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ECOLOGY, EPIDEMIOLOGY AND CONTROL OF SAND FLIES FROM KOLHAPUR REGION, INDIA

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ABSTRACT

Kolhapur region of Maharashtra, India provides very rich biodiversity due to high rainfall (700-6000 mm), temperature range 8° - 41°C, several types of water bodies and decaying organic matters of both plant and animal origin. The environment of Kolhapur thus, is very conducive for sand fly populations. The sand flies suck the blood of humans, domestic and wild animals and transmit diseases like Leishmaniasis (kala-azar), three day fever, yellow fever, oroya fever etc. in different parts of the world. Therefore, ecology and control measures of *Phlebotomus* sand flies (Diptera : Psychodidae) have been studied from Kolhapur, India. A total of 15 species of the genus *Phlebotomus* and *Sergentomyia* belonging to 9 sub genera have been reported. The seasonal abundance and life cycles in *P. (E.) argentipes, P. (E.) glaucus* and *P. (I.) tubifer* have been reported at 27±1°C, 70-80% R.H. and 12 hr photoperiod in the laboratory. They completed their life cycles from egg to adult within 25 days, 45 days and 52 days respectively. All above 3 species were very abundant in monsoon season than in winter and summer. *Sergentomyia (S.) smithi, S.(N.) chalarni, S.(N.) malbarica, S.(G.) poonaensis* and *P.(E.) glaucus* were mostly found in plains and in relatively less rainfall area. While, *P.(I.) tubifer, S. (G.) indica, S.(N.) dhandai, S.(N.) chakravarti, S.(N.) hodssoni* and *S.(N.) iyengari* were found mostly in forests and heavy rain areas. Hence, Kolhapur region is supposed to be a high risk area for sand fly borne diseases. Therefore, preventive and curative control measures are given in the text.

KEY WORDS: Sand flies, ecology, epidemiology, control, Kolhapur, India.

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INTRODUCTION

The sand flies (Diptera : Phlebotomidae) are small sized, brownish hairy and small moth like insects which suck the blood of humans, cats, dogs and other domestic animals and transmit diseases such as *Leishmaniasis* (Kala Azar), three day fever, oroya fever, yellow fever, etc. to different parts of the world. They are related to landscape epidemiology as vectors of viruses, bacteria and protozoa, and widely distributed in the tropics and other warm mainland areas including Europe, America, Australia, Asia and Africa. In India, Leishmaniasis was first occurred in 1869 in Assam and then in 1882 in Bihar. It has been also reported from Tamil Nadu, West Bengal, Gujarat, J. & K. and Himachal Pradesh[6]. More than 700 species of *Phlebotomids* have been reported from the world[14]. From India 78 species have been reported[27]. Review of literature indicates that Mitra and Roy[22], Fairchild [10], Abonnenc[1], Alder & Theodor[3], Theodor[29, 30], Barnett[5], Belova[4], Cahili[7], Disney[8], Hertig and Johnson[13], Lee *et al*.[16], Lewis[17,18,19,20]; Lewi *et al*[21], Sinton[28], Williams[32], Kaul *et al*.[15], etc. worked on biodiversity, ecology, epidemiology and control of sand flies from different parts of the world including India. Uncertain and changing environment may lead dissemination of Leishmaniasis and other sand fly borne diseases to virgin states of India. Therefore, survey, resurvey, ecology and ecofriendly control strategies of sand flies are essential components of disease management. Keeping in view all above facts, the present work was carried out.

MATERIALS AND METHODS

Ecological studies of sand flies have been carried out during the years 2011-2013 from Kolhapur district of Maharashtra, India including six tahasils Shirol, Kolhapur, Gargoti, Ajra, Radhanagari and Gaganbawda based on climatic and geographical conditions by collecting sand flies fortnightly. Kolhapur is situated between 15^{o} to 17^{o} North latitude and 73^{o} to 74^{o} East longitude acquiring 1, 46,575 hectares land and uneven rain from 700 mm to 6000 mm. Sand flies were collected with the help of insect net made up of muslin cloth by one man one hr search method from human dwellings/ houses / cattle sheds. The sand flies collected were time being kept in 70% alcohol and later, slides were prepared by adopting standard method of dehydration. Weekly count of sand flies was made from study spots till the disappearance of the flies. Spot observations of sand flies were also made on sitting behaviour on walls of houses, 5 x 5 sq.ft. area and their biting behaviour to humans and other vertebrate hosts. Mating and oviposition behaviours were also recorded by spot observations. For life cycle studies, 10 sand flies were confined in glass cage, 25 x 25 x 25 cm for mating and further oviposition on wet and dry soil layer of ½ inch of decaying organic matter, placed at bottom of the cage. Eggs were laid on decaying organic matter. Newly hatched larvae fed on decaying organic matter, within three instars larvae pupated in soil and then became adults. Within 25 days, *P. (E.) argentipes* developed from egg to adult. The biting behaviours were noted at day and night time by spot observations. Mating behaviour was observed at night with the help of red light since sand fly read red as black. However, mating was also studied at day time by spot observations.

RESULTS

Results recorded in tables 1 to 4 and figs 1 to 3 indicated that 15 species of the genera *Phlebotomus* and *Sergentomyia* belonging to seven subgenera namely *Euplebotomus*, *Neophlebotomus*, *Grassomyia, Idiophlebotomus, Sintonius Parrotamyia* and *Spelaeomyia* were prevalent in Kolhapur district. *P.(E.) argentipes* was found throughout the year Out of 15 species, *P.(E.) argentipes* and *S.(N.) iyengari* were prevalent in all study spots. *P.(I.) tubifer* and other two (unknown) species were abundant in the forest ecosystem and reported from Kolhapur, Radhanagari, Gaganbawda and Ajra while, *P.(E.) glaucus, S.(G.) poonaensis, S.(N.) chalarni, S. (S.) sirohi* and *S.(S.) smithi* were mostly found in plains of Kolhapur region. The abundance of *P.(E.) argentipes, P.(E.) glaucus* and *P.(I.) tubifer* during the year is recorded in table-2. The populations of sand

flies were decreased in heavy rain period. However, the species were more abundant during monsoon season than winter and summer season (Table-2). The diversity, abundance, distribution and life cycles of sand flies are recorded in tables 1 to 3 while, geographical and metrological features of study spots are given in table-4. Under laboratory conditions (27±1^{o}C, 70-80% R.H. and 12 hr photoperiod) *P. (E.) argentipes, P.(E.) glaucus* and *P.(I.) tubifer* completed their life cycles from egg to adult within 25 days, 45 days and 52 days respectively (Table-3). The field sex ratio (m:f) of sand flies was favoring females (1:3.24). Mating occurred at night from 7.00 p.m. to 12.00 p.m. Both, males and females were polygamous, mated frequently. Mating period ranged from 2-4 minutes (average 3.5). The sexes also mated at day time but rarely. Mated females oviposited in wet and dry soil containing decaying organic matter of plants. A single mated female laid 60-100 eggs. The females were more fecund in monsoon than summer. During day time *A. (E.) argentipes* were sitting on dump shady places and on the walls of bath rooms, latrines and houses. They were also noted under piles of dump bricks or stones. On the boundary walls of houses, sand flies were sitting in thousands in cracks and crevices both at night and day time at Panchgaon, Kolhapur. At night in human dwellings sand flies were found sitting and walking on mosquito net and crawling in beds for a bite. Sand flies preferred babies and children for biting and mostly ankles and the 1.5 ft leg area from foot side. The bites resulted red swelling and intense irritations. However, the intensity of bites of sand flies was less than mosquitoes. The consultancy to medical practitioner reported no Leishmaniasis case from Kolhapur region. However, greater diversity and very high population of sand flies suspected Kolhapur region of India as a high risk area for sand fly borne diseases. Clinical features of Kala-azar include general malaise, high fever, loss of weight, hepatosplenomegaly, anaemia, dark skin and extreme emaciation.

Table – 1

Diversity, occurrence and distribution of sand flies from Kolhapur region

S. No.	Species	Place					
		Kolhapur	Radhanagari	Gaganbawda	Gargoti	Ajra	Shirol
1.	*P. (Euphlebotomus) argentipes* (A. & B.)	+	+	+	+	+	+
2.	*P. (Euplebotomus) glaucus* (Mitra & Roy)	+	+	-	+	-	+
3.	*P. (Idiophlebotomus) tubifer* (Lewis & Lane)	+	+	+	-	+	-
4.	*S. (Grassomyia) indica* Theodor	+	+	+	+	+	-
5.	*S. (Grassomyia) poonaensis* Mitra & Mitra	+	-	-	+	-	+
6.	*S. (Neophlebotomus) dhandai* (Lewis)	+	+	+	+	+	
7.	*S. (Neophlebotomus) chakravarti* (Mitra)	+	+	+	+	+	-
8.	*S. (Neophlebotomus) hodgsoni* (Sinton)	+	+	+	-	+	-
9.	*S. (Neophlebotomus) iyengari* (Sinton)	+	+	+	+	+	+

S. No.	Species	Place					
		Kolhapur	Radhanagari	Gaganbawda	Gargoti	Ajra	Shirol
10.	*S.(Neophlebotomus) malbarica* (Annandale)	+	-	-	-	-	+
11.	*S. (Neophlebotomus) chalarni (*Young & Chalam)	+	-	-	+	-	+
12,	*S. (Sintonius) sirohi* Kaul, Dhanda & Modi	+	-	-	-	-	+
13.	*S. (Parrotamyia)* sp.1	+	+	-	+	+	-
14,	*S. (Parrotomyia*) sp. 2	+	+	-	+	+	-
15	*S. (Spelaeomyia) smithi* Mitra & Roy	+	-	-	+	-	+

Table - 2

Abundance of sand flies in Kolhapur

S.No.	Species	Occurrence (months)											
		June	July	Aug	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May
1.	*P.(E.) argentipes*	++	+++	+++	+++	+++	++	++	++	+	+	+	+
2.	*P. (E.) glaucus*	++	++	++	++	++	+	+	+	+	+	+	+
3.	*P.(I.) tubifer*	++	++	+++	++	++	+	+	+	+	+	+	+

Low +, Moderate ++, High +++.

Table – 3
Life cycle in sand flies

Sr. No.	Species	Egg. Hatching period (days)	Larval period (days)	Pupal Period (days)	Formation of adult period (days)
1	*P. (E.) argentipes*	5	14	6	25
2	*P. (E.) glaucus*	7	28	10	45
3	*P. (I.) tubifer*	7	35	10	52

Table – 4
Geographical and metrological features of Kolhapur region (Study spots)

Sr. No.	Place	Altitude (m)	Latitude (^{o}N)	Longitude (^{o}E)	Humidity (%)	Temp. range (^{o}C)	Rain fall (mm)
1	Kolhapur	545.6	16.42	74.16	65.87	8-41	1000-1100
2	Radhanagari	585.00	16.41	73.99	65-90	8-38	2500
3	Gaganbawda	900.00	16.545	73-88	65-94	8-37	5000-6000
4	Gargoti	559.00	16.31	74.14	60-85	9-39	1400-1500
5	Ajra	648.00	16.10	74.20	63-87	8-38	2000-2200
6	Shirol	555.00	16.73	74.60	65-84	10-40	750-800

. **Figure 1**
Sergentomyia sp

Figure 2
Sand flies on unplastered wall

Figure 3
Unplastered bathroom walls, habitat of sand flies

Figure 4
Cattle shed with buffalos, habitat of sand flies

Control strategies for Sand flies
Preventive control

i. Earthling of soil frequently for exposing egg, larvae and pupae of sand flies for natural mortality factors viz. biotic and abiotic.
ii. Disposal of decaying organic matter of plants associated with human dwellings and cattle sheds.
iii. Plastering of walls of human dwelling s and cattle sheds. Curative control:
i. Use of attractants and killing of flies.
ii. Use of repellents like DEET.
iii. Burning population of sand flies sitting on walls.
iv. Spraying breeding places i.e decaying organic matter with following insecticides, 0.03% DDVP/ Malathion/ Azadirachtin/ phosphamidon or Baygon 10- 20 %.

DISCUSSION

Lewis[19] studied the taxonomy, distribution, physiology, host preference and resting habits of sand flies. According to him over 530 species of Phlebotomids (sand flies) were prevalent from old and new world, probably more than half of above from the New world. He distributed sand flies under three genera namely *Phlebotomus* Rondani and *Sergentomyia* Franca & Parrot from old world and *Lutzomyia* Franca from New world. The

status of above genera greatly influenced the epidemiology of the disease Leishmaniasis. Taxonomy and classification of sand flies were studied by Abonnenc[1], Fairchild[10], Lewis[18,19,20], Theodor[29, 30], etc. According Seccombe[27] 78 species of Phlebotomine sand flies were prevalent in India. Out of which 15 species, were recorded from Kolhapur region of Maharashtra. *Phlebotomus papatashi* Seopoli and *P. (E.) argentipes* were very common throughout India. From Kolhapur district we found *P. argentipes* and *P. glaucus* very common in occurrence. *P. argentipes* was dominant over others and found throughout the year in Kolhapur region but its peak was in July and then its population steadily continued with more or less same status up to November. Sand flies were widely distributed in the tropics and other warm main land areas, and extended northwards to latitude in the region of 50^{o} N, such as 48^{o} in Soviet Union, Savignies near Beauvais in France, Jersey in the English Channel and Kamloops in Canada[19]. He reported *Phlebotomus* as the dominant genus in the south of the northern temperate part of the old world while, *Sergentomyia* and *Lutzomyia* in the tropics of the old and new worlds respectively. In USA, there were few species of sand flies but, none of them were medical importance[19]. Kolhapur is situated at 15-17^{o} North latitude and 73-74^{o} East longitude with uneven rainfall 700 mm to 6000 mm wherein 15 species of sand flies have been reported. Out of which *P. argentipes* was very common. A one year long study (March 1979-March 1980) was carried out by Feliciangeli[11] at San Esteban, an endemic focus of cutaneous Leishmaniasis in Northern Venezuela with the aim of observing the seasonal fluctuation of the local Phlebotomine sand fly species. The influence of climatic factors (temperature, relative humidity and rainfall) on population dynamics was analyzed in three collecting sites - a house, a peridomestic area and sylvatic region. Among anthropophilic species, *Lutzomyia panamensis* behaved as a wet season species, the mean minimum relative humidity was the critical factor influencing the total number of individuals. When the population density of this fly decreased, it was successfully replaced by *L. ovallesi*, a dry season species. On the other hand, seasonal variations of *L. gomezi* were more strongly affected by the temperature. Elnaiem[9] studied ecology and control of the sand fly as vector of *Leishmania donovani* in East Africa with special emphasis on *Phlebotomus orientalis*. *P. orientalis* Parrot and *P. martini* Parrot was the principal vectors of *L. donovani* in Sudan, Ethiopia and Kenya and *P. celiae* Minter was the secondary vector of the parasite in one focus in Ethiopia. Findings on sand fly fauna and other circumstantial evidence indicated that *P. martini* was also responsible for transmission of *L. donovani* in VL endemic foci of Somalia and Uganda. It has been observed that *P. orientalis* occupied distinct habitat characterized by black cotton soil and *Acacia seyal - Balanites aegyptiaca* vegetation, where as *P. martini* and *P. celiae* were associated with termite mounds. Ozbel *et al*.[24] performed an entomological survey to determine the distribution of probable vector species. They collected sand flies from the districts of dry, wet high land and wet coastal belt zones of Sri Lanka using CDC light traps, sticky traps and cattle - baited net traps during July, 2005 and resurveyed in February 2006. Over all, 2 species of the genus *Phlebotomus* and 8 species of *Sergentomyia* were collected. From Southern part of Sri Lanka higher numbers of Phlebotomus flies were collected probably due to different ecological aspects. *P. (E.) argentipes* was widely distributed in Sri Lanka but, *P. stantoni* was found only in 4 districts. As like Kolhapur, India, *P. (E.) argentipes* was vector of *L. donoveni* in Sri Lanka. According to Rotureau *et al*.[25] in French Guiana, at least 5 *Leishmania* species were known to be symptomatically transmitted in Sylvatic ecotypes. During 20 years period of recent survey many ecological changes have occurred. Sand fly collections were conducted with CDC light traps in five stations representing the main ecotypes of French Guaina. A total of 817 sand flies belonging to 2 genera 18 sub-genera and 46 different species were identified. The most common species in the region refer to *Lu. umbratilis, Lu. Infraspinosa, Lu ininii* and *Lu. flaviscutellato* with collection percentage 16.6%, 12.7%, 8.00% and 6.1% respectively. From South east Iran a total of 9925 sand flies representing 22 species were collected in hilly and mountainous area, while 1500 specimens

including 13 species were captured in plain region [14]. *Sergentomyia dentata, S. dreyfussi, S. arekovi, S. iranica, S. mervynae, S. palestinensis, P. eleanorae, P. halepensis, P. keshishiani* and *P. mesghali* were collected only in mountainous area, while *P. salehi* alone was found in plain area. *Phlebotomus alexandri* (98%), *P. bergeroti* (92.3%), *P. kazeruni* (99.4%), *P. papatasi* (65.2%), *P. sergenti* (97%), *S. africana* (83.3%), *S. baghdadis* (98.7%), *S. christophersi* (99.7%), *S. clydei* (62.5%), *S. hodgsoni* (99.7%), *S. mervynae* (98.7%) and *S. tiberiadis* (99.3%) were mainly distributed in the hilly and mountainous areas. Kaul *et al*.[15] reported 14 species of sand flies from arid and semi arid areas of Rajasthan, India. Out of which *P. papatasi, P. sergentii, S. clydei, S. baghdadis* and *S. christophersi* were also reported from Iran parts adjoining to India[14]. A complex disease Leishmaniasis caused by the protozoan *Leishmania* spp. has been reported from 60 countries of the world but, 90% of them occurred in India, Nepal, Bangladesh and Sudan[6]. In India the disease manifests in two forms. The cutaneous (dry and wet) and visceral (Kala Azar) variety. Leishmaniasis may be sporadic, endemic or epidemic. About 500 species of *Phlebotomus* have proven/ suspected vectors transmitting *Leishmania* spp. (protozoan parasite) from animal to animal, animal to man and man to man. At present, Kala-azar is a serious problem in Bihar, West Bengal and eastern Uttar Pradesh[6]. In 1996, Bihar and west Bengal had 33 and 10 affected districts respectively. The districts located north of river Ganges were affected by Kala- azar. West Bengal had two foci of Kala-azar in the out breaks of 1992. The districts affected were Malda and Murshidabad[2]. Lymphadenopathy was common presenting feature of Kala-azar in the Mediterranean region where the vector differed from the one in India. About 5% of Kala-azar patients in Iran developed Lymphadenopathy and recently, *Leishmania tropica* has been isolated from Lymph node during the outbreak. The reports of Lymphadenopathy as a measure presenting feature in India raised the possibility of new vector. Nandy et al. indicated wide spread co-existence of Malaria and Kala-azar in Bihar [23]. In India, the conditions favoring epidemic of Kala-Azar were from rural areas more than 600 meters above sea level, heavy annual rainfall, mean humidity above 70%, a temperature range of 15^{o}C to 38^{o}C with a diurnal variation of more than 7^{o}C, abundant vegetation, subsoil water and alluvial soil[6]. More or less the same situation was noticed in Kolhapur region and 15 species of sand flies were prevalent in the region. Out of which *P.(I.) tubifer* and two unknown species were abundant in the forest ecosystems from Kolhapur, Radhanagari, Gaganbawda and Ajara. *P. (E.) glaucus, S. (G.) poonaensis, S. (N.) chalarni, S. (S.) sirohi* and *S.(S.) smithi* were mostly found in plains of Kolhapur region. The prevalence of *P.(E.) argentipes* throughout the year in Kolhapur (Panchgaon) was very risk factor. The occurrence of *P. (I.) tubifer and P.(E.) glaucus* with highest population was also risk factor for Kolhapur region. However, 3 species were more abundant during monsoon months viz. from June to October. In India, Kala-azar was believed to occur in the endemic north east part of the country[26] and was not known to occur in Western part of Maharashtra except some immuno compromised or migrants from north-east. However, from Gondia Maharashtra, a visceral Leishmaniasis have been recently reported[31]. In addition, some isolated cases have also been reported from Mumbai city. Similarly, Gawade et al.[12] showed a case of HIV+VL association in a Nepali national working in Kolhapur, Maharashtra. The nearest state to Kolhapur suspected but unproven endemic area is Goa. All above situations in Maharashtra and prevalence of 15 species of sand flies including *P. (E) argentipes* in Kolhapur region suggest that the Kolhapur region is suspected risk area for Leishmaniasis and sand fly borne diseases. Therefore, control measures against Sand flies suggested in the text would worthwhile for disease management.

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