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# Population dynamics of mosquitoes and malaria vector incrimination in district Charsadda, Khyber Pakhtunkhwa (KP) Pakistan

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ABSTRACT

This study aimed to obtain updated information on mosquito diversity and malaria vector incrimination in Charsadda Khyber Pakhtunkhwa to help in devising effective control and preventive measures in the area. Monthly survey of indoor mosquitoes for one year was carried out in three villages, Dhaki Totaqi and Mathra. Female Anopheline were used to detect Circumsporozoites protein (CSP) using Enzyme Linked Immunosorbent Assay. Among 17 mosquito species, *Culex quinquefasciatus, Anopheles splendidus, Anopheles stephensi, Anopheles fluviatilus, Anopheles culicifacies* and *Culex tritaeniorhynchus* were predominant. Dhaki village had the highest mosquito species diversity (1.015) and similar species richness (0.7) and evenness (0.5) with village Mathra. Slide positivity rate (SPR) shows that the rate of malaria transmission increases with mosquito population. Four anopheline species i.e. *A. stephensi, A. fluviatilis, A. splendidus*, and *A. culicifacies* were CSP positive. The CSP rate was 0.8%, where two specimens of *A. splendidus* and one of *A. fluviatilus* were positive for *Plasmodium falciparum, Plasmodium vivax* was represented by two variants 210 and 247. Among the 20 CSP positive specimens, variant 210 was found in 12 (one belonging to *A. culicifacies*, two each of *A. stephensi* and *A. fluviatilus*, seven specimens of *A. splendidus*) and 247 in 8 specimens (two of *A. stephensi*, three each of *A. fluviatilus* and *A. splendidus*). The number of infected mosquitoes collected from animal sheds was higher (15) though non-significant ($P > 0.05$) than that from bedrooms (8).

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## 1. Introduction

Mosquitoes are the most abundant and widely distributed insects responsible for human and animal morbidity and mortality on large scale worldwide (Versteirt et al., 2013). Vector-borne infectious diseases, such as malaria, dengue fever, yellow fever, and plague, cause a significant fraction of the global infectious disease burden; indeed, nearly half of the world's population is at risk of being infected with at least one type of vector-borne pathogen (Stanley et al., 2008; WHO, 2004). Vector-borne plant and animal diseases, including several newly recognized pathogens, reduce agricultural productivity and disrupt ecosystems throughout the world. These diseases profoundly restrict socioeconomic status and development in countries with the highest rates of infection, many of which are located in the tropics and subtropics (Stanley et al., 2008).

Before implementation of vector born diseases control, they caused millions of deaths worldwide (Crosby, 2005). In order to prevent and control the transmission and possible outbreaks of mosquito borne diseases like malaria and arboviruses, intensive insect population studies are essential requirement (Versteirt et al., 2013). The issue of development of co-relation between vector species composition and malaria transmission has been neglected in Pakistan. To develop successful control measures the proper recognition of vector species, their habits (feeding and resting) and habitat play a key role (WHO, 2004). Determining the biting behavior, parous rate, sporozoite rate and behavior of infected and uninfected mosquitoes are also very important information, for assessing the role of vectors in the epidemiology of malaria in the area (Taye et al., 2006).

The advent of Enzyme Linked Immuno Sorbant Assay (ELISA) for the detection of *Plasmodium* sporozoites has helped a lot in the identification of vector species and estimation of malaria burden in areas of low to moderate malaria transmission (Wirtz et al., 1987). This technique has been largely used for the detection of

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**Table 1**
Species composition and abundance of mosquitoes in three villages of district Charsadda.

| Species | Locality | | | |
|---|---|---|---|---|
| | Dhaki no. (%) | Totaki no. (%) | Mathra no. (%) | Total no. (%) |
| *C. quinquefasciatus* | 2961 (63.65) | 7991 (91.93) | 3588 (66.71) | 14,540 (77.66) |
| *A. splendidus* | 910 (19.56) | 69 (0.80) | 524 (9.74) | 1503 (8.03) |
| *A. stephensi* | 219 (4.71) | 294 (3.38) | 738 (13.72) | 1251 (6.68) |
| *A. fluviatilus* | 457 (9.82) | 137 (1.58) | 207 (3.8) | 801 (4.3) |
| *A. culicifacies* | 28 (0.94) | 107 (1.23) | 158 (2.93) | 293 (1.56) |
| *C. tritaeniorhynchus* | 15 (0.51) | 38 (<0.5) | 79 (1.47) | 132 (0.7) |
| *Ar. subalbatus* | 32 (0.69) | 31 (<0.5) | 46 (0.85) | 109 (0.58) |
| Total mosquitoes | 4652 | 8692 | 5378 | 18,722 |

*A. subpictus, A. annulari, A. maculatus, A. nigerimus, A. pulcharimus, C. Vishnui, C. mimeticus, C. theileri, C. biteaniorhynchus* and *Mansonia uniformis* were less that 0.5% of total collection. Total mosquitoes also include number of mosquitoes less than 0.5%.

circumsporozoite protein (CSP) in mosquitoes. The present study was also focused to identify the malaria vectors by performing ELISA test of the blood fed Anopheline mosquitoes to determine the vector species of malaria and estimating the status of malaria in the district Charsadda. This study could be used as a guideline for any malaria vector control strategy applied in this district.

Few published work is available regarding mosquito fauna in different parts of KP Province of Pakistan. Like in KP (Azakhel refugee's village) a study was conducted to determine transmission and control of *vivax* malaria in Afghan refugee settlement in Pakistan, where nine species of *Anopheles* mosquitoes were found positive for CSP by using ELISA (Rowland et al., 1997). Similarly eight species of *Anopheles* were reported positive for *Plasmodium falciparum* and *Plasmodium vivax* using circum-sporozoite protein (CSP) in Eastern Afghanistan to study Anopheline vector and malaria transmission (Rowland et al., 2002). Among infected mosquitoes 46% contained *P. falciparum*, 45% *P. vivax* VK-247 and 9% *P. vivax* PV-210.

The present study is the first attempt to determine the mosquito fauna of district Charsadda, its species composition, relative abundance and seasonal variation as well as identifying malaria vectors, which may assess in control strategies of the targeted species.

## 2. Study area

Three villages Mathra, Dhaki and Totaqi were selected from Charsadda district of KP Pakistan to carry out mosquito survey from January to December 2007 once a month.

Village Mathra is a fertile agricultural plain with plenty of water supply from river Swat. The main crops of the village are sugar cane and maize. Village Dhaki has well-developed irrigation system, where main crops are tobacco, maize and sugar cane. Village Totaqi is a fertile area with plenty of water Supply. The village is surrounded by sugarcane fields.

To have maximum coverage of the whole district mosquito fauna, the distances among the focal points were kept large. Moreover, the selection of focal points comprised of potentially malarious villages of the region (based on clinical record of the district) that could represent all its possible anopheline and other mosquito species composition. Additionally, the selection of study areas was also based on their geographical position in order to represent the whole district as was recommended by Reisen and Milby (1986).

The selected houses in each village have adjacent marshes and water pools, surrounded by agricultural fields and vegetation which are potential breeding sites for outdoor resting mosquitoes. Ten houses were selected from each village, which had animal sheds (suitable for indoors resting). Collection was then made from the bedrooms and animal sheds. The bed rooms were made of cement blocks or mud with majority having wooden roofs however; few were also having cemented roofs. The animal sheds were all made of mud walls and wooden roofs.

## 3. Mosquito collection

Spray catch method was used for the collection of mosquitoes. Before the pyrethroid insecticide spray, windows and ventilators were closed. Bedrooms and animal sheds were sprayed for 10–15 s depending upon the size of the room and were left closed for 15 min. Forceps and a torch were used to pick up the fallen mosquitoes and were stored in tubes containing silica gel (Herrel et al., 2004; Reisen and Milby, 1986; Tyagi and Yadav, 2001). The mosquitoes were identified by using taxonomic key compiled from fauna of British India by (Barraud, 1934; Christopher, 1933).

After the identification and sexing of collected mosquitoes, female Anopheline mosquitoes were transferred to the tubes containing silica gel. Head with thorax of these mosquitoes was cut down to avoid any chance of contamination with the blood present in the abdomen (Somboon et al., 1993). Both the pieces were kept in separate eppendorf tubes and were kept at 4 °C. Head with thorax of each mosquito was used for testing the Circumsporozoites protein (CSP), Pv247, Pv210 and Pf-CSP.

All ratios were analyzed statistically applying Chi-square using SPSS v 16.0 for Windows. The value for statistically significance was kept at 0.5.

## 4. Results

Regular collections from the selected three villages were made in the last week of each month. A total of 18,722 mosquitoes were collected of which 11,071 were female and 7608 male individuals. Taxonomic study revealed seventeen species of four genera i.e. *Anopheles, Culex, Armigeres* and *Mansonia* (Table 1). *C. quinquefasciatus* was the most numerous species (77.66%) found in all the three villages, followed by *Anopheles splendidus* (8.03%) and *Anopheles stephensi* (4.3%), while other species were relatively low in number.

*Anopheles nigerimus, Anopheles pulcharimus, Culex biteaniorhynchus* and *Mansonia uniformis* were not found in Dhaki. Similarly *Culex mimiticus, Culex theileri* and *M. uniformis* were not collected from Totaki while all the species identified in this survey except *C. biteaniorhynchus* made their representation in village Mathra. Among the 17 species, 10 species abundance was less than 0.5% of the total collection. Maximum number of species (9) was found in Genus *Anopheles* followed by genus *Culex* (6), while a single species each of both genera *Armigeres* and *Mansonia* was observed. Highest density of mosquitoes was found in the village

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