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Prevalence of *Culex, Aedes, Anopheles* and *Armigeres* mosquitoes at selected localities of district Peshawar Khyber Pakhtunkhwa Pakistan

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Abstract
A survey from April to December 2016 regarding prevalence, relative abundance and preferred breeding habitats of mosquito species was conducted in three union councils (Pajaggi, Dag and Larama) of District Peshawar, Khyber Pakhtunkhwa, Pakistan. A total of 4086 adults mosquitoes (1806 male and 2280 female) were identified, comprising 11 species belonging to four genera; *Culex, Aedes, Anopheles* and *Armigeres*. Among all species *Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cx. vishnui, Ae. albopictus* and *Ar. subalbatus* were identified as dominant. The maximum mosquito population was recorded in Pajaggi union council (1549/4086) followed by Dag (1473/4086) and Larama union councils (1064/4086) respectively. In September and October highest mosquitoes density (966/4086 and 864/4086) was recorded while lowest in June (179/4086) and December (212/4086) respectively. Catch basins were the most preferred breeding site from which 4 species comprising 1299/4086 mosquitoes were collected.

Keywords: Relative abundance, *Culex, Anopheles, Aedes, Armigeres,* Peshawar

1. Introduction
Mosquitoes being vector of many diseases transmit infections like malaria, dengue, yellow fever etc. to millions of people annually [1]. Mosquitoes being small biting insects belongs to family Culicidae, suborder Nemertocera and order Diptera [2]. Approximately 3,500 mosquito species have been classified into 41 genera [3]. Mosquito species belonging to genera *Culex*, *Aedes*, *Armigeres* and *Anopheles* serves as vectors for many infectious diseases [4, 5] because of their abundance, vector capability, recurrent infection and diversity [6]. According to WHO (2010) report [7] mosquito borne diseases caused approximately one million deaths and 247 million reported cases in subtropical and tropical areas of the world. Dengue and malaria is caused by mosquitoes of genera *Aedes* and *Anopheles*. In 2015 around the globe 50 to 200 million dengue incidences occurred with approximately 20,000 deaths [8]. In Pakistan the disease is reported from both rural and urban areas with outbreak in central areas of Lahore, Peshawar and in Northern areas of the country [9]. Vectors of filariasis and Japanese encephalitis are mosquito of genera *Culex* [10]. Genus *Armigeres* mosquitoes are reported vectors of lymphatic filariasis and animal filariasis [11] and dog heartworm in urban areas [12].
Mosquitoes breed in water bodies at different sites either natural as rivers and lakes or man-made as discarded tyres, water tanks, bottles, cups, ant traps being their most dominant breeding sites [13, 14]. Factors including water temperature, vegetation, water currents, water sources, water quality affect mosquito distribution [13]. Pakistan a sub-tropical country has rich fauna of vectors including mosquitoes, sand flies and other Dipterans. The presence of vast agricultural lands, open networks of irrigation channels and rivers provide natural breeding sites for these vectors [2]. Few studies have been conducted on different aspects of mosquitoes including the prevalence, seasonal distribution, abundance and stratification of *Aedes*, *Culex*, *Anopheles* and *Armigeres* in different habitats of Khyber Pakhtunkhwa including District Peshawar, District Buner and Landi Kotal [15, 16, 17].
Our research was aimed to find out the prevalence, distribution, relative abundance, seasonal variation and stratification of selected areas in Peshawar for *Aedes*, *Culex*, *Anopheles* and *Armigeres* in different habitats for the first time. This work also describes mosquito's active habitats for breeding being important for vector management and effective control strategies in

selected areas of Peshawar KP, Pakistan. Good knowledge of vectors is an important key on basis of which steps can be taken for control measures.

2. Materials and Methods

2.1 Study area

Peshawar being capital of Khyber Pakhtunkhwa province is the largest and most urbanized district with population of 2,982,816 according to 1998 census report. Peshawar with area 1257 square kilometer lies between 71°-22' to 71°-42' E latitudes and 33-44' to 34-15' N longitudes. While our selected study area in Peshawar lies between at 71°-25' to 71°-36' E latitudes and 33-59' to 34-3' N longitudes. Study area of present research was different Mouza in three union councils i.e., UC Pajaggi, Larama and Dag of district Peshawar. UC Pajaggi includes six mouza i.e., Pajaggi, Faqir Ghari, Ghari Mir Tayab, Ahadi Pora, Mander Kheil and Isa Khel. UC Larama includes two mouza i.e., Larama and Ghari Baluuhabad. UC Dag includes eight mouza i.e., Dar Mangi, Dag, Garhi Fazal Haq, Terai Bala, Terai Payan, Jabba Jangal, Mahausalu and Shagindkal. The map for study areas was prepared using *ArcGIS* version *10.3.1* (Figure 1).

2.2 Study type and period

The nature of this study is descriptive with objective to study the prevalence, distribution and stratification of selected areas for four mosquito's genera (*Aedes*, *Anopheles, Armigeres*, *Culex*). The study was carried out from April to December 2016. Each mouza of the three selected union councils was visited three days per week. The collection was made twice a day, in morning from 7:00 am to 9:00 am and in evening from 5:00 pm to 7:00 pm regularly.

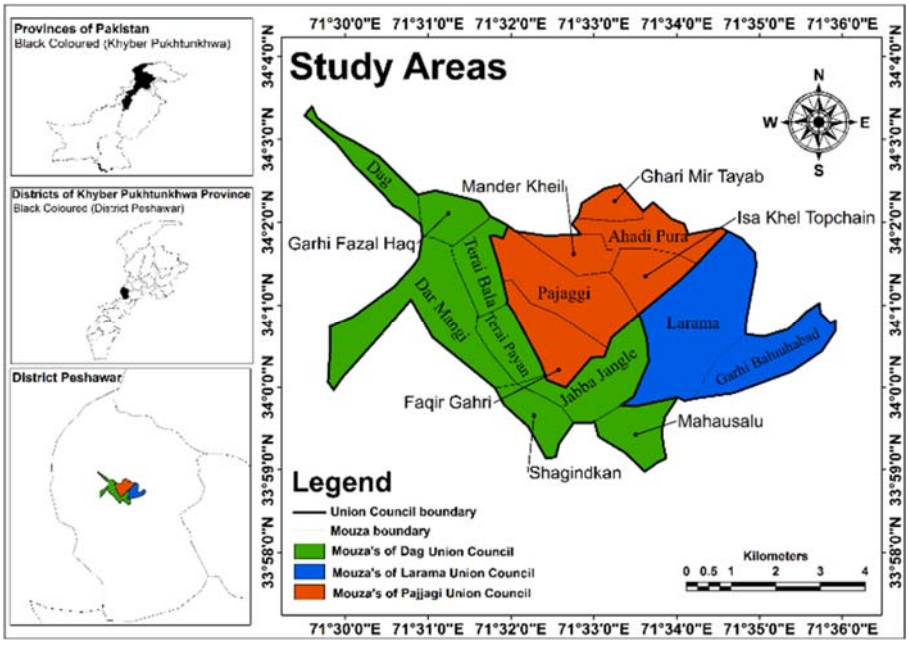

Fig 1: Map for the mosquito sampling areas.

2.3 Sampling strategies

Adult mosquitoes were collected using hand nets, light trap, back pack aspirator from catch basins, ditches and open areas with standing water. Larvae were collected using Pasteur pipette and iron dipper (14x6 cm) from tree holes, water containing plastic bottles, inside ditches, discarded automobile tyres and defrost water collection tray in refrigerator [18, 19]. All larvae and pupae collected in plastic jars were covered with net to prevent escape of adult mosquitoes upon their emergence. For collection of adult mosquitoes a small hole was made in the net and kept close to cotton swab. No artificial food was given as the water (300-500 ml) collected with larvae had sufficient food material. The jars with larvae were kept at room temperature up to emergence of adults [20].

2.4 Killing and preservation of mosquitoes

The collected and emerged adult mosquito's species were killed in the killing jar with the help of cotton swab impregnated with ethyl acetate. These specimens were segregated sex wise and mounted using entomological pins in collection boxes containing naphthalene balls. The collected mosquito species were morphologically identified using binocular microscope while taxonomic identification was carried out using taxonomic keys provided in "The fauna of British India, including Ceylon and Burma" by Christophers (1933) and Barraud (1934) [2]. Relevant information from the time of sample collection was recorded with great care and precision for final analysis and conclusion.

2.5 Data Analysis

Laboratory reared and captured adult mosquitoes were used for analysis. Variations of selected mosquito genera in different seasons were analyzed in terms of distribution and relative abundance by using the given formula [15].

$$Relative\ abundance = \frac{n}{L} \times 100 \quad (1)$$ Where

n = number of each species specimens

L = total number of specimens.

Different mosquito species were classified in different relative abundance classes given in Table 1.

Table 1: Classification of species/genus based on their relative abundance

Relative abundance (%)	Classification
Less than 1	Satellite species
Less than 5	sub-dominant species
More than 5	Dominant species

Following formula was used for identification of percent distribution of all mosquito species/Genus in various habitats [20].

Distribution $(C) = \frac{n}{N} \times 100$ (2) Where

n = number of those habitats where species was found,

N = total number of habitats

After %age distribution different classes of mosquito's species/genus were also analyzed being illustrated in Table 2.

Table 2: Different classes of species/genus based on their distribution

Classes	Distribution (%)	Classification
C1	0-20%	Sporadic
C2	20.1-40%	Infrequent
C3	40.1-60%	Moderate
C4	60.1-80%	Frequent
C5	80.1-100%	Constant

3. Results

The results of the present study showed that all selected localities were found positive with presence of mosquitoes irrespective of their income species. The current study yielded a total of 4086 adults with 1806 male and 2280 female mosquitoes (Table 3 and Figure 2).

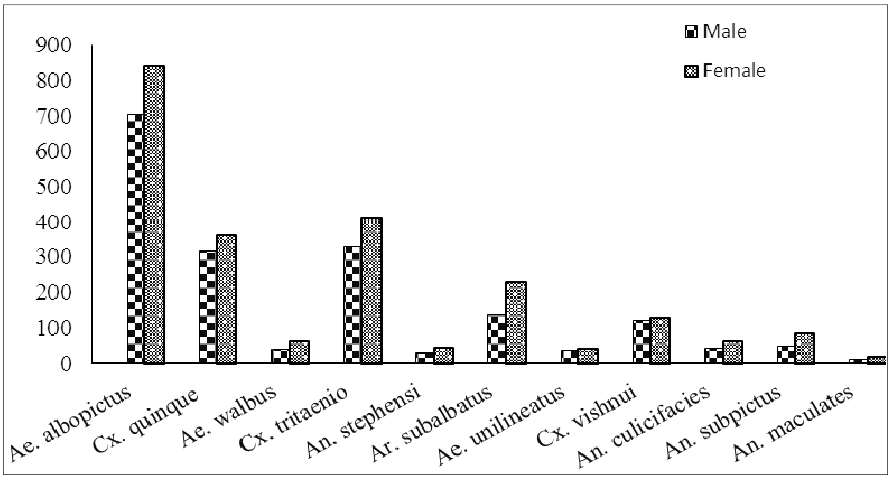

Fig 2: Total number of males and females mosquitoes collected during the survey.

Taxonomic identification revealed 11 mosquito species belonging to four genera *Culex*, *Anopheles*, *Aedes* and *Armigeres*. Genus *Culex* was represented by three species *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus* and *Cx. vishnui*; genus *Armigeres* by *Ar. subalbatus;* genus *Aedes* by *Ae. albopictus, Ae. unilineatus* and *Ae. walbus* and genus *Anopheles* by *An. culicifacies*, *An. maculatus, An. subpictus* and *An. stephensi* (Table 3).

The highest number of mosquitoes i.e., 966 (23.64%) and 864(21.14%) were collected in month of September and October respectively while lowest in June 179(4.38%). During September and October distribution of different mosquito species remained high indicating its regular occurrence (Table 3).

Significant differences were observed among various species. The activities of *Aedes* species started in March, increased till April and May, decreased during months of June and July. It went higher again in August to November and decreased afterwards till December. *Culex* and *Anopheles* were highest in September and October. *Anopheles* species started in February, increased up to May, decreased during June-July and increased again in August-October but further decreased in November-December. *Armigeres* were high during September-October, decreased during December-August (Table 3).

Most preferred breeding site was catch basins where 4 species and 1299 mosquitoes were collected accounting for 31.79% of the total mosquitoes sampled. Ditches and tree holes were found to be second preferred habitats inhibited by 4 species and total 780(19.08%) and 696 (17.03%) mosquitoes respectively. From water collection tray in refrigerator 4 species and total 573(14.02%) mosquitoes were recovered. Plastic bottles and tyres were least preferred habitats inhibited by 3 species with total of 393(9.61%) and 345(8.44%) mosquitoes respectively (Table 3 and Table 4).

In terms of distribution genus *Armigeres* were moderate, genus *Anopheles* were frequent and genus *Culex* and *Aedes* were constant. On the basis of relative abundance, all four genera were dominant (Table 5). Among eleven species belonging to these four genera; distribution of seven species were found to be sporadic, two moderate, one frequent and one constant. On the basis of relative abundance five species were dominant, five sub-dominant while one was identified as satellite specie (Table 6).

The results of regional distribution of mosquitoes illustrated highest mosquito occurrence of (1549/4086) in six mouza of Pajaggi union council followed by eight mouza of Dag union council (1473/4086) and two mouza of Larama union council with density of (1064/4086).The difference in the density of mosquitoes in different union councils may be due to availability of suitable breeding sites and conduciveness of local environmental conditions (Table 7).

Table 3: Monthly variation in species composition and abundance of mosquitoes.

Sites	Species	Sex	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Tree holes	*Ae. albopictus*	M	11	7	4	3	14	19	20	15	9	102
		F	14	9	6	5	17	27	23	24	14	139
	Cx. quinquefasciatus	M	1	19	6	2	5	19	18	5	5	80
		F	4	23	4	3	4	21	24	3	5	91
	Ae. walbus	M	2	3	4	1	7	9	11	1	1	39
		F	4	5	6	3	8	14	14	3	4	61
	Cx. tritaeniorhynchus	M	2	21	1	5	2	29	21	5	2	88
		F	2	25	1	2	4	34	22	4	2	96
	Total		40	112	32	24	61	172	153	60	42	696
Small Bottles	*Cx. quinquefasciatus*	M	8	21	3	4	3	31	27	5	9	111
		F	12	22	1	1	0	34	30	12	14	126
	An. stephensi	M	1	3	2	0	0	9	11	1	2	29
		F	3	5	2	1	0	11	12	3	5	42
	Cx. tritaeniorhynchus	M	3	2	1	3	1	8	13	2	1	34
		F	1	4	4	5	3	11	10	9	4	51
	Total		28	57	13	14	7	104	103	32	35	393
Defrost water collection tray in Refrigerator	*Ae. albopictus*	M	12	7	4	2	4	23	18	15	4	89
		F	16	11	3	1	2	29	21	19	3	105
	Ae. unilineatus	M	2	2	5	2	7	6	12	2	3	41
		F	2	4	2	5	5	9	5	1	2	35
	Cx. tritaeniorhynchus	M	2	22	3	1	4	25	33	12	1	103
		F	3	25	5	2	5	32	39	24	4	139
	Ar. subalbatus	M	3	2	2	1	2	4	4	3	2	23
		F	1	2	5	4	3	6	7	7	3	38
	Total		41	75	29	18	32	134	139	83	22	573
Catch basins	*Cx. quinquefasciatus*	M	1	31	3	6	3	34	38	4	4	124
		F	3	33	8	8	5	39	43	3	3	145
	Ae. albopictus	M	51	54	12	15	16	52	58	36	21	315
		F	63	59	17	17	13	58	65	47	24	363
	Cx. vishnui	M	3	12	6	8	4	47	33	6	2	121
		F	3	9	2	7	15	52	32	5	3	128
	An. culicifacies	M	2	8	1	3	5	9	7	3	3	41
		F	2	11	3	5	11	11	10	5	4	62
	Total		128	217	52	69	72	302	286	109	64	1299
Ditches	*Ae. albopictus*	M	27	23	6	8	20	28	14	2	4	132
		F	31	29	4	8	12	28	16	7	4	139
	Cx. tritaeniorhynchus	M	3	21	2	5	8	35	27	3	1	105
		F	4	24	7	9	13	39	23	5	2	126
	An. subpictus	M	1	8	7	9	2	7	9	3	1	47
		F	2	12	6	9	25	11	13	4	1	83
	Ar. subalbatus	M	2	1	2	8	13	16	11	7	1	61
		F	2	3	5	9	17	24	15	10	2	87
	Total		72	121	39	65	110	188	128	41	16	780
Tyres	*Ae. albopictus*	M	9	7	4	6	4	7	11	8	10	66
		F	15	11	2	8	6	15	13	12	14	96
	An. maculates	M	0	0	0	1	3	3	1	0	2	10
		F	1	0	0	1	5	5	1	1	3	17
	Ar. subalbatus	M	1	2	3	5	17	5	11	6	1	51
		F	2	5	5	7	27	31	18	7	3	105
	Total		28	25	14	28	62	66	55	34	33	345
Grand total			337	607	179	218	344	966	864	359	212	4086
Percentages %			8.25	14.86	4.38	5.34	8.42	23.64	21.15	8.79	5.19	100

Table 4: Habitat specificity of different mosquito (Larvae/Adults) species.

Species	Tree holes	Small Bottles	Collection tray in Refrigerator	Catch Basins	Ditches	Tyres
Ae. albopictus	+		+	+	+	+
Cx. quinquefasciatus	+	+		+		
Ae. walbus	+					
Cx. tritaeniorhynchus	+	+	+		+	
An. stephensi		+				
Ar. subalbatus			+		+	+
Ae. unilineatus			+			
Cx. vishnui				+		
An. culicifacies				+		
An. subpictus					+	
An. maculates						+

Table 5: Relative abundance and distribution of four selected mosquito genera

Genus	Total	Relative Abundance	Distribution	Relative abundance status	Distribution class
Culex	1668	40.82	83.33	Dominant	Constant
Aedes	1722	42.14	83.33	Dominant	Constant
Anophles	331	8.10	66.67	Dominant	Frequent
Armigeres	365	8.93	50.00	Dominant	Moderate

Table 6: Relative abundance and distribution of mosquito species

Species	Total	Relative Abundance (Species)	Distribution	Relative abundance status	Distribution class
Cx. quinquefasciatus	677	16.57	50.00	Dominant	Moderate
Cx. tritaeniorhynchus	742	18.16	66.67	Dominant	Frequent
Cx. vishnui	249	6.09	16.67	Dominant	Sporadic
Ae. albopictus	1546	37.84	83.33	Dominant	Constant
Ae. walbus	100	2.45	16.67	Sub dominant	Sporadic
Ae. unilineatus	76	1.86	16.67	Sub dominant	Sporadic
An. stephensi	71	1.74	16.67	Sub dominant	Sporadic
An. culicifacies	103	2.52	16.67	Sub dominant	Sporadic
An. subpictus	130	3.18	16.67	Sub dominant	Sporadic
An. maculates	27	0.66	16.67	Satellite	Sporadic
Ar. subalbatus	365	8.93	50.00	Dominant	Moderate

Table 7: Population of different mosquito species in study areas

Mosquito species	Survey areas (Union council)			Total species
	Dag	Pajaggi	Larama	
Ae. albopictus	434	700	412	1546
Cx. quinquefasciatus	323	231	123	677
Cx. tritaeniorhynchus	32	43	25	100
Ae. walbus	306	254	182	742
An. stephensi	30	27	14	71
Ar. subalbatus	133	101	131	365
Ae. unilineatus	41	15	20	76
Cx. vishnui	67	89	93	249
An. culicifacies	28	41	34	103
An. subpictus	67	41	22	130
An. maculates	12	7	8	27
Total	1473	1549	1064	4086

4. Discussion

As mosquito eggs, larvae and pupae are dependent on aquatic habitats [20] hence knowledge about type of breeding habitats is important for an effective control of mosquito [21]. There is no published information regarding prevalence of *Culex*, *Aedes*, *Anopheles* and *Armigeres* mosquitoes in selected localities of Peshawar which subsequently led to the foundation of present study.

Present research was focused on collection of mosquito adults and larvae from different breeding habitats to study the prevalence, species composition, frequency, breeding habitats, relative abundance and seasonal variation of mosquitoes [19]. In present survey total of 11 mosquito species belonging to 4 genera (*Aedes, Armigeres, Anopheles and* Culex) were recorded. Maximum frequency was observed for *Ae. albopictus* followed by *Cx. tritaeniorhynchus, Cx.*

quinquefasciatus, and then others. In contrast study conducted by Khan *et al* [8, 16] recorded 15 species in Swat Ranizai and 21 species in district Buner, KP respectively. The more probable reason of difference in species recovered is comparatively smaller area of the present study than afore studied areas. Variation in ecological conditions in different study areas can be another possible cause of this difference [15, 19].

Naz *et al.,* [19] studied population dynamics of mosquitoes in various breeding habitats at University of Peshawar, KP Pakistan and recovered 6 mosquito species of four genera *Aedes*, *Culex*, *Armigeres* and *Anopheles* with maximum frequency of occurrence for *Ae. albopictus* followed by *Cx. quinquefasciatus, Ar.subalbatus, Ae. walbus*. Likewise, Ali and Rasheed [15] recorded 9 species of two genera *Culex* and *Anopheles* in Palosai stream, Peshawar. Out of 9 species; *Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cx. vishnui*, *An. culicifacies, An. maculatus, An. subpictus*, and *An. stephensi* are similar with species recorded in our study. The maximum similarity of species in afore studied areas might be similar environmental conditions *i.e.,* temperature, humidity and habitats.

In present study fluctuation in mosquito population with seasonal variation was observed. An increase in mosquito population was noticed in the successive months from April-May and September-October (Table 3). Akram *et al.,* [22] reported suspended mosquitoes activity during June and July due to high relative humidity (70%) while in August the mosquito population began to increase with rising temperature. Khan *et al*., [23] reported activity of mosquitoes throughout the year being most active during May, September and October. Likewise Naz *et al.,* [19] reported highest number of mosquitoes during October (481/2419) while the lowest in December (224/2419).

In the present study various habitat specificity of mosquitoes were observed. The catch basins appeared to be the most favorable breeding habitat as maximum mosquitoes were recovered. Our findings are similar to results of Harding *et al.,* [24] who recorded larvae in catch basins and concrete water tanks. While tyres was preferred breeding site in the study conducted by Khan *et al.,* [16] in District Buner KP Pakistan. Suleman and Khan [25] recovered 90% of *Ae. albopictus* from water containing tree holes which is congruent with our study results with tree holes the second most preferred breeding site. Ecological as well as urban nature of Peshawar might be the reason of difference in breeding sites.

5. Conclusion and Recommendations

Mosquito fauna of study areas at district Peshawar is represented by genera *Aedes, Culex, Anopheles* and *Armigeres* respectively. It is thus concluded that species belonging to genera *Aedes* and *Culex* are abundant compared to *Anopheles* and *Armigeres* species. Human activities contributing to availability of mosquitoes breeding sites may lead to establishment of pathogens and increased risk of transmission of vector borne diseases. The finding of this study necessitates further entomological investigations of mosquitoes using different sampling methods to describe their feeding, resting behaviors and role in disease transmissions.

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