated from females by their smaller size. The sandflies have a very limited flight range (estimated to be 250 m), are active during warm months, and depending on the particular species, feed on mammals, birds or reptiles (animal reservoirs for the parasite). The feeding is mostly at dusk and within the flying range. The females lay eggs on the

ground and the larvae require constant temperature, complete dryness and organic material to mature.

The recently recognized leishmaniasis endemic area in Himachal Pradesh is spread across villages in the valley of the River Satluj on both banks; along Pooh subdivision of Kinnaur district towards north-east border, Kumarsain subdivision of Shimla district towards south-west, Rampur division and adjoining villages (Shimla district) towards southern side, and Nirmand subdivision of Kullu district on the northern side (924–2900 m above mean sea level). To understand the epidemiology and effectively contain leishmaniasis in this region, it became essential to obtain information on the sandfly bionomics in this endemic focus. However, information available on sandflies of the region is rather scanty. An attempt was made to collect sandflies, study their distribution, habitat, and identify species with an aim to delineate their role in the transmission of leishmaniasis in the region. In this paper we present our observations on the sandfly data thus collected.

Material & Methods

During June 2003 to September 2007, relatively warmer months of the year, sandflies were collected from highly endemic pockets of cutaneous leishmaniasis which comprised five villages each of Kinnaur and Shimla districts from the already delineated endemic area[1]. The sites identified for trapping sandflies resting indoors were houses and cattlesheds, and outdoors were tree holes and burrows in the peridomestic areas of previously treated patients of cutaneous leishmaniasis. The present endemic focus lies in Himachal Pradesh, a north-western hill state of India (30ºN, 70ºE) that shares its north-eastern border with China. Different geographical formations such as mountains, plains, rivers and rivulets are characteristics of the area and climatic conditions vary greatly across one district to another. The terrain of Kinnaur is mostly mountainous with dry, loose strata and the average temperature is 16.5ºC (summers) and 1.5ºC (winters) at its district headquarters (Rekong Peo). It receives rains (average 4.9–84 mm) during February to October and snow (64–1399 mm) during October to April. In contrast, winters are mild, and summers are warm and humid in Rampur area of District Shimla. Most of the endemic area comprises rural setup with agriculture and cattle rearing as main occupations of the inhabitants. In most instances the cattlesheds are poorly ventilated and situated on the ground floor/basement with loose wooden planks separating the living rooms above.

Attempts were made to collect sandflies by using sticky traps or aspirators. The sticky traps, plastic sheets smeared with vaseline, were hung overnight inside the houses and cattlesheds in front of doors and windows, and near tree holes and rodent burrows in the fields belonging to patients. Aspirators, made of small glass or plastic tube attached to rubber tubing at one end for aspiration, were used to collect sandflies indoors. Sandflies resting inside the crevices in cattlesheds or inhabited rooms were driven out with the help of twigs, located under torchlight and trapped in aspirators. Time of collection was from 1700–1900 hrs in eight locations, 1500–1700 hrs in nine locations, and 0900–1200 hrs in five locations. The collected sandflies were stored in screw capped plastic tubes which were labeled for the area, name of the village and head of the family, location (indoor/outdoor, inhabited room/cattleshed, etc.) and number of sandflies caught.

Male and female sandflies were differentiated from their size. Gut contents from 40 female sandflies were used either for preparing direct smears or culture for *Leishmania* in modified NNN medium (Novy, MacNeal and Nicoll's medium containing RPMI 1640 and 10% heat inactivated fetal bovine serum). The smears were stained with Giemsa. Sixty-two sandflies were sent for identification to three different institutes— (i) National Institute of Virology (Medical Entomology Department), Pune, India; (ii) National Institute of Communicable Diseases, Delhi, India; and (iii) National History Museum, London. The sandflies were identified by their morphological (mouth parts and spermathecae) charac-

teristics and PCR for mitochondrial gene *cytochrome-b*, by methods reported previously[2]. The sandflies were also subjected to trypanosomatid specific ITS-γDNA based PCR[3] for presence and identification of any *Leishmania* spp in them.

Results

Attempts to use sticky-traps were altogether unsuccessful. We could trap 142 (M–22, F–120) sandflies with aspirator. The sandflies were collected with ease between 1700–1900 hrs from cattlesheds, grass/fuel wood stores or a few from inhabited rooms of 18 houses in 10 villages spread across Kinnaur and Shimla districts (Fig. 1). Thirty-three sandflies were identified by Medical Entomology Department of the National Institute of Virology, Pune, seven by National Institute of Communicable Diseases, Delhi, and 22 at National History Museum, London where PCR studies for mitochondrial gene *cytochrome-b* and trypanosomatid specific ITS-γDNA were also carried out simultaneously. The 62 sandflies subjected to identification were of the genus *Phlebotomus*. Table 1 shows details of sandflies collected from different villages and their species identity. One of the eight *Phlebotomus longiductus* sandfly, collected from village Shaneri (Rampur) was detected to have *Leishmania tropica* and *Trypanosoma theileri* on PCR studies. None of the Geimsa stained smears prepared from gut contents of sandflies showed the presence of promastigotes, and all the cultures developed bacterial or fungal contamination perhaps because of inability to maintain sterile environment in field conditions.

Table 1. Distribution and identified species of Phlebotomine sandflies of Himachal Pradesh

Village (No. of sandflies collected)	*Phlebotomus chinensis* (*Adlerius*) *longiductus*	*P. major*	*P. kandelakii* (*Larroussius*) *burneyi*
District Shimla			
Shingla (5)	5	–	–
Shaneri (25)	8	–	2
Khopari (1)	1	–	–
Racholi (11)	–	–	–
Kumarsain (9)	1	2	2
District Kinnaur			
Telangi (10)	2	1	–
Pooh (8)	1	–	1
Spillo (6)	2	–	–
Kilba (21)	7	2	1
Chagaon (46)	19	3	2
Total (142)	46	8	8

Note: Only 62 of the 142 collected sandflies were identified. One of the sandflies collected from Village Shaneri was identified to be *Phlebotomus longiductus* and detected to have *Leishmania tropica* and *Trypanosoma theileri*. Thirty (M–6, F–24) sandflies were not subjected to identification and gut contents of others were used for smear and culture of the parasite.

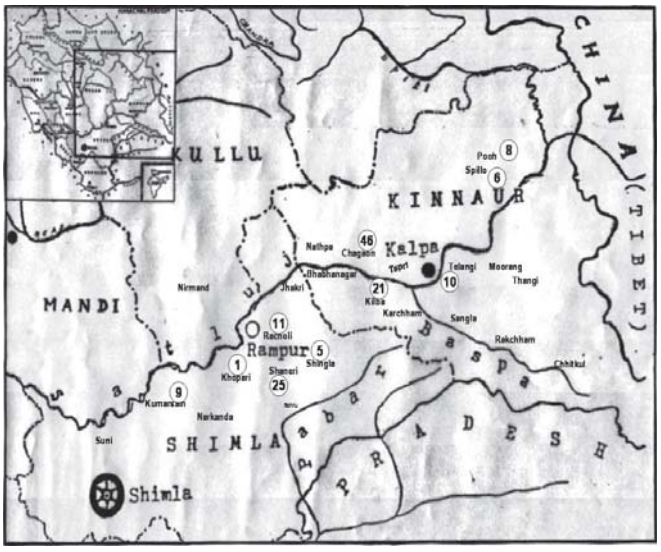

Fig. 1: Geographic distribution of sandflies in Satluj river valley, endemic focus of human leishmaniasis (Himachal Pradesh), India. Encircled figures depict number of sandflies collected from that location (All locations are approximate)

Discussion

The human leishmaniasis in endemic focus of Satluj river valley of Himachal Pradesh (India) has many features similar to those of the disease present in Mediterranean countries; such as localized cutaneous leishmaniasis (CL) co-exists with visceral leishmaniasis (VL), and *Leishmania donovani* is predominant pathogen for CL whereas only a few cases have

been due to *Leishmania tropica*[1,4,5]. Similarities are also evident in the topography of the endemic area as well. However, not many studies are available on mapping of Phlebotomid sandfly fauna of Himachal Pradesh, India. Kalra & Lewis[6] could identify four sandflies as *P. salehi* evidently infected with *Leishmania*. These were obtained from burrows of the Indian desert gerbil *Meriones hurrianae* that is believed to be a reservoir of *L. tropica* in Rajasthan. However, there is no serious attempt made subsequently to identify the vector(s) for CL in India except for an occasional suggestion incriminating either or both *P. papatasi* and *P. sergenti*. Kaul & Jain[7] attempted to arrange 44 known species of *Phlebotomine* sandflies seen in India in five physiographic divisions. According to this arrangement, Himachal Pradesh falls in the 2500 km long belt of northern mountain ranges which comprises three parallel ranges from Brahmputra in the east to the Indus in the west. They identified 21 species of *Phlebotomine* sandflies in this Himalayan belt, six in the genus *Phlebotomus* and 15 of the *Sergentomyia*. However, the parts of the valley having difficult to approach higher altitudes have not been studied by them in sufficient details.

Kulkarni *et al*[8] conducted largest ever survey between 1966 and 1970 in subtropical and temperate areas of Western Himalayan regions of Himachal Pradesh including parts of the Satluj river valley, Uttar Pradesh, Jammu & Kashmir, and hill districts of West Bengal. They collected 1103 sandflies comprising 649 *Phlebotomus* spp (M–146, F–503) and 454 *Sergentomyia* spp (M–262, F–192). Although they could mark the presence of *P. argentipes* (the only proven vector for VL in the Indian subcontinent) in Uttar Pradesh, *P. chinensis (Adlerius) longiductus* Parrot (M–2, F–26), *P. major (Larroussius)* Annandale (M–36, F–45), *Sergentmyia babu* (M–34, F–79), *S. baghdadi* (M–15, F–5), *S. khawi hodgsoni* (M–5, F–0), *S. zeylanica* (M–7, F–2), *S. hospitii* (M–3, F–2) , *S. bailyi* (M–3, F–3), and *S. montana* (M–12, F–8) were the only sandflies found in Himachal Pradesh. The presence of *P. longiductus* and *P. sergenti* has also been observed in the Ladakh region of Jammu & Kashmir in an another study[9] which has eco-geographical features similar to the present endemic area in Himachal Pradesh. We tend to agree with Kulkarni *et al*[8] that *P. longiductus* along with *P. major* and *S. montana* are predominantly mountainous species as we too have found *P. chinensis longiductus* predominantly, 46 of the 62 identified sandflies, in this endemic focus. None of the studies thus far carried out, however, have incriminated these sandflies as vector(s). The identification of the main vector species of sandfly in this region is complicated because it is still uncertain which *Leishmania* species is causing the local disease. It seems, however, that *P. chinensis longiductus* is a main vector in this endemic focus especially with the demonstration of *L. tropica* in one of them. However, incriminating a particular sandfly species is difficult despite the presence of *Leishmania* stages in the gut as it does not necessarily mean that the insect is able to transmit the disease.

The possibility of *P. chinensis longiductus*, a known vector for *L. infantum* being the primary vector for human leishmaniases in this endemic focus also appears more plausible in view of its predominant presence in this focus, *L. donovani infantum* causing both cutaneous and visceral leishmaniasis, and K39 seroprevalence in dogs (known reservoir for *L. infantum*) in the region[1,4,5,10]. However, this is purely conjectural at the moment and we recommend a more elaborate study to establish the identity of the vector(s) of human leishmaniasis in this endemic focus. The cattlesheds seem to provide ideal environment for sandfly resting and breeding while their proximity to the residence perhaps ensure continuous transmission of infection to humans.

Acknowledgement

We thank Dr P.V.M. Mahadev, Deputy Director, National Institute of Virology, Pune, India, Dr Kaushal Kumar, Joint Director, National Institute of Communicable Diseases, Delhi, India, and Dr Paul Ready, National History Museum, London for their technical help extended for identification of

sandflies. Dr Paul Ready also performed PCR studies for mitochondrial gene *cytochrome-b* and trypanosomatid specific ITS-γDNA. The study has been partly funded by a grant from Tribal Development Department of Govt. of Himachal Pradesh.

References

1. Sharma NL, Mahajan VK, Negi AK. Epidemiology of a new focus of localized cutaneous leishmaniasis in Himachal Pradesh. *J Commun Dis* 2005; *37:* 275–80.
2. Hodgkinson VH, Birungi J, Quintana M, Deitze R, Munstermann LE. Mitochondrial *cytochrome-B* variation in populations of the visceral leishmaniasis vector *Lutzomyia longipalpis* across eastern Brazil. *Am J Trop Med Hyg* 2003; *69:* 386–92
3. Torri AF, Englund PT. A DNA polymerase-β in the mitochondrion of the trypanosomatid *Crithidia fasciculata*. *J Biol Chem* 1995; *270:* 3495–7.
4. Sharma RC, MahajanVK, Sharma NL, Sharma A. A new focus of cutaneous leishmaniasis in Himachal Pradesh (India). *Indian J Dermatol Veneorol Leprol* 2003; *69:* 170–2.
5. Sharma NL, Mahajan VK, Kanga A, Sood A, Katoch VM, Mauricio I, *et al*. Localised cutaneous leishmaniasis due to *Leishmania donovani* and *Leishmania tropica:* preliminary findings of the study of 161 new cases from a new endemic focus in Himachal Pradesh, India. *Am J Trop Med Hyg* 2005; *72:* 819–24.
6. Kalra NL, Lewis DJ. The identity of the probable vector of *Leishmania tropica* among rodents in India. *Trans R Soc Trop Med Hyg* 1975; *69:* 522.
7. Kaul SM, Jain DC. Distribution of *Phlebotomine* sandflies (Diptera: Psychodidae) according to the Physiographic Divisions of India. *J Commun Dis* 1995; *27:* 155–63.
8. Kulkarni SM, Bhat HR, Modi GB. Survey of Phlebotomid sandflies from the Himalayan region, India (Diptera: Phlebotomidae). *Indian J Med Res* 1978; *67:* 583–8.
9. Kaul HN, Shetty PS. Sandflies of Jammu region with a review on their records in Jammu & Kashmir state, India. *Indian J Med Res* 1983; *78:* 643–6.
10. Sharma NL, Mahajan VK, Negi AK, Verma GK. The rK39 immunochromatic dipstick testing: a study for K39 seroprevalence in dogs and human leishmaniasis patients for possible animal reservoir of cutaneous and visceral leishmaniasis in endemic focus of Satluj river valley of Himachal Pradesh (India). *Indian J Dermatol Venereol Leprol* 2009; *75:* 51–4.

Corresponding author: Dr N.L. Sharma, Department of Dermatology, Venereology & Leprosy, R.P. Govt. Medical College, Kangra, Tanda–176 001 (HP), India.
E-mail: nandlals@hotmail.com

Received: 19 June 2008 *Accepted in revised form:* 1 May 2009