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Sand Flies Distribution and Bionomics, in Al-Madinah Al-Munawarah

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Abstract: An entomological survey was carried out in four provinces representing the surroundings of Al-Madinah Al-Munawarah, Western Saudi Arabia from May 2007 to April 2008, to determine diversity, seasonal variations and bionomics of sand flies, for better planning of their control strategies and prevention of leishmaniasis. Sand flies were collected using CDC light traps and sticky traps. A total of 4411 sand flies were collected. Six species belonging to two genera were identified; *P. papatasi*, *P. sergenti*, *P. bergeroti*, *S. antennata*, *S. sergenti* and *S. clydei*. *P. papatasi* was the predominant anthropophagic species (>80%). Sand flies were abundant intra-domiciliary (>75%) (mainly *Phlebotomus*) than extra-domiciliary with highly significance (mainly *Sergentomyia*), intra-domiciliary abundance was inflated by a greater proportion of female flies. Of the 845 dissected *Phlebotomus* females, *Leishmania* infection rate was ranged from 0.3 to 1.5%, mainly June and sometimes May and October which give spotlight on infectivity of sand flies and planning of control strategies, 362 (42.8%) were gravid, 417 (49.3%) were blood fed; Regarding parity and fecundity, the striking finding was that none of the dissected females were parous in January, it shed light on the mating season habits of sand flies in Almadinah region and to design novel control strategies.

Key words: Al-Madinah Al-Munawarah, sand fly, *Phlebotomus*, *Sergentomyia*

INTRODUCTION

Sand flies (Diptera: Psychodidae: Phlebotominae) are of considerable public health importance in the tropics and subtropics. Species of the old world genus *Phlebotomus* or American genus *Lutzomyia* are vectors of vertebrate pathogens, including zoonotic *Leishmania* sp. that affect people in more than 80 countries. There are an estimated 12 million cases of leishmaniasis worldwide, 2 million new cases occur each year and 350 million people are at risk. The disease can present itself in four different forms in humans, all with devastating consequences: cutaneous, diffuse cutaneous, mucocutaneous and visceral. Visceral leishmaniasis is fatal if left untreated and the cutaneous forms are often disfiguring and mutilating. Cutaneous Leishmaniasis (CL) is endemic in some countries in the Middle East, on the Mediterranean coast and in Central Asia (Desjeux, 2004).

Sand flies could carry and transmit other zoonoses such as the bacterium *Bartonella bacilliformis* (Birtles, 2001) and several arboviruses, including phleboviruses (Tesh, 1988) those causing sand fly fever and certain flaviviruses, orbiviruses and vesiculoviruses those causing vesicular stomatitis (Ashford, 2001). In addition, sand fly bites may cause allergic reactions and substantial irritation in sensitive people.

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A micro-geographical study was done a year before by the authors in one of Al-Madinah Provinces (El-Badry *et al*., 2008), its results was the impetus to study sand flies populations involving a more wider geographical areas including a panel of four different areas representing the surrounding of Al-Madinah Al-Munawarah, Western Saudi Arabia, considered to have a prevalent cases of leishmaniasis according to the Ministry of Health annual reports, to clearly indicates diversity of sand flies populations and fully document their seasonal variations and bionomics in Al-Madinah geographical region of the kingdom. This is of importance in determining the period of maximum risk of *Leishmania* transmission, thus avoid and prevent sand flies biting and successfully plan and implement control programs.

MATERIALS AND METHODS

This study was carried out to determine sand flies distribution and bionomics through 12 months duration, from May 2007 to April 2008.

Study Area

The entomologic survey was carried out in region covered by Al-Madinah, including four different surrounding housing areas; Mondasa, Agool, Abyar Almashy and Almaliliah, considered to have a relatively high prevalence of leishmaniasis according to the Ministry of Health annual reports, since 129 and 163 cases of cutaneous leishmaniasis (most of them were *Leishmania major*) were discovered in northern part of Al-Madinah Al-Munawarh including Al-Agool in year 2005 and 2006, respectively (unpublished data of Ministry of Health, Al-Madinah Al-Munawarh) and the parasite identification was not fully studied. The chosen sites situated at the margins of Al-Madinah (Fig. 1), at 24°46' N, 39°61' E and about 631 m above sea level.

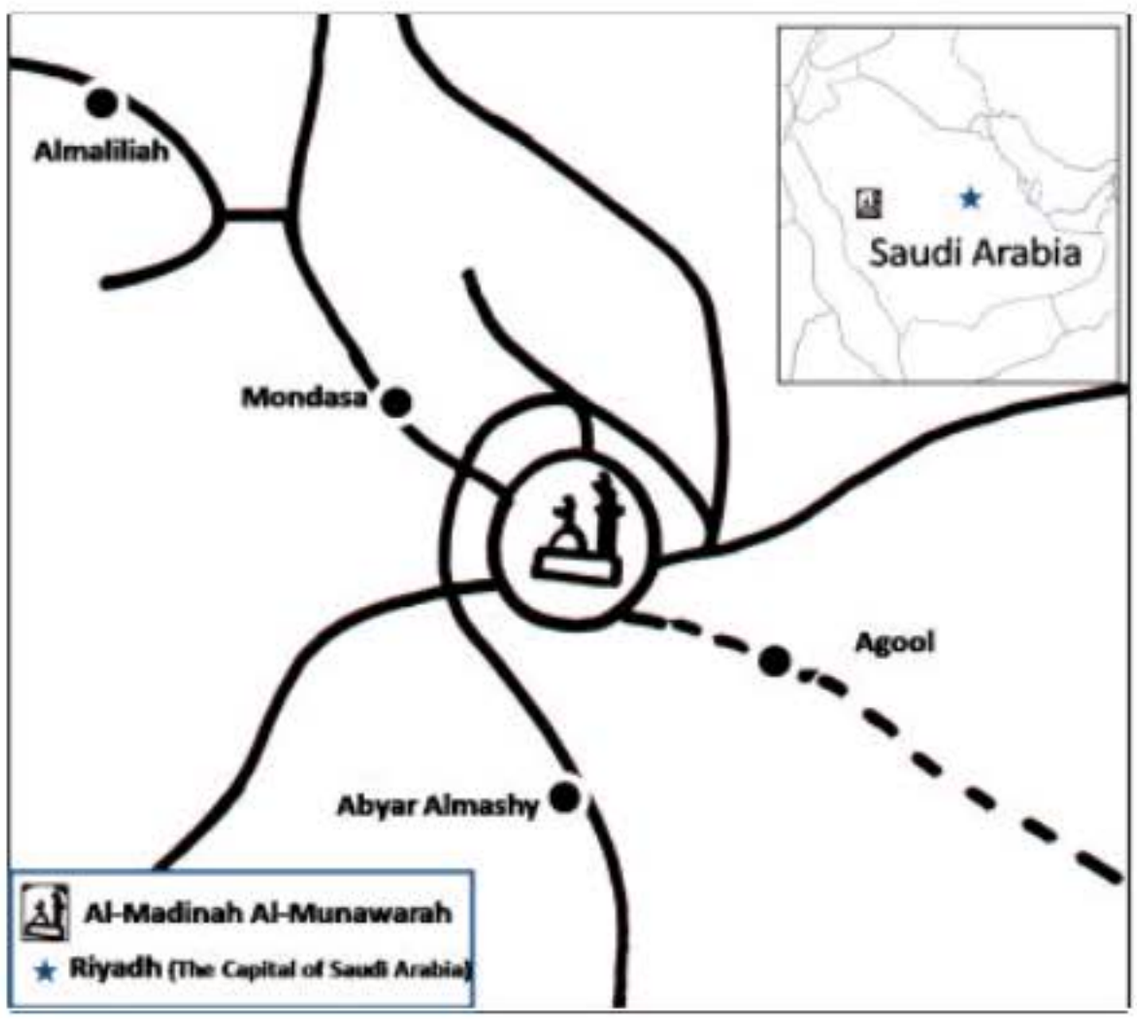

Fig. 1: Al-Madinah Al-Munawarah map showing study areas

They are semi-cultivated desert areas with farms containing sheep and poultry breeding houses, irrigated by underground water, in addition houses of the villages which has created a rich habitat supporting endemic animals, including desert rodents and sand flies populations. The desert surround the villages has little caves inhabited by the desert rodent (e.g., *Psammomys obesus*) the main reservoir of cutaneous leishmaniasis. Average temperature is high during the year (August mean 38°C) and winter generally moderate (January means 25°C). Sand flies were collected from four representative sites comprising twelve stations.

Sand Flies Collection

Sand flies were collected from the study area (twelve stations) using twelve CDC (Centers for Disease Control) miniature light traps (John W. Hock Co., Gainesville, FL, USA) with small-mesh collecting bags and one hundred Sticky traps for each station at each night for two consecutive nights each month during the period May 2007 to April 2008 (a total of forty-eight nights). Equal number of traps distributed evenly allover the intra-domiciliary and extra-domiciliary stations of the study sites close to wall cracks, crevices, in front of animal burrows, at the bottom of large trees or dense bushes, preferably near reptile and mammals holes as well as sheep and poultry housings. Centers for Disease Control light traps were placed at 50-70 cm above ground level. Papers of sticky traps were of standard A4 page size (21×29.7 cm), coated with castor oil and held upright on wooden dowels to permit ground clearance and rotation with wind direction, they were fixed at a height of 30 cm above ground level. Traps were set one hour before the sunset and collected before/at the sunrise of the next morning. Individual code names, consisting of a letter(s) taken from the collection area followed by a number were assigned for collection site and station then day and month of collection.

Sand Flies Processing and Identification

Flies collected by sticky traps were removed with a needle, rinsed in water then placed in 70% ethanol to be transferred to the laboratory for further processing. At laboratory; sand flies were dissected on sterilized microscope slide and mounted in Berlese's medium (Gun Charcoal Mountant-Asco, Manchester, UK) with the head ventral-side up and the reminder of the specimen placed laterally under a single cover slip (Sawalha *et al*., 2003). Flies captured in light traps were brought to the laboratory in special cages. At laboratory; flies anesthetized in the freezer for 5 min, placed in sieve, washed and rinsed in distilled water then sand flies were separated and sorted by sex under a dissecting microscope. Immediately after segregation, sand flies were dissected on sterilized microscope slides, in sterile physiological saline, their head and genitalia were mounted in Berlese's medium for species identification (Mutinga and Odhiambo, 1986). The female *Phlebotomus* species were further dissected to determine reproductive status as either parous or nulliparous (based on the presence of follicular relics in the ovaries) (Magnarelli *et al*., 1984), their abdominal and thoracic gut were examined for flagellates then the mid-gut contents were cultivated according to Van Zandbergen *et al*. (2002) at 26°C for 8 days in RPMI (Roswell Park Memorial Institute) 1640 medium (PAA Laboratories, Germany) supplemented with L-Glutamine and 5% FCS (PAA Laboratories, Germany) in a humidified atmosphere containing 5% CO_2 and examined microscopically for 2 weeks for promastigotes.

Taxonomic identification of cleared, slide-mounted sand flies was based on external and internal morphology using several taxonomic keys (Kirk and Lewis, 1951; Lewis, 1982; Lewis and Büttiker, 1980; Büttiker and Lewis, 1983; Lane *et al*., 1988; Young and Duncan, 1994), taking into consideration the following morphological characters: size and color of insect, measures of palps, antennal ascoids, setae and spines of the male reproductive apparatus and leg.

Statistical Analysis

All the results were tabulated and presented by figures and histograms, descriptive statistics were performed using Statistical Package for the Social Sciences (SPSS for Windows Version 13).

RESULTS

All monitored sites (Mondasa, Agool, Abyar Almashy and Almaliliah) were positive for sand flies. A total of 4411 (100%) sand flies specimens were collected, Abyar Almashy yielded the highest number of sand flies; 1760 (37.9%), followed by Agool, 1501 (34%), then Mondasa, 757 (17.2%). Almaliliah yielded the smallest number of collected sand flies, 483 (10.9%) (Table 1-4, Fig. 2).

Species Composition

Six species, were identified and presented in each surveyed region, three of which belonging to the *Phlebotomus* genus and three to the *Sergentomyia* genus. *Phlebotomus papatasi* (Scopoli) was the most abundant species (3612/4411 = 81.9 %), followed by *P. sergenti* (Parrot) (213/4411 = 4.8 %); *S. clydei* (Sinton) (189/4411 = 4.3 %); *S. antennata* (Newstead) (150/4411 = 3.4%) then *S. serg*enti (*theodori*, Parrot) (104/4411 = 2.4 %); *P. bergeroti* (Parrot) (17/4411 = 0.4%) (Table 1-4, Fig. 2-4).

Seasonal Variation

Monthly activity of collected sand flies showed a pattern of seasonal variation in all monitored sites and within each detected sand flies species (Table 1-4, Fig. 2-4). They peak at June in Abyar

Table 1: Sand flies population densities and seasonal variation at Mondasa, Madinah, Saudi Arabia (May 2007 to April 2008)

	P. papatasi		*P. sergenti*		*P. bergeroti*		*S. sergenti*		*S. antennata*		*S. clydei*		Total		
Months	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	Total
May 07	83	167	12	14	0	0	6	13	1	6	0	5	103	205	308
Jun. 07	11	35	21	5	2	1	3	5	0	1	2	3	39	50	89
Jul. 07	19	15	6	3	0	1	2	1	0	0	3	1	30	21	51
Aug. 07	17	14	0	0	0	0	2	0	0	0	0	2	19	16	35
Sep. 07	21	7	0	0	0	0	2	0	0	0	6	2	29	9	38
Oct. 07	16	14	4	1	0	1	0	2	0	0	2	5	22	23	45
Nov. 07	16	10	0	0	0	0	0	0	0	0	0	0	16	10	26
Dec. 07	10	4	0	0	0	0	0	0	0	0	0	0	10	4	14
Jan. 08	3	4	0	0	0	0	0	0	0	0	0	0	3	4	7
Feb. 08	9	7	0	0	0	0	0	1	0	1	1	2	10	11	21
Mar. 08	26	18	0	0	1	0	1	0	0	0	5	2	33	20	53
Apr. 08	31	17	5	8	0	0	2	0	0	0	3	5	41	30	71
Total															
No. (%)	262.0 (34.6)	312.0 (41.2)	48.0 (6.3)	31.0 (4.1)	3.0 (0.4)	3.0 (0.4)	18.0 (2.4)	22.0 (2.9)	1.0 (0.1)	8.0 (1.1)	22.0 (2.9)	27.0 (3.6)	354.0 (46.8)	403.0 (53.2)	757 (100)

Table 2: Sand flies population densities and seasonal variation at Agool, Madinah, Saudi Arabia (May 2007 to April 2008)

	P. papatasi		*P. sergenti*		*P. bergeroti*		*S. sergenti*		*S. antennata*		*S. clydei*		Total		
Months	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	Total
May 07	6	23	0	0	0	0	0	0	6	1	0	0	12	24	36
Jun 07	100	32	9	15	0	0	0	0	4	1	6	3	119	51	170
Jul 07	19	32	5	2	0	0	3	1	3	3	1	2	31	40	71
Aug 07	85	28	3	0	0	0	0	2	58	22	3	0	149	52	201
Sep 07	125	42	23	9	0	0	0	1	13	2	18	5	179	59	238
Oct 07	127	39	14	12	0	0	0	0	5	0	1	0	147	51	198
Nov 07	89	134	0	0	0	0	0	0	0	0	9	5	98	139	237
Dec 07	132	41	5	12	0	0	13	8	0	4	6	14	156	79	235
Jan 08	18	15	5	8	0	0	0	0	1	0	1	2	25	25	50
Feb 08	1	5	2	0	0	0	4	1	1	2	0	0	8	8	16
Mar 08	3	5	2	0	0	0	2	0	3	2	0	0	10	7	17
Apr 08	4	21	0	2	0	0	0	0	2	3	0	0	6	26	32
Total															
No. (%)	709.0 (47.2)	417.0 (27.8)	68.0 (4.5)	60.0 (3.9)	0 (0)	0 (0)	22.0 (1.5)	13.0 (0.9)	96.0 (6.9)	40.0 (2.7)	45.0 (2.9)	31.0 (2.2)	940.0 (62.6)	561.0 (37.4)	1501 (100)

Table 3: Sand flies population densities and seasonal variation at Abyar Almashy, Madinah, Saudi Arabia (May 2007 to April 2008)

	P. papatasi		*P. sergenti*		*P. bergeroti*		*S. sergenti*		*S. antennata*		*S. clydei*		Total		
Months	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	Total
May 07	86	116	2	0	0	0	0	0	1	2	5	2	94	120	214
Jun 07	285	102	0	2	1	0	0	0	0	0	8	0	294	104	398
Jul 07	135	58	0	1	0	0	0	0	0	1	0	0	135	60	195
Aug 07	147	58	0	0	0	0	0	0	0	0	0	0	147	58	205
Sep 07	66	30	0	0	0	0	1	2	0	1	0	2	67	35	102
Oct 07	80	73	0	0	0	0	10	4	0	0	1	3	91	80	171
Nov 07	96	32	1	0	0	1	1	4	0	0	1	0	99	37	136
Dec 07	14	10	0	0	0	3	4	1	0	0	2	5	20	19	39
Jan 08	7	2	0	0	0	0	0	0	0	0	0	0	7	2	9
Feb 08	9	7	0	0	0	0	0	0	0	0	0	0	9	7	16
Mar 08	51	21	0	0	0	0	0	2	0	0	2	3	53	26	79
Apr 08	81	25	0	0	0	0	0	0	0	0	0	0	81	25	106
Total															
No. (%)	1057 (63.3)	534 (32)	3.0 (0.2)	3.0 (0.2)	1.0 (0.1)	4.0 (0.2)	16 (1)	13.0 (0.8)	1.0 (0.1)	4.0 (0.2)	19.0 (1.1)	15.0 (0.9)	1097.0 (65.7)	573.0 (34.3)	1670 (100)

Table 4: Sand flies population densities and seasonal variation at Almaliliah, Madinah, Saudi Arabia (May 2007 to April 2008)

	P. papatasi		*P. sergenti*		*P. bergeroti*		*S. sergenti*		*S. antennata*		*S. clydei*		Total		
Months	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	Total
May 07	29	25	0	2	21	1	0	0	2	3	0	1	52	32	84
Jun 07	42	44	0	3	13	3	0	0	0	2	11	13	66	65	131
Jul 07	20	14	4	5	6	0	0	0	7	1	3	8	40	28	68
Aug 07	16	9	0	0	3	2	0	0	2	4	0	3	21	18	39
Sep 07	11	6	1	0	2	1	0	1	0	0	4	1	18	9	27
Oct 07	26	2	0	5	0	2	0	0	2	0	2	2	30	11	41
Nov 07	14	9	0	0	0	0	0	0	1	1	0	1	15	11	26
Dec 07	2	0	0	1	0	0	0	0	0	0	0	0	2	1	3
Jan 08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Feb 08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mar 08	9	4	1	0	0	0	0	0	0	2	0	0	10	6	16
Apr 08	26	13	2	1	1	1	0	0	1	2	1	0	31	17	48
Total															
No. (%)	195.0 (40.4)	126.0 (26.1)	8.0 (1.6)	17.0 (3.5)	46.0 (9.5)	10.0 (2.1)	0 (0)	1.0 (0.2)	15.0 (3.1)	15.0 (3.1)	21.0 (4.3)	29 (6)	285 (59)	198 (41)	483 (100)

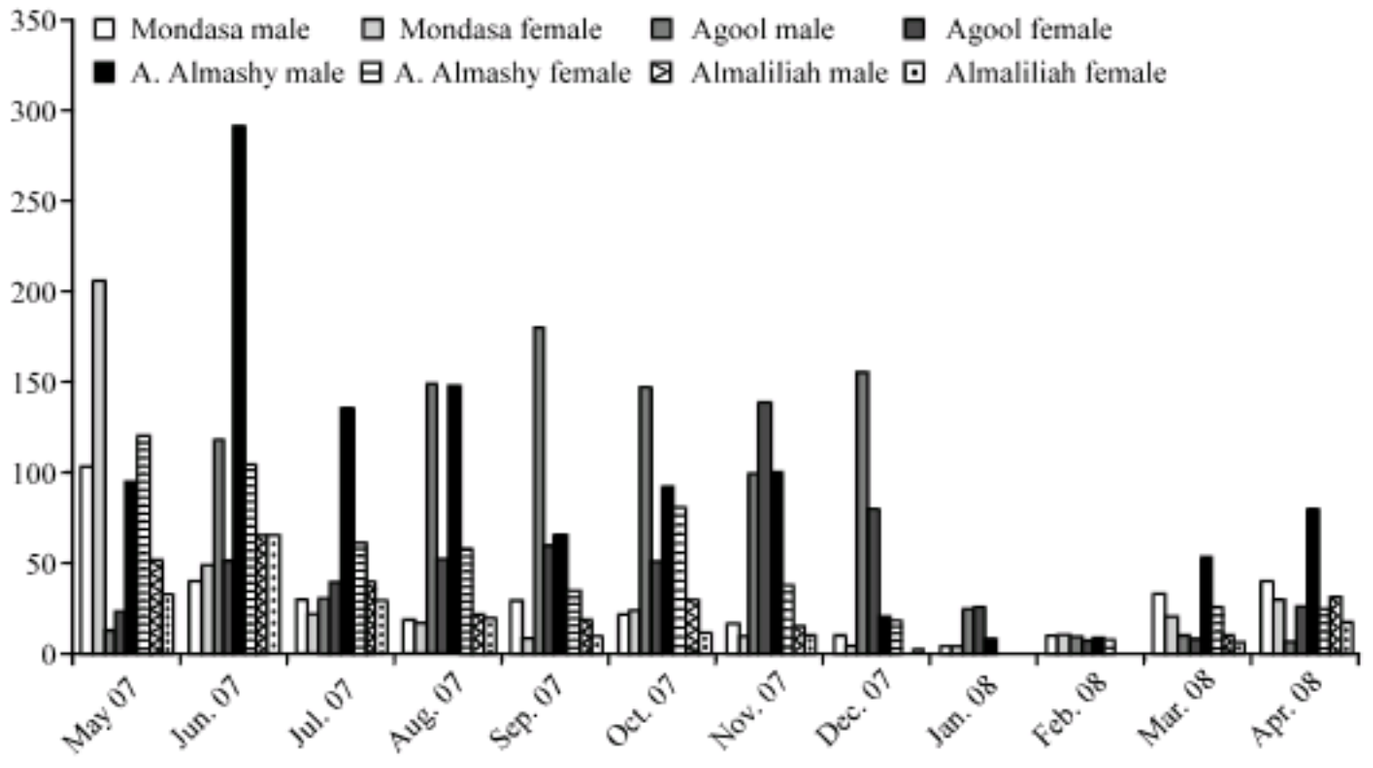

Fig. 2: Sand flies seasonal variation from all the study areas from May 2007 to April 2008

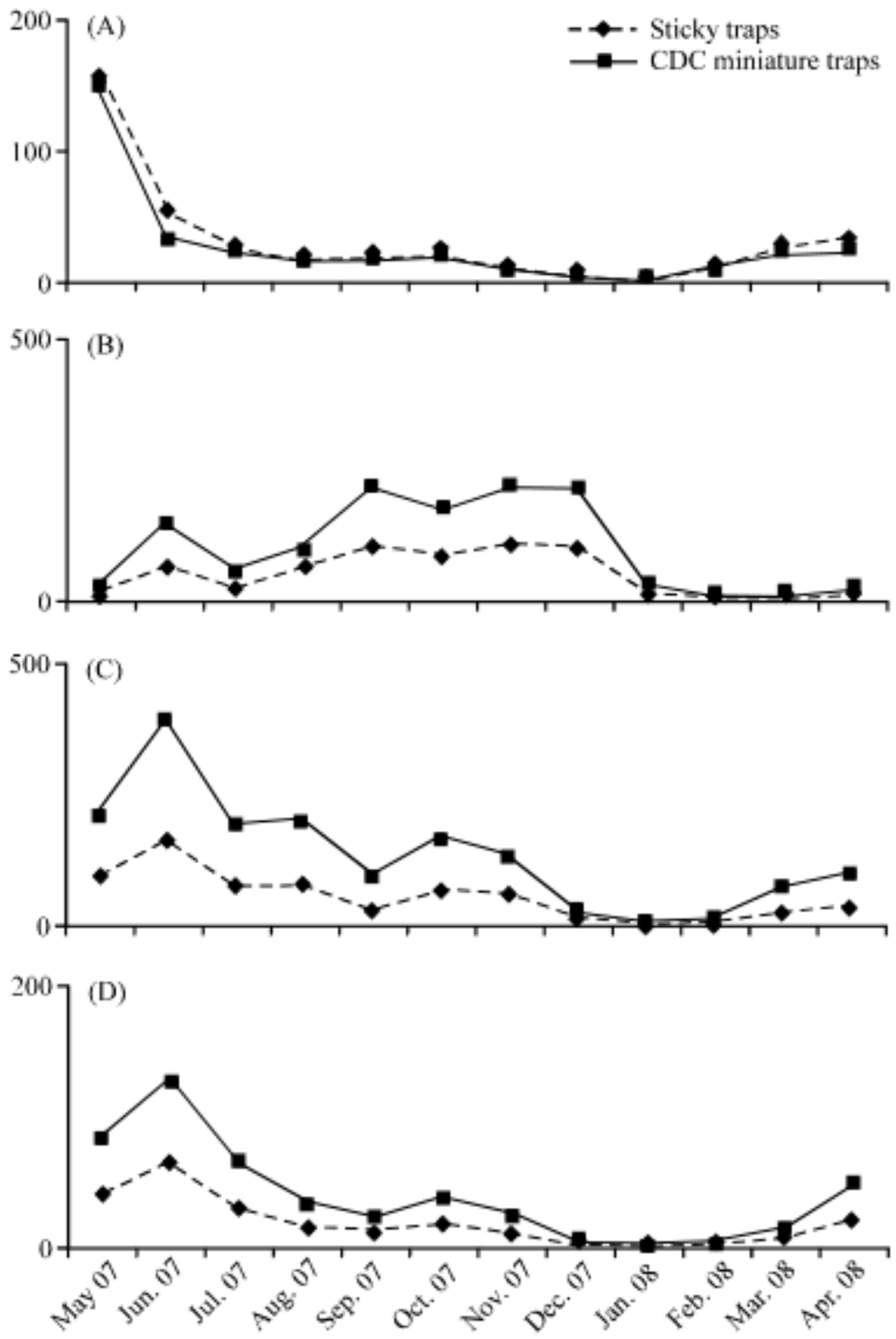

Fig. 3: Panel showing seasonal profiles of sand fly density in (A)Mondasa, (B) Agool, (C) Abyar Almashy and (D) Almaliliah, Al-Madinah, Saudi Arabia (May 2007 to April 2008), expressed as monthly, sticky traps and CDC miniature light trap rates

Almashy and Almaliliah, at May in Mondasa and at December in Agool with highest number of captured flies and sand flies per trap. They decrease to become apparently absent in January and February. There was no significant difference between the used two methods of collecting sand flies (Sticky traps and Light traps) both were efficient but light traps capture live sand flies (Fig. 3A-D).

Sex Ratio

There were seasonal variations in male/female (sex) ratio of different species of the same area and within the same species of different areas. Collected males 2676/4411 (60.7%) were significantly higher than collected females sand flies 1735/4411 (39.3%). Abyar Almashy yielded the highest number of sand flies (37.9%), showed the highest sex ratio (1.9) followed by Agool (1.7) then Almaliliah (1.4). Mondasa showed a less number of females with sex ratio, 0.9 (Table 5).

Domiciliary Abundance

There was dominance of intra- and peri-domiciliary abundance (>75%, mainly *Phlebotomus* species [*P. papatasi*]) of sand flies than extra-domiciliary (mainly *Sergentomyia* species), in all study

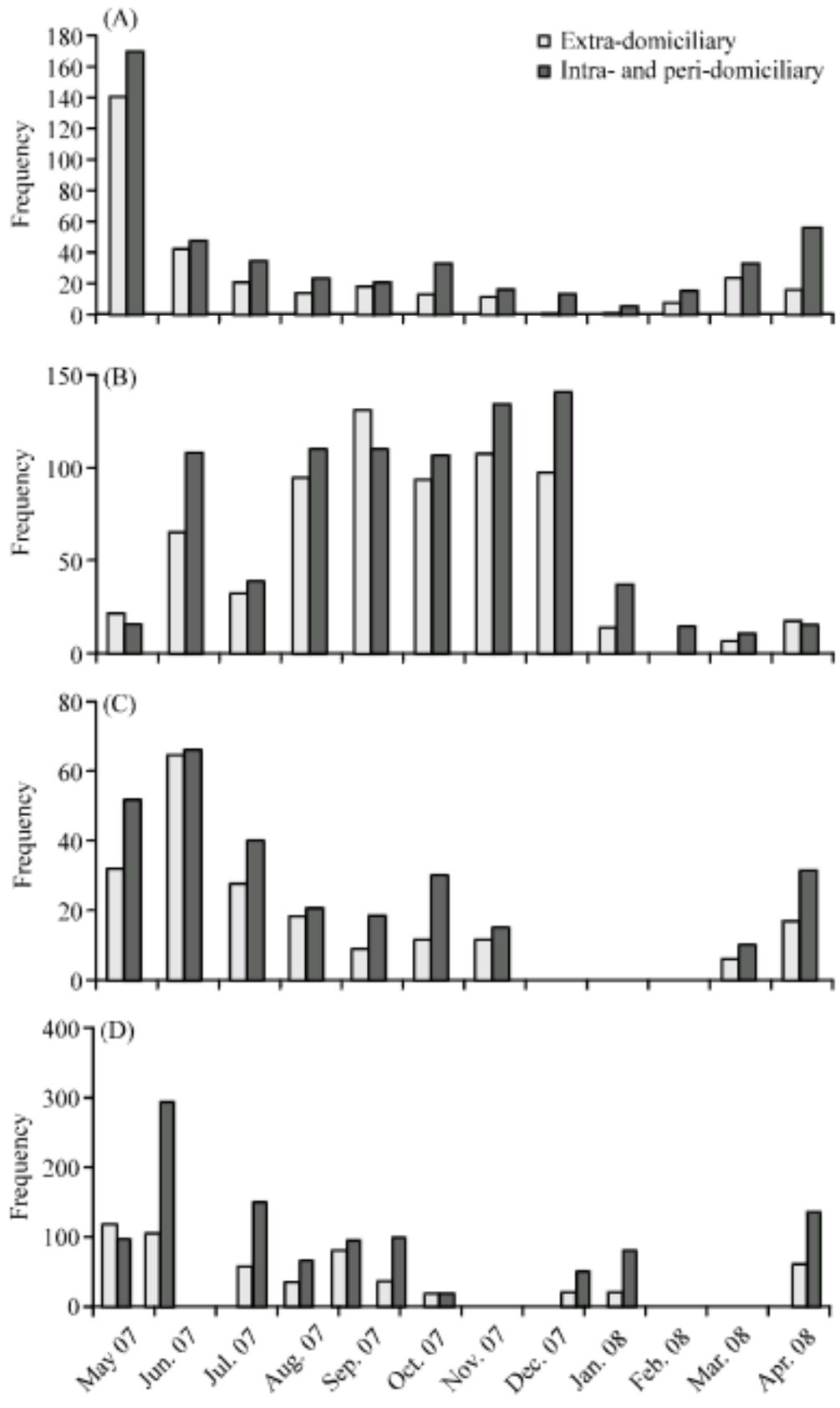

Fig. 4: Seasonal domiciliary distribution of sand flies species in (A) Mondasa, (B) Agool, (C) Abyar Almashy and (D) Almaliliah, Al-Madinah, Saudi Arabia (May 2007 to April 2008)

areas of high significance ($p<0.01$). Intra- and peri-domiciliary abundance were 454, 826, 988 and 264 in Mondasa, Agool, Abyar Almashy and Almalilah, respectively and extra-domiciliary abundance were 303, 675, 682, and 219, respectively (Fig. 4A-D).

Reproductive Status, Feeding Status and Leishmania Infections of Females

Of the 845 dissected *Phlebotomus* females; 138 from Mondasa, 381 from Agool, 260 from Abyar Almashy, 66 from Almaliliah. Blood were found in gut of 417 (49.3%) females. The *Leishmania* infection rate by culture of sand flies intestinal contents was 0.3% in Agool in June, 1.2% in Abyar Almashy in May, June and October and 1.5% in Almaliliah. No *Leishmania* parasites were found in any dissected females in Mondasa (Table 6).

Table 5: Species composition, species density, No. of ♂, No. of ♀ and sex ratio of sand flies collected (May 2007 to April 2008)

	P. papatasi	*P. sergenti*	*P. bergeroti*	*S. sergenti*	*S. antennata*	*S. clydei*	Total
Mondasa							
No. of ♂	262.0	48.0	3.0	18.0	1.0	22.0	354.0
No. of ♀	312.0	31.0	3.0	22.0	8.0	27.0	403.0
Sex ratio (♂/♀)	0.8	1.4	1.0	0.8	0.1	0.8	0.9
Agool							
No. of ♂	709.0	68.0	0.0	22.0	96.0	45.0	940.0
No. of ♀	417.0	60.0	0.0	13.0	40.0	31.0	561.0
Sex ratio (♂/♀)	1.7	1.1	0.0	1.7	2.4	1.5	1.7
Abyar Almashy							
No. of ♂	1057.0	3.0	1.0	16.0	1.0	19.0	1097.0
No. of ♀	534.0	3.0	4.0	13.0	4.0	15.0	573.0
Sex ratio (♂/♀)	2.0	1.0	0.3	1.2	0.3	1.3	1.9
Almaliliah							
No. of ♂	195.0	8.0	46.0	0.0	15.0	21.0	285.0
No. of ♀	126.0	17.0	10.0	1.0	15.0	29.0	198.0
Sex ratio (♂/♀)	1.5	0.5	4.6	0.0	1.0	0.7	1.4
Total							
No. of ♂	2223.0	127.0	56.0	50.0	113.0	107.0	2676.0
No. of ♀	1389.0	111.0	17.0	49.0	67.0	102.0	1735.0
Sex ratio (♂/♀)	1.6	1.1	3.2	1.0	1.7	1.0	1.5

Table 6: Female sand flies population dissected and their results from all the study areas from May 2007 to April 2008

Area	No. of dissected females	Blood fed females	Parous females	Leishmania natural infection in gravid females
Mondasa	138	59	56	0
Agool	381	208	185	1
Abyar Almashy	260	123	98	3
Almaliliah	66	27	23	1

DISCUSSION

An entomological survey was carried out in a panel of four different provinces (Mondasa, Agool, Abyar Almashy and Almaliliah) representing the surrounding of Al-Madinah Al-Munawarah, Western Saudi Arabia. Two different sampling methods (Stick traps and CDC light traps) were used from May 2007 to April 2008. All monitored sites were positive for sand flies. A total of 4411 sand flies specimens (2676 [60.7%] males and 1735 [39.3%] females) were collected. Abyar Almashy yielded the highest number of sand flies and Almaliliah yielded the smallest number (Table 1-4, Fig. 2). This is may be explained by the nature of Abyar Almashy, it is a semi-arid area with fallow irrigated fields and stabilized sand habitat which made it had more moisture. Six species, were identified and presented in each surveyed region, belonging to two genera. Three of which belonging to the *Phlebotomus* genus, potential vector of leishmaniasis, *Phlebotomus papatasi* (Scopoli) was the most abundant species (81.9%), the main human biting species, recognized main vector of *L. major* (rural CL) (Al-Qurashi *et al*., 2000), followed by *P. sergenti* (Parrot) (4.8 %), recognized vector of *L. tropica* (urban CL) (Al-Zahrani *et al*., 1988), then *P. bergeroti* (Parrot) (0.4 %). Three belong to *Sergentomyia* genus; *S. clydei* (Sinton) (4.3%); followed by *S. antennata* (Newstead) (3.4%) then *S. sergenti* (*theodori*, Parrot).

A preliminary study was done by El-Badry *et al*. (2008) at a micro-geographical level in only one of Al-Madinah province, a total of 621 sand flies were collected from March 2006 to February 2007. *P. papatasi*, *P. sergenti*, *P. bergeroti*, *S. antennata*, *S. sergenti* and *S. shewtzi* species were identified with population peak at June. *P. papatasi* was the predominant species (~70%). Thus, the main impetus of this study was to involve a more wider geographical areas of Al-Madinah to clearly indicates diversity of sand flies populations and fully document their seasonal variations and bionomics in Al-Madinah geographical region of the kingdom.

In the present study, five species; *P. papatasi*, *P. sergenti*, *P. bergeroti*, *S. antennata* and *S. sergenti* were already detected in our previous study (El-Badry *et al*., 2008), while *S. clydei* species was recorded for the first time.

Dominance of one or two species of sand flies is a feature in many places of Saudi Arabia (Lewis and Büttiker, 1980; Al-Gindan *et al*., 1984; Morsy *et al*., 1992; El-Sibae *et al*., 1993; Bakr, 1995; Ibrahim and Abdoon, 2005; Ibrahim *et al*., 2005; El-Badry *et al*., 2008). Regarding monthly activity of sand flies was the same for all detected species, there was absence of adult sand fly population with the onset of cold weather (period of January and February), since, sand fly larvae undergo diapauses, permitting them to survive the winter and emerge as adults in the following spring with a population increase by April to peak at May/June and declining in December, however in Agool they peaked at September, October and December in addition to June (Table 1-4, Fig. 2-4). Agool has a relatively different pattern, this is may be due the nature of Agool with a fallow field of stabilized sand beside irrigated field near Agool valley land which mad it had more moisture especially in late summer. Such pattern of seasonal abundance in Abyar Almashy, Almaliliah and Mondasa was observed by El-badry *et al*. (2008) in El-Nekheil and by other studies in Asir (Ibrahim *et al*., 2005), Hail (Bakr, 1995) in Saudi Arabia and Egypt (Hanafi *et al*., 2007). Knowing the monthly activity of sand flies of importance in determining the period of maximum risk of *Leishmania* transmission and for the successful implementation of control program.

There was intra- and peri-domiciliary abundance of sand flies (>75%), mainly *Phlebotomus* species [*P. papatasi*]) than extra-domiciliary, mainly *Sergentomyia* species in all study areas of high significance ($p<0.01$) (Fig. 3). Similar patterns of domiciliary abundance were recorded by earlier studies (Quate, 1964; Lane *et al*., 1988). Since *Sergentomyia* species are more adapted to open and dry habitats while *Phlebotomus* species prefer housing areas (intra- and peri-domiciliary) with more breeding sites. There is a great risk of being bitten inside the houses than outside away especially in late spring and early summer.

There were seasonal variations in male/female (sex) ratio of different species of the same area and within the same species of different areas, which implicate more study on the geographical and ecological systems and fecundity of Al-Madinah sand flies.

Regarding parity and fecundity, the major striking finding was that none of the collected females were parous in January 2008 (Table 6). This had also been preceded by a decline in parity in the previous and next two months then followed by a surge in parity in May and June to slightly decline in following 4 months. This is likely to shed light on the mating season habits of sand flies mosquitoes in Almadinah region and may be implimented to design novel control strategies. The proportions of gravid flies may be a valid indicator of the physiological age and of epidemiologic importance at this focus. More than 42% of dissected females were blood fed mainly intra- and peri-domiciliary ones. No *Leishmania* parasites were found in any dissected *Phlebotomus* females in Mondasa (Table 6) which showed the lowest sex ratio (0.9) (Table 5). The *Leishmania* infection rate by culture of sand flies intestinal contents in other areas ranged from 0.3 to 1.5% in June, May and October which shed light on infectivity of sand flies and planning of control strategies of cutaneous leishmaniasis.

There was no significant difference between two methods of collecting sand flies (Sticky traps and Light traps) both were efficient but light traps capture live sand flies which are important foe dissection for *Leishmania* detection. Health workers in Al-Madinah geographical area can benefit from the results of this study for better planning of sand flies control strategies aiming to prevent cutaneous leishmaniasis.

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