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Distribution and Population Dynamics of *Phlebotomus* Sandflies (Diptera:Psychodidae) in an Endemic Area of *Cutaneous leishmaniasis* in Asir Region, Southwestern Saudi Arabia

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Abstract: *Phlebotomus* sandflies (Diptera: Psychodidae) were surveyed, for two successive years (June, 1999 to May, 2001) in six *Cutaneous leishmaniasis* (CL) endemic provinces lying within 3 topographically different zones of Asir region, southwestern Saudi Arabia using sticky paper traps. A total of 1896 flies (1326 males and 570 females) belonging to six *Phlebotomus* species was collected. *Phlebotomus bergeroti* Parrot was the most abundant species regarding density and/or distribution (85.65% of the total *Phlebotomus* flies collected), followed by *P. sergenti* Parrot (10.97%) then *P. alexandri* Sinton (1.11%); *P. papatasi* Scopoli (0.95%); *P. orientalis* Parrot; (0.84%) and *P. arabicus.* (0.48%). Dominance of *P. bergeroti* in all surveyed sites and at different altitudes and the recorded increase in the number of leishmaniasis cases after few months of its beak of abundance may highlight its role as a probable vector of leishmaniasis in this area. The distribution of sandfly species encountered according to areas and altitudes and its relation to environmental conditions were investigated. The highest fly abundance (62.72%) was found at costal plains and foothills (0 to 800 meter above sea level), whereas 5.12 and 1.85% of flies were collected from Sarawat mountains (1600 to 3100 m) and Asir Plateau (1220 to 1750 m) above sea level, respectively. The highest fly abundance and the period of maximum risk of *Leishmania* transmission were recorded during the spring and summer seasons (period from March to September). The results obtained in the present study are important for the successful implementation of control programs against leishmaniasis.

Key words: *Phlebotomus* sand flies, insect ecology, *Cutaneous leishmaniasis*, Asir, Saudi Arabia

INTRODUCTION

Cutaneous leishmaniasis (CL) is an endemic disease in many areas of Saudi Arabia[1-3]. The disease is more common on the foothills and high plateau of Asir region in the south-west of the Kingdom[4]. The distribution of *Cutaneous leishmaniasis* (CL) is influenced by little-known geographical and climatic factors that determine the distribution of different sandfly vectors[5-8], parasites[1,2,4] and reservoirs[9,10].

The study of *Phlebotomus* sandfly of Asir area is important because this area has been a subject for various development and tourist projects as well as resettlement programs. Such activities seem to be closely associated with disease epidemics[11]. The *Phlebotomus* sandfly of Asir region has been poorly studied in comparison with other areas of the Kingdom, though CL has long been recognized as an important public health problem in the area[4]. A survey of *Phlebotominae* sandfly of Saudi Arabia including Asir region was carried out between 1975 and 1981[9]. Further research were made on sandfly ecology, taxonomy and leishmaniasis of Saudi Arabia including Asir region[12-14]. Intensive research programs are still needed to clarify different aspects of this serious disease. Therefore, the aim of this study, besides updating knowledge of prevalent local *Phlebotominae* sand fly species, was to analyze aspects related to the distribution and seasonal fluctuation of CL vectors compared to CL cases recently reported in the region.

MATERIALS AND METHODS

The study area: Asir lies in the southwestern part of the Kingdom of Saudi Arabia between latitude 17:27-21:00 and longitude 41:23-44:33. Its population exceeds one and a half million, representing 10% of the kingdom population and one quarter of the Kingdom's total area. The region is a mountainous area and is divided into 3 distinct topographical zones based on geographical characteristics as follows:

Sarawat Asir: This is a narrow belt of mountain series that extends north-south along the coastal plains of the Red Sea, with an altitude of about 1600 and 3000 meters

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above sea level (asl). The climate is moderate with an average temperature of 17.7°C and an average relative humidity (RH) of 53.1%. The rainfall is throughout the year with an annual average of about 342 ml. These highlands are covered mainly by forest vegetation which mainly include *Rhanterium epapposum, Juniperus* trees, *Olea chrysophylia, Acacia* spp. and *Dodonea* trees.

Asir Plateau: This plateau lies on the eastern part of the mountain series of Sarawat Asir and their level of elevation decrease when moving towards the east away from the mountain series. Generally, it is a plain plateau with an elevation level range between 1220 and 1370 m asl in the far east and between 1600 and 1750 m asl at the west near the mountain range. The climate is moderate with an average temperature of 19.5°C and 44.4 %RH. The average annual rainfall is about 250 ml. The temperature rises when moving towards the eastern direction away from the mountains and this is accompanied with a decrease in rainfall. The plateau is crossed by several Wadis originating from the high mountains in the west. The vegetation cover and grass in the plateau is rather poor but they become fairly dense at the edge of the mountains. The main types of plant include *Achillea* sp. and *Anthemis tigrensis*.

Tihamat Asir (Tihama lowlands): This is a local name to describe the coastal plains that extend from north to south along the Red Sea and the Sarawat mountain series. Many mountains are scattered over these plains, particularly at the foothills of Sarawat mountains. The altitude along the coastal plains ranges between sea level up to 200 m asl, whereas at the foothills in the east it ranges well between 300 and 800 m asl. Tihama plains are crossed by many Wadis as a result of floods from heavy rains on Sarawat mountains series. The eastern part of Tihama plains along the mountains is characterized by semi-arid semi-humid mountain climate; whereas the western part along the Red Sea, lies within the humid hot belt of the coastal desert. But generally, these lowlands are characterized by high temperature (the average, maximum and minimum temperature recorded were 30.3, 39.4 and 20°C, respectively) and high relative humidity which might reach up to 90% in winter season at the coast. The average annual rainfall is about 98 ml. A Fairly lush growth of *Acacia* spp. in the plains and on the bottom of hill sides is seen. In wadis, vegetation is dense consisting of *Acacia* trees, *Ziziphus spina-christe* and Dom trees.

Methodology: Sandflies were collected by using sticky traps. White printing papers (21x29.7 cm) painted with castor oil were pasted to thin wooden rectangular frame with handle. At each collection site, thirty sticky traps were set randomly in intra-domiciliary and extra-domiciliary settings close to wall cracks and crevices in front of rodents' burrows as well as animal housings. Traps were placed at the site of collection before sunset (18:00 h) and collected the following morning before sunrise (06:00 h). Flies were removed from the sticky papers and placed in 70% ethanol and then transferred to the laboratory for further processing. Preserved sandflies were examined and samples belonging to the genus *Phlebotomus* were separated, cleared in chloral hydrate and mounted in Puri's medium for identification. The identification of sandfly species were based on the criteria mentioned by Lewis and Buttiker[9], Kirk and Lewis[15]. Data were statistically analyzed using the SPSS computer program.

RESULTS

The species composition, relative abundance, sex ratio as well as the distribution of various species of *Phlebotomus* sandflies in 6 health sectors of Asir region, were reported case of CL throughout the period from June, 1999 to May 2001, are present in Table 1 and 2. Six *Phlebotomus* species were identified throughout the study period, namely: *Phlebotomus bergeroti* Parrot; *P. sergenti* Parrot; *P. alexandri* Sinton; *P. papatasi* (Scopoli) , *P. orientalis* Parrot and *P. arabicus* Theodor. *P. bergeroti* was the most abundant species regarding density and/or distribution. This species was encountered in all surveyed sites and represented 85.65% of the *Phlebotomus* sandflies collected. *P. sergenti* was second in abundance but much lower than *P. bergeroti* (10.97%). The species was captured from most sampling sites. As for *P. papatasi* the collection was extremely low and was only collected from Abha and Mohayel sectors. *P. orientalis* and *P. alexandri* revealed also very low density. Only 6 males of *P. arabicus* were captured during the whole period of study from Abha Sector . The results also show that 1230 males and 547 females were collected throughout the study period (Table 1).

Table 1: Relative abundance and species composition of *Phlebotomus* sandflies collected from different areas of Asir, Saudi Arabia, throughout the period from June, 1999 to May, 2001

	Number of flies collected/Visit			
Species	♂	♀	Total	Percentage
P. bergeroti	1081	543	1624	85.65
P. sergenti	90	25	115	10.97
P. alexandri	20	1	21	1.11
P. papatasi	16	3	19	0.95
P. orientalis	14	2	16	0.84
P. arabicus	9	0	9	0.48
Total	1230	574	1804	100

Table 2: Geographical distribution of *Phlebotomus* sandflies collected from various localities of Asir Region, Saudi Arabia, throughout the period from June, 1999 to May, 2001

		Number of flies collected locality													
		P. bergeroti		*P. sergenti*		*P. orientalis*		*P. papatasi*		*P. alexanderi*		*P. arabicus*		Total	
Locality	Type of collection	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	No.	%
Abha	Fixed	143	78	27	6	5	1	14	3	6	0	6	0	289	
	Non-fixed	12	3	1	0	7	1	1	0	0	0	1	0	26	
	Total	155	81	28	6	12	2	15	3	6	0	7	0	315	17.46
Al-majarda	Fixed	274	146	21	4	1	0	0	0	0	0	0	0	446	
	Non-fixed	54	30	2	4	1	0	0	0	1	0	0	0	92	
	Total	328	176	23	8	2	0	0	0	1	0	0	0	538	29.82
Mohayel	Fixed	306	180	21	7	0	0	1	0	6	0	0	0	521	
	Non-fixed	135	69	0	0	0	0	0	0	0	0	0	0	204	
	Total	441	249	21	7	0	0	1	0	6	0	0	0	725	40.19
Rijal Alma`a	Fixed	142	32	3	0	0	0	0	0	0	0	0	0	177	
	Non-fixed	13	4	1	1	0	0	0	0	0	0	0	0	19	
	Total	155	36	4	1	0	0	0	0	0	0	0	0	196	10.86
Tannoma	Fixed	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Non-fixed	0	0	0	0	1	0	0	0	0	0	0	0	1	
	Total	0	0	0	0	1	0	0	0	0	0	0	0	1	0.06
Sabt Alalaya	Fixed	2	1	14	2	0	0	0	0	7	1	2	0	29	
	Non-fixed	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Total	2	1	14	2	0	0	0	0	7	1	2	0	29	1.61
Total		1081	543	90	24	15	2	16	3	20	1	9	0	1804	

Table 3: Attitudinal distribution of *Phlebotomus* sandflies in Asir region, Saudi Arabia throughout the period from June, 1999 to May, 2001

			Number of flies collected/visit (30 traps)							
Attitudinal zone	Area of collection	Altitude (meters)	*P. bergeroti*	*P. sergenti*	*P. orientalis*	*P. papatasi*	*P. alexanderi*	*P. arabicus*	Total	%
Coastal plains	Habeil and Marabah	0-350	338	9	1	8	4	1	361	30.31
Foothills	Majarda and Mohayel	500-800	688	55	1	0	3	0	747	62.72
Assir Plateaux	Al-Faraein	1220-1750	9	3	4	3	1	2	22	1.85
Sarawat Mountain	Abel-Balahmar	1600-3100	35	10	10	2	1	3	61	5.12

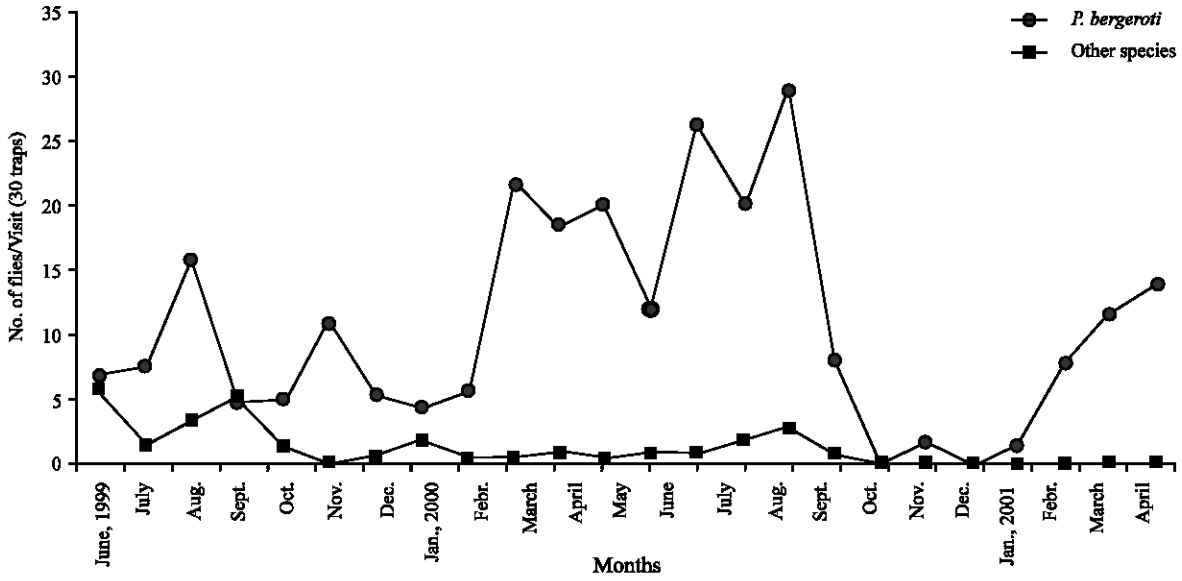

Fig. 1: Monthly abundance of *Phlebotomus* sandflies in Asir region, Saudi Arabia, throughout the period from June, 1999 to May, 2001

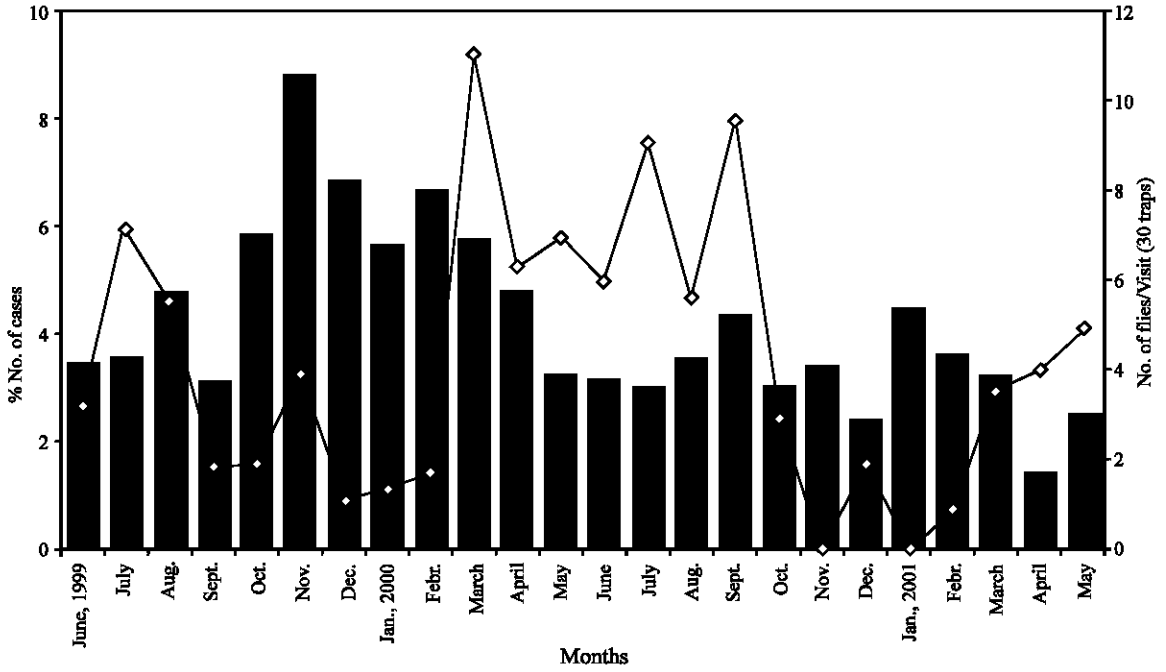

Fig. 2: Distribution of *Cutaneous leishmaniasis* in relation to *P. bergeroti* density in Asir region, Saudi Arabia throughout the period from June, 1999 to May, 2001

A high significant difference ($p>0.005$) was found between the abundance of *Phlebotomus* sandflies at low and high altitudes (Table 3). The highest fly abundance was found in low land of Tihama foothills and the costal plains (62.72 and 30.31% of the total flies collected, respectively). Low sandfly densities were observed at higher altitudes of Sarawat mountains and Asir plateau (5.12 and 1.85% of the total flies collected). The results also show that the composition of the *Phlebotomus* sandfly species is not affected by altitude.

The highest abundance of *Phlebotomus* sandflies (Fig. 1) was recorded during the spring and summer seasons (period from March to September) when the average atmospheric temperature and RH ranged between 24 to 34°C and 30 to 45%, respectively. The lowest fly abundance was observed throughout the period from November to February. The statistical analysis of data shows a significantly positive correlation ($p>0.05$) between fly density and temperature at the foothills, where the maximum number of flies was recorded ($r = 0.401$ and 0.478), during the 1st and 2nd years of study, respectively. A significant ($p>0.05$) negative correlation was found between the density of flies and relative humidity ($r = -0.536$ and- 0.734) during the 1st and 2nd years of study, respectively. In general, *P. bergeroti* showed higher density during the months of relatively high temperature and low relative humidity which seemed to be the most important factors governing sandfly abundance. The present data also suggest that the seasonal density of sandflies can be related to the amount and the distribution pattern of rainfall.

Cases of CL in Asir region were reported throughout the whole year (Fig. 2). More cases were reported in the 1st year of study. The high abundance of *P. bergeroti* during the months of relatively high temperature and low relative humidity (March to September), was followed by high numbers of CL cases in winter months, when the density of sandflies were minimum.

DISCUSSION

Dominance of one or two species of sandflies is a feature of many places in Saudi Arabia such as Houfuf, Al Khubar, Al Ula, Wadi Sheib and Wadi Khumra[12], Riyadh[21], Gassim[6] and Hail[7]. Dominance of *P. bergeroti* and *P. sergenti* in our collection could be attributed to high altitude and/or vegetation, topographic, climatic conditions in the area.

Most investigators reported *P. papatasi* as a peridomestic and dominant species in Saudi Arabia and in many other parts of the old world[7,12,14,16-20]. In our collection, *P. bergeroti* was the dominant species (85.65% of *Phlebotomus* flies collected), whereas *P. papatasi* was a scarce species (only 0.95%). These findings confirms the marked affinity of sandfly fauna of Asir and southern Sinai, Egypt, where,

P. bergeroti and *P. sergenti* constituted the highest percentage of sandflies collected, whereas *P. papatasi* was a scarce species[5].

The present results show that 1230 males and 547 females were collected throughout the study period. This male biased sex ratio was also observed in Asir[9,14], Jordan valley[22] and Sinai, Egypt[23]. This biased ratio may be due to an effect of larval environment or larval competition or due to the method of collection. A male biased ratio was observed in most *Paraphlebotomus* species collected using sticky traps in Saudi Arabia[9].

Our results show that the altitude has an apparent effect on sandfly density. The highest fly abundance was found in low land of Tihama foothills and the costal plains, whereas low fly densities were observed at higher altitudes of Sarawat mountains and Asir plateau. This may indicate that the foothills of Tihama is the most favorable site for the breeding and activity of *Phlebotomus* sandflies followed by the costal plains. Lower fly abundance at higher altitudes, may be due to low temperature, fog and strong winds. Present results confirmed what have been found by earlier investigators in Asir region[14].

Although a high significant difference was found between the abundance of *Phlebotomus* sandflies at low and high altitudes, it is of interest to note that the composition of the *Phlebotomus* sandfly species is not affected by altitude. These results contradict what have been found by other investigators who found that the species spectrum shows a tendency for more species to occur at higher altitudes than in the Tihama districts[14]. Again our findings disagree with what have been found by the Buttiker *et al.*[14] as they stated that it is evident that *P. papatasi* and *P. bergeroti* prefer lower altitudes whereas, *P. orientalis* and *P. arabicus* are species of higher altitudes. Collection of most species from all altitudes also disagrees with what have been reported in Sinai, Egypt, where a remarkable difference in sandfly species composition was observed at different altitudes[5].

Sandfly population dynamics are largely regulated by the meteorological conditions, geomorphology, altitude and vegetation[5,7,13,14,23-28]. Knowing the monthly activity of sandflies is of great value in determining the period of maximum risk of *Leishmania* transmission and for the successful implementation of control programs. The present results indicate that the highest abundance of sandflies were recorded during the spring and summer seasons, whereas the lowest fly abundance was observed throughout the period from November to February. A similar pattern of abundance was observed in an earlier study in Asir region[14], in Hail[7], Saudi Arabia and in Sinai, Egypt[23]. On the other hand, present findings mostly disagree with the findings of other researchers who confined the abundance of *P. bergeroti* to sites with lower temperature (25 and 27°C) and higher RH (40 to 60%) in southern Sinai, Egypt[26]. In general, *P. bergeroti* showed higher density during the months of relatively high temperature and low relative humidity which seemed to be the most important factors governing sandfly abundance. The present data also suggest that the seasonal sandfly density can be related to the amount and distribution pattern of rain fall. In the foothills and costal plains dry season's rains were invariable followed by a rapid and temporary increase in fly density. This correlation is not clear in Asir plateau or Sarawat mountains where rains are heavy and fall mostly throughout the whole year. The greatest decrease in density or disappearance of sandflies during the period from November to February is probably due to low temperature, high RH and strong wind. Similar findings were also recorded in Asir and Hail, Saudi Arabia [7,14] and in Sinai, Egypt[23]. The recorded pattern of sandfly abundance in Asir area is probably affected by the intensive use of insecticides inside and outside the houses for the control of sandflies and other insects.

The significance of some sandfly species which occur in Saudi Arabia and are or may be vectors of CL, was discussed by Büttiker *et al.*[14]. The study has enlarged or changed the known distribution of several species which may be associated with leishmaniasis in the study area. *P. papatasi* was proved as a vector or suggested as a possible vector of CL in certain urban areas of many countries as Yemen, SSR, Iraq, Egypt and Saudi Arabia[12,17,9,29]. This species was assumed as the vector of dermal leishmaniasis in the Tihama districts of Asir region[14]. Present study shows that *P. papatasi* is a rare species and is not concentrated near towns. This may indicate that this species has little or no role in *Leishmania* transmission in Asir region. This assumption agrees with what has been found in Kuwait where *P. papatasi* did not appear as an urban species and was not regarded as a possible vector of leishmaniasis in that country[30]. *P. sergenti* which was collected from all sampling sites and at all altitudes is a proven vector of CL in the area[31] and in other parts of the world[5,11,14,23]. *P. bergeroti* previously seemed a rare species in Saudi Arabia[30]. In an earlier study, this species represented from 8 to 32% of the total sandflies collected and was assumed as the secondary vector of leishmaniasis on the Asir mountain plateau[14]. In this study *P. bergeroti* numbered 85.65% of the total sandflies collected. Dominance of this species in all surveyed sites and at different altitudes and the recorded increase in the number of leishmaniasis cases after few months of its

beak of abundance may highlight its role as a probable vector of leishmaniasis in this area. *P. bergeroti* is a suspected vector of CL in some African countries as Chad, Djibouti and Mauritania as well as in Yemen Republic[11,30]. Other researchers reported *P. bergeroti* as a probable vector of *L. tropica* to man in restricted areas as Makka Al Mokarama[30]. However, till now none of the researchers in the field of leishmaniasis proved this suspicion. So, intensive research programs are still needed to clarify the possible role of this highly abundant species in *Leishmania* transmission in southwestern provinces of Saudi Arabia, where this disease is widely spread.

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