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ORIGINAL ARTICLE

Anopheline species and their *Plasmodium* infection status in Aligarh, India

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Abstract Malaria is a global issue and India contributes substantially to global malaria incidence. Information related to malaria vectors is very limited in Aligarh. The environmental and climatological situations permit the continual breeding of vectors in permanent breeding sites. This study was designed with the aim to screen all the anophelines species and possible malaria vectors in three different localities of Aligarh. *Anopheles* mosquitoes were collected from three different localities (Fort, Jalali and Tappal) during peak malaria transmission season (July to November) by using mouth aspirator and CDC light traps. Enzyme-linked immunosorbent assay (ELISA) was done to detect *Plasmodium falciparum*, *Plasmodium vivax*-210 and *P. vivax*-247 circumsporozoite proteins (CSP) from the collected female species. A total of 794 female anopheline mosquitoes belonging to 7 species were collected by different methods. Circumsporozoite protein–enzyme-linked immunosorbent assay was performed with 780 anopheline mosquitoes out of which 13 mosquitoes were positive in CSP–ELISA. Thus, the overall infection rate was 1.66% (13/780). Four (0.51%) mosquitoes belonging to three species were positive for *P. falciparum*, 7 (0.89%) mosquitoes belonging to three species were positive for VK 210 and 2 (0.25%) mosquitoes belonging to *Anopheles culicifacies* and *Anopheles stephensi* species were positive for VK 247. No mixed infection was found in this study. According to species, the highest infection rate was observed in *An. culicifacies* (7/288, 2.43%) followed by *An. stephensi* (2.40%) and *Anopheles annularis* (1.98%). *An. culicifacies* and *An. stephensi* were previously incriminated as malaria vectors in Aligarh. There was, however, no previous report in favor of infections in *An. annularis* in Aligarh. The on-going Malaria Control Program in India needs up to date information on malaria vectors. A major challenge is the lack of knowledge about vectors and their role in malaria transmission. Findings of this study suggested that in the absence of major malaria vectors there is a possibility that other *Anopheles* species may have been playing a role in malaria transmission in Aligarh.

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1. Introduction

Malaria remains one of the leading causes of morbidity and mortality in the tropics. Malaria is an entirely preventable and treatable mosquito-borne illness. In 2014, 97 countries and territories had ongoing malaria transmission. An

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estimated 3.2 billion people are at risk of malaria, of whom 1.2 billion are at high risk. There were an estimated 198 million cases of malaria worldwide ranging between 124 and 283 million (World Malaria Report, 2014). Even though Africa accounts for 90% of the mortality burden for malaria, South-East Asia region such as Thailand, Nepal, Bhutan, Bangladesh, Myanmar, Indonesia, Sri Lanka and India still suffers considerable mortality and morbidity. According to the first report of Roll Back Malaria (RBM) South-East Asia has the highest rate of drug resistance in the world, and multi-drug resistance has contributed to the re-emergence of malaria in many areas, especially along international borders (WHO, 2013). Among member countries of South East Asian Region Office (SEARO) of WHO, India contributes >85% cases annually. These malaria cases were highly under-reported. The estimates of WHO indicate about 81 million cases (Korenromp, 2004). Malaria is endemic in India with varying levels of endemicity. Most regions in the country have an unstable malaria situation except in the North-Eastern region where stable malaria situation prevails. In India 58 species of *Anopheles* are recorded, out of which six are considered as primary vectors and four as secondary vectors. The primary vectors include *Anopheles culicifacies*, *Anopheles stephensi*, *Anopheles fluviatilis*, *Anopheles dirus*, *Anopheles minimus* and *Anopheles sundaicus* whereas *Anopheles annularis*, *Anopheles philippiensis* (*nivipes*), *Anopheles varuna* and *Anopheles jeyporiensis* are considered as secondary vectors (Rao, 1984). Among the primary vectors *An. culicifacies* has been established as a major vector of malaria in rural and periurban areas of India (Subbarao et al., 1988). In India *Plasmodium vivax* and *Plasmodium falciparum* cause benign and malignant tertian malaria and 60–65% of the malaria infections are reported to be due to *P. vivax* and 30–35% due to *P. falciparum* (Adak et al., 1998). A three years survey conducted by Saifi et al. (2010) showed that in Aligarh district the slide positivity rates for *Plasmodium* were 26.82%. The prevalence of *P. vivax* was 17.18% and for *P. falciparum* it was 9.6%.

Aligarh has a tropical monsoon type of climate with its highly attributed seasonal variations including the North-East and North-West monsoons. The North-East monsoon occurs from December to mid June and is distinguished by high temperatures and dry winds of continental region. In the Aligarh district a variety of mosquito breeding grounds are present which facilitates the emergence of different anopheline species. Although enough literature is available on mosquito fauna and vectors from many parts of India very little published information on screening and vector incrimination of malaria in Aligarh is available. Wajihullah and Saifi (2001) incriminate *An. culicifacies* as a predominant vector of malaria. The present study was, therefore, undertaken to obtain some information regarding prevalent anopheline species and their possible malaria vectors in Aligarh district.

2. Materials and methods

2.1. Study areas

Aligarh, where the proposed work was carried out is situated in the upper Ganga–Yamuna Doab and it extends from 27°29′ N to 28°11′ N latitudes and 77°29′ to 78°38′ longitudes. It has a tropical monsoon type of climate typified by seasonal variations including the North-East and North-West monsoons. The North-East monsoon occurs from December to mid June and is distinguished by high temperatures and dry winds of continental origin. Clear skies, dust and low humidity are observed from time to time. Mid June to October is marked by humid winds of oceanic origin, including cloudy weather, rainfall and high relative humidity. Aligarh experiences extreme cold during winter as there is a subsequent fall in temperature. The average maximum temperature in November is 29.5 °C while in January which is the coldest month of the year, the mean maximum and minimum temperatures are 20 and 4 °C, respectively. The onset and retreat time of the monsoon differ considerably every year. Usually the rain starts by the mid of June and continues till the end of September or early October. During this period of the year 90% of the total annual precipitation occurs. Excess rain in this part of the year may cause the low-lying areas or depressions and ditches to be water-logged. Relative humidity varies considerably throughout the year. With the onset of monsoon, the relative humidity increases ranging from 65% to 77%. January records the highest percentage of relative humidity of about 82%.

2.2. Collection of Anopheles mosquitoes

For vector, screening mosquitoes were collected from Aligarh from July to November 2012. Mosquito collection was made from three different localities of Aligarh. These localities were Fort, Dhorra and Sasni Gate. *Anopheles* mosquitoes were collected from (18.00–24.00 h) both indoors and outdoors by human landing catches (HLC) methods by using mouth aspirators (WHO, 1975) and the resting collection was conducted in 20 households. After completion of HLC and resting collection, CDC miniature light trap model 512 (origin: John W. Hock Inc, USA) was also used for entomological investigation. Mosquitoes were collected from four houses per night on each of five successive nights once within the peak malaria transmission season (July to November). Four volunteers collected mosquitoes at each house two indoors and two outdoors. Every night the house was shifted randomly. Thus, in each study area for entomological surveillance, human landing catches (HLC), resting collection and CDC miniature light trap were conducted in 20 households. Each CDC trap was installed for at least 12 h (6 pm to 6 am). Each night four trappings were conducted for five days of a week for each of the areas alternatively once in the peak season.

2.3. Ethical consideration

A written consent was obtained from the houses where entomological collections were made. The mosquito collectors were monitored up to three months with a provision for treatment in case they got malaria, but such a case did not occur during the reported period.

2.4. Mosquito sample preparation

Next morning, mosquitoes were sorted and identified. Identifications were made on the basis of their morphological features with the help of standard regional pictorial keys (Wattal and

Kalra, 1961). After identifying the species each mosquito was preserved in a cryo-vial in silica gel in order to prevent microbial growth that can result in high background values.

3. CSP–ELISA

The field caught mosquitoes were tested by ELISA for the presence of circumsporozoite protein (CSP) by using an enzyme-linked immunosorbent assay (ELISA), as described previously (Wirtz et al., 1987a,b). Enzyme-linked immunosorbent assay was used to detect *P. falciparum*, *P. vivax*-210 (VK 210), and *P. vivax*-247 (VK 247) CSP in field caught mosquitoes. In each test, for positive control *Plasmodium* species were used and for negative control field caught male *Anopheles* mosquitoes were used. Monoclonal antibody (MAB) was obtained from the National Institute of Malaria Research (NIMR) which was produced by Kirkegaard and Perry Laboratories (Atlanta, GA). Same batches of capture monoclonal antibodies were used in all tests. The absorbance of the solution at 410 nm was determined 60 min after adding the substrate to Biorad ELISA plate reader. The cut-off point of absorbance was calculated by multiplying twice with the mean value of negative controls in respective tests. For ELISA positive mosquito tests were repeated to confirm it a positive.

4. Results

Seven hundred and ninety-four female anopheline mosquitoes belonging to 7 species were collected by different methods (Table 1). One hundred and twenty-three mosquitoes were collected by CDC light trap and 671 mosquitoes by other methods (HLC and resting). Majority of the mosquitoes collected by other methods were found resting in the cattle shed, indoors or outdoors of human dwellings. *An. culicifacies* was the dominant species (36.94%) followed by *An. stephensi* (21.15%), *Anopheles subpictus* (19.64%), and *An. annularis* (13.09%) while *Anopheles vagus* (4.78%), *Anopheles lindesayi* (2.51%) and *Anopheles barbirostris* (2.14%) were recorded in less numbers.

Circumsporozoite protein–enzyme-linked immunosorbent assay was performed with 780 anopheline mosquitoes (remaining mosquitoes were kept as voucher specimen). Thirteen mosquitoes were positive CSP–ELISA (Table 1). Thus, the overall infection rate became 1.66% (29/780). Four (0.51%) mosquitoes belonging to three species were positive for *P. falciparum*, 7 (0.89%) mosquitoes belonging to three species were positive for VK 210 and 2 (0.25%) mosquitoes belonging to *An. culicifacies* and *An. stephensi* species were positive for VK 247. No mixed infection was found in this study. *P. falciparum* positive *Anopheles* species included two *An. culicifacies*, one *An. stephensi* and one *An. annularis*. VK 210 positive species included four *An. culicifacies*, two *An. stephensi* and one *An. annularis* while VK 247 positive species included one *An. culicifacies* and one *An. stephensi*. The highest infection rate (Table 2) was observed in *An. culicifacies* (7/288, 2.43%) followed by *An. stephensi* (2.40%) and *An. annularis* (1.98%). From Fort 6 CSP-positive (2.75%) mosquitoes had been identified in three species including *An. culicifacies*, *An. stephensi* and *An. annularis* (Table 3). In Jalali, 4 mosquitoes were identified CSP positive (1.42%) belonging to two species including *An. culicifacies*, and *An. stephensi*. In Tappal three mosquitoes were found CSP positive (1.06%) belonging to three species *An. culicifacies*, *An. stephensi* and *An. annularis*.

Table 2 CSP-ELISA positive mosquito infection rate according to anopheline species.

Species	No	Pf	Pv-210	Pv-247	Total (%)
An. culicifacies	288	2 (0.69)	4 (1.38)	1 (0.34)	7 (2.43)
An. stephensi	166	1 (0.60)	2 (1.20)	1 (0.60)	4 (2.40)
An. subpictus	154	0	0	0	0
An. annularis	101	1 (0.9)	1 (0.9)	0	2 (1.98)
An. vagus	37	0	0	0	0
An. lindesayi	18	0	0	0	0
An. barbirostris	16	0	0	0	0
N	780	4 (0.51)	7 (0.89)	2 (0.25)	13 (1.66)

5. Discussion

The on-going Malaria Control Program in India, stresses the fact of up-to-date information on malaria vectors. Understanding the multifaceted determinants of malaria transmission is important as many factors play a role in malaria transmission. A major challenge is the lack of knowledge about vectors, their seasonal abundance, infection rates and role in malaria transmission. Further, little information is available about the local patterns of malaria infection, the annual and seasonal changes in the prevalence with each *Plasmodium* species and the effect of antimalarial measures. Presently current vector control programmes are being

Table 1 Anopheline species collected from three different localities of study area by different methods.

Species	Fort			Jalali			Tappal			Total (%)		
	LT	Others	Total	LT	Others	Total	LT	Others	Total	LT	Others	Total (%)
An. culicifacies	12	93	105	9	87	96	7	83	90	28	263	291 (36.94)
An. stephensi	11	41	53	9	51	90	9	47	56	29	139	168 (21.15)
An. subpictus	14	21	35	4	61	65	13	43	56	31	125	156 (19.64)
An. annularis	3	14	17	6	29	35	9	43	52	18	86	104 (13.09)
An. vagus	2	4	6	4	9	13	3	16	19	9	29	38 (4.78)
An. lindesayi	1	3	4	2	7	9	2	5	7	5	15	20 (2.51)
An. barbirostris	1	2	3	1	4	5	1	8	9	3	14	17 (2.14)
N	44	178	222	35	248	283	44	245	289	123	671	794 (100)

Table 3 Area wise CSP–ELISA positive rates.

Species	Fort					Jalali					Tappal				
	LT	Others	Total	Positive	Pre (%)	LT	Others	Total	Positive	Pre (%)	LT	Others	Total	Positive	Pre (%)
An. culicifacies	12	91	103	4	3.88	8	87	95	2	2.10	7	83	90	1	1.1
An. stephensi	10	40	50	1	2.0	9	51	60	2	3.33	9	47	56	1	1.78
An. subpictus	14	21	35	0	0	4	61	65	0	0	13	41	54	0	0
An. annularis	3	14	17	1	5.8	6	29	35	0	0	9	40	49	1	2.04
An. vagus	2	4	6	0	0	4	9	13	0	0	3	15	18	0	0
An. lindesayi	1	3	4	0	0	2	5	7	0	0	2	5	7	0	0
An. barbirostris	1	2	3	0	0	1	4	5	0	0	1	7	8	0	0
N	43	175	218	6	2.75	34	246	280	4	1.42	44	238	282	3	1.06

implemented on little reliable report involved in malaria transmission. Successful implementation of a vector control programme in India will help to pinpoint the main vectors and to develop knowledge on the bionomics of the species involved in the disease transmission.

Anopheline mosquitoes were collected from the three study sites of Aligarh district in India. *An. culicifacies* and *An. stephensi* were previously incriminated as malaria vector in Aligarh. There was, however, no previous report in favour of infections in *An. annularis* in Aligarh as Wajihullah and Saifi (2001) incriminate *An. culicifacies* and *An. stephensi,* vectors of malaria. This study was conducted within a short period of time and mosquitoes were not collected on a seasonal basis. Thus, there might be a chance to miss some of existing anophelines species. In the Fort area, the highest prevalence rate of 2.75% of CSP in *Anopheles* mosquitoes was recorded whereas in Jalali and Tappal CSP prevalence rate was found 1.42% and 1.06% respectively.

A total of three species was found CSP-positive in the present study. The result of this study was compared with a recent study conducted in Assam state of North-Eastern India, where there was evidence of CSP infection in *Anopheles karwari*, *Anopheles maculatus*, *Anopheles nigerrimus*, *An. barbirostris* and *An. subpictus* (Prakash et al., 2004). Korgaonkar et al. (2012) reported a prevalence rate of 1.3% of CSP in *An. stephensi* from Goa. Alam et al. (2012) in rural Bandarban, Bangladesh reported 15 (0.6%) female anophelines belonging to eight species were found to be positive for *Plasmodium* infection by CSP–ELISA. Of those, 11 (0.4%) mosquitoes were positive for *P. falciparum* and four (0.2%) for Pv-210. No mosquito was found positive for Pv-247. *An. maculatus* (2.1%, 2/97) had the highest infection rate followed by *Anopheles umbrosus* (1.7%, 2/115) and *An. barbirostris* (1.1%, 2/186).

The presence of CSP in *An. annularis* species has been reported for the first time in Aligarh, which is an important finding of this study. *An. culicifacies* was highest in this study collection and also in CSP infection (7/288, 2.43%): this species has been incriminated as a predominant malaria vector in Aligarh. *An. stephensi* was second in this study collection and also in CSP infection (4/166, 2.40%) whereas *An. annularis* was lowest in CSP infection (2/101, 1.98%). Although *An. subpictus* and *An. vagus* and *An. barbirostris* also appeared to have vector potential but CSP was not detected in these species in the present findings.

6. Conclusion

Findings of this study suggested that anopheline species other than *An. culicifacies*, *An. stephensi* and *An. annularis* might have a role in the transmission of malaria in Aligarh. The detection of CSP in some anopheline species should be taken into consideration for further studies to investigate their possible role in malaria transmission in Aligarh district of India. There is still remaining controversy for CSP–ELISA particularly due to its false positive results in previous studies. Thus, positivity in a CSP–ELISA should not be taken as the only criterion in confirming the vector status of an *Anopheles* species (Beier et al., 1987; Lochouarn and Fontenille, 1999; Somboon et al., 1993).

Conflict of interests

The authors declare that there is no conflict of interests in this paper.

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