Short Communication

SAND FLY FAUNA OF PUNE CITY, MAHARASHTRA, INDIA: REVISITED AFTER 40 YEARS

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ABSTRACT

Background: Leishmaniasis, a sand fly-borne disease, is a major health concern with an estimated 350 million people under threat in 98 tropical countries. Sand flies are also the incriminated vectors of several viruses of public health importance including encephalitis causing ChandipuraVirus, Toscana Virus etc. Present study was initiated to determine the sand fly fauna of Pune city,

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which has shown dramatic changes in its infrastructural development recently.

Methods: Twenty-six localities covering new townships and recently annexed fringe villages were surveyed and collected sand flies using hand-held aspirators during March to November 2018. Sand flies were identified morphologically, pooled according to the species, gender and locality and processed for virus isolation in a cell culture system.

Results: During the study, 5,387 sand flies were collected comprising four species, *i.e.*, *S. punjabensis* (39.3%), *S. babu* (30.4%), *S. bailyi* (27.2%), and *P. argentipes* (3.2%). *Phlebotomus papatasi,* however*,* was strikingly absent in the collection. Most of collections (89.8%) were made from areas in close proximity to humans, *viz.*, living rooms, toilets, bathrooms etc. Though per man hour density in the fringe villages was found high; the newly urbanized areas also had a substantial abundance of sand flies. All the sand flies were processed for virus isolation, but no virus could be isolated. There is an urgent need to find the probable reason for the decline in *Phlebotomus* sand fly population as this could help better management of sand fly-borne infections.

Conclusion: An upsurge in the population of *Sergentomyia* spp. and a shift of their breeding/resting to those in close proximity to human settlements were observed. This poses a concern as these sand flies have a history to harbour leishmania parasites as well as viruses like Chandipura, Toscana and other viruses of public health importance.

Keywords: Chandipura virus, Pune, sand flies, *Sergentomyia*, urbanization

INTRODUCTION

Phlebotomus sand flies, belonging to subfamily *Phlebotominae* transmit leishmaniasis, a disease estimated to impact >350 million people and approx.

60,000 deaths annually.[1] In India, *P. argentipus* and *P. papatasi* are the principal vectors of *leishmania* parasites and Bihar, Jharkhand, Uttar Pradesh and West Bengal together contribute to 2/3rd of the total global cases.[2] Sand flies are also associated with maintenance and transmission of several viruses of public health importance, *viz*., Chandipura virus, Vesicular Stomatitis virus, Toscana virus, Sand fly Fever virus etc.[3-7] Chandipura virus, (Vesiculovirus: Rhabdoviridae), first reported from India, is the most pathogenic sand fly-borne viral disease as far as case fatality rate (CFR) is concerned.[6,7] An explosive outbreak was reported in central India comprising parts of Maharashtra, Telengana and Gujarat, in 2003, which killed >300 children with CFR ranging from 55% to 78%.[6,8] The disease progression is so fast that the victims (children <15 years) succumb to infection within 24-48 hr of manifestation of symptoms.[7,8] This region has become endemic since 2003 and, recently, outbreaks and seroprevalence of Chandipura virus (CHPV) were reported from Odisha and Uttar Pradesh.[7-9] The only other sand fly-borne virus reported from India is the Sand fly Fever virus (SFV) that caused an outbreak of febrile illness in Aurangabad district of Maharashtra.[10] Investigations yielded isolation of Sicilian and Naples strains from humans and sand flies as well as detection of neutralizing antibodies in human sera.

Earlier studies conducted in Pune district have documented a rich fauna of sand flies.[11,12] Despite its proximity to Aurangabad district from where both CHPV and SFV strains were isolated, no activity of these viruses was ever reported from Pune.[6,7] In recent years, Pune witnessed tremendous infrastructural development, *i.e*., construction of wide roads, residential and commercial complexes etc. The present study was, therefore, designed to determine the species composition, abundance and distribution of sand flies in certain newly urbanized areas of Pune city.

MATERIAL & METHODS

The study area comprised 26 sites, all within 30 km of Pune city which included a few newly urbanized areas and fringe villages with almost similar climatic conditions. The newly urbanized areas have undergone vast developmental activities that included construction of multi-storeyed buildings, commercial buildings and wide roads at the expense of agricultural and grazing lands. On the

contrary, majority of the fringe villages still retained the rural culture and had typical old type houses with cattle sheds in close proximity. Though concrete housing complexes are scanty in these fringe areas, connectivity is good due to good roads and street lights.

RESULTS AND DISCUSSION

Weekly collections of adult sand flies were made from all the possible breeding/resting habitats using hand-held mouth aspirators. Indoor collections were made mainly from living rooms, store rooms, pump houses, bath rooms and toilets while outdoor collections were made by searching rodent burrows, tree holes, decaying vegetation, cow dung heaps and termite mounds etc. Collections were brought live to the laboratory, where sand flies were identified using keys provided by Lewis[13] and pooled according to species, gender, and locality. *Sergentomyia* spp., generally a peri-domestic breeder was found predominantly breeding/resting indoors (89.8%). Toilets and bathrooms contributed to 54.4% of the collection while living rooms, store rooms, abandoned houses, cattle-sheds, tree holes, rodent burrows etc. contributed for the rest (Fig 1). Cattle-sheds, generally considered the major breeding sites, were less productive (6.4%). Tree holes/buttresses contributed 2.2% while rodent burrows contributed to 1.6%. The collection time was recorded and per man hour density (PMHD) determined as described earlier.[11,12]

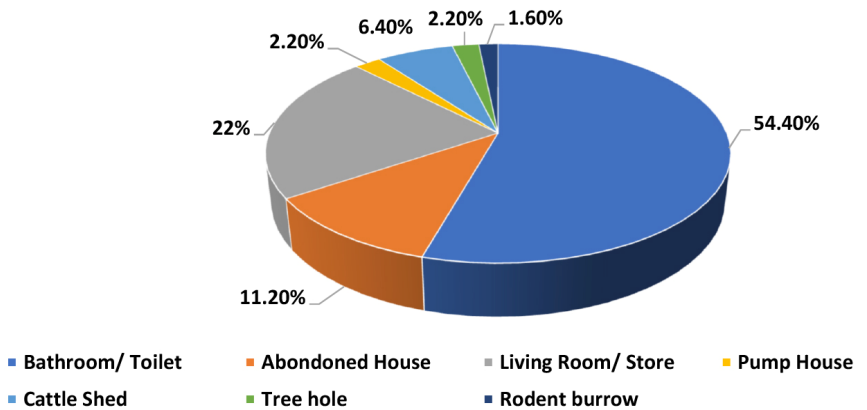

Fig. 1. Breeding/resting sites of sand flies in the study area.

A total of 5387 sand flies, belonging to four species under two genera, were collected with an average per man hour density (PMHD) of 31.6. Sand flies belonging to genus *Sergentomyia* were found predominant and were represented by three species, *viz., S. punjabensis* (39.25)*, S. babu* (30.4) and *S. bailyi* (27.15). Genus *Phlebotomus* was represented only by *P. argentipes* and constituted 3.2% of the total collection. They were mainly found breeding/resting in cattle-sheds. Head and genitalia of 10% of the collections were preserved in Hoyer's medium as voucher specimens.

As expected, comparatively very low prevalence of sand flies was seen in the newly established townships with developmental activities in comparison to fringe villages (Fig. 2). In the new townships, the PMHD varied from 1.2 to 10.7 with an exception of Aundh area where the PMHD remained high with 28.4. This was probably due to the inclusion of Aundh gaon within the Aundh township. Aundh gaon still retained the village culture and had a number of cattlesheds. In fringe villages, the PMHD varied from 9.4 to 45.6 (Rautwadi) and remained consistent throughout the study. The study revealed an inversely proportional relationship of sand fly abundance with infrastructural developmental activities. Absence of *P. papatasi* in the study area was conspicuous despite having mud walled houses in certain study sites.

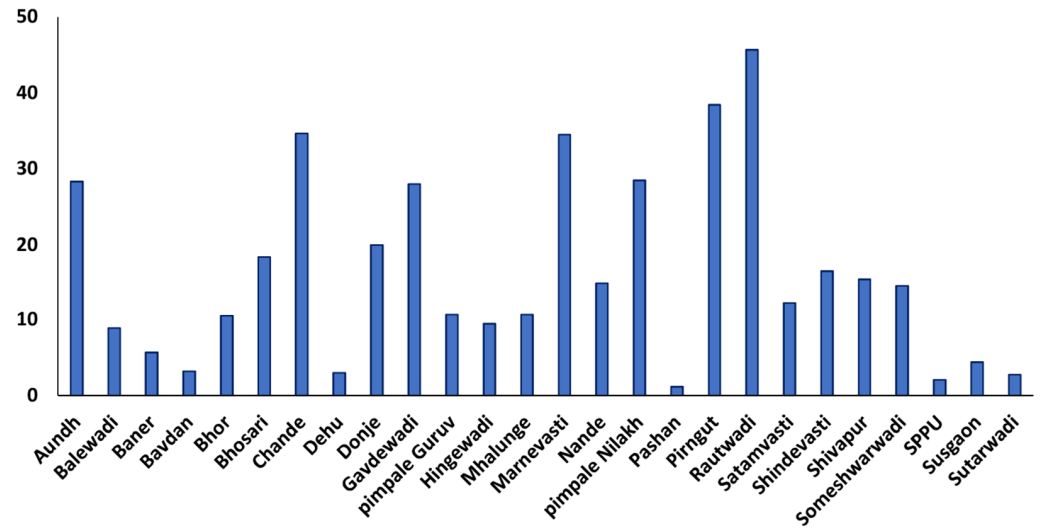

Fig. 2. Average PMHD of sand flies in different collection sites

All the sand flies were processed for virus isolation as described by Sudeep *et al.*[8] In brief, individual sand fly pools were homogenized in 2% minimum essential medium (Himedia, India) using a hand-held battery operated homogenizer (Sigma, USA), sterilized by filtration (Millipore, 0.22µm) and inoculated over confluent monolayers of Vero E6 cells. Inoculated cultures were incubated at 37 ^{o}C in a 5% CO_2 atmosphere and observed daily for cytopathic effects. Despite processing all the sand flies, no virus could be isolated. All the samples were passaged at least twice before declaring them as negative. In an earlier study, we have isolated Chandipura virus from a pool of two *Sergentomyia* sand flies using the cell line.[8] Inability to isolate virus(es) during the study could be due to the absence of active virus circulation within the ecological niche, but clearly more investigations are required in this field.

Though we could not conduct a year round study, the available data alluded to the prevalence of sand flies during late winter, summer, monsoon and post monsoon seasons (Fig. 3). Sand fly density was found very low in March and April 2018 but increased in May with the onset of pre-monsoon showers. Monsoon season was found productive (PMHD >36); however, post-monsoon period had the highest abundance as the PMHD increased to 40, 42.5 and 40, for September, October and November, respectively.

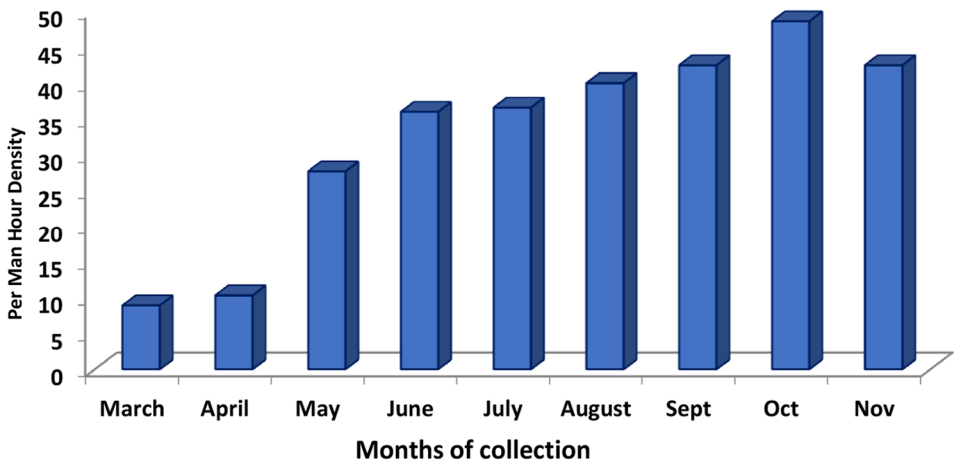

Fig. 3. Seasonal prevalence of sand flies during the study.

Sand flies belonging to genus *Phlebotomus* are endophilic, mainly breeding/resting in close proximity to human habitation while *Sergentomyia* spp., are peridomestic breeders.[1,14] Present study has revealed four species; the absence of *P. papatasi* and the drastic reduction of *P. argentipes* (3.2%) in the study area being noteworthy. Earlier studies in Pune district had recorded nine species of sand flies; three belonging to genus *Phlebotomus* and six to genus *Sergentomyia.*[11,12] *Phlebotomus argentipes* was the most abundant and highly prevalent species found in Pune and had a proportion of 58:42 with *P. papatasi*. It is difficult to point out the exact reason for the decline of *P. argentipes* and a complete absence of *P. papatasi* in the region but it can be safely surmised that a cumulative effect of urbanization, climate change and extensive application of insecticides/pesticides could be behind this. On the other hand, the present study highlighted a surge in the population of *Sergentomyia* sand flies and their shift towards human settlements as 89.8% of the *Sergentomyia* sand flies were collected from indoor resting areas (Fig. 1). Due to urbanization, most of their breeding habitats, *i.e*., tree holes, tree buttresses, rodent burrows etc. have been disturbed and this possibly has forced them to adapt to more safe breeding/resting habitats close to human habitats.

Not much attention was earlier given to *Sergentomyia* species as vectors of pathogens of public health importance due to their zoophilic nature. However, recent studies have shown human blood in their blood meals.[14] Detection of *Leishmania major* RNA in *S. minuta, S. sintoni* and *S. darlingi* sand flies, presence of *L. tropica* in *S. ingrami* and *S. hamoni* species in Ghana as well as isolation of *L. major* and *L. donovani* parasites from *S. garnhami* in Kenya and *S. babu* in India confirms their potential role as vectors.[14] In addition, detection/isolation of Chandipura virus, Toscana virus, Saboya virus, Sicilian-like virus, Tete virus and several unidentified viruses from different members of the genus recently, demonstrate their probable role in the maintenance/ transmission of these infections.[3-6]

In summary, the present work hints towards a natural selection occurring among sand fly species in response to environmental factors. Understanding this could lead to a better management of sand flies and sand fly-borne viruses. Conversely, an upsurge of *Sergentomyia* sand flies and a change in the exophillic and exophagic behaviour by drifting to breed/rest near human habitats warrants an in depth investigation as it will have epidemiological considerations and lead to an increased risk of sand fly-borne arbovirus transmission.

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Conflict of Interest: None.

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