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Malaria epidemiology in changing scenario and anopheles vector in Ghaziabad district, Uttar Pradesh, India

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Abstract
In the past few decades, there have been change in the malaria distribution pattern due to several environmental and manmade conditions including developmental activities. Ghaziabad district, Uttar Pradesh adjoining the capital of India, Delhi once has rural malaria epidemic, but due to developmental activities by urbanization the breeding patterns of Anopheles is changing resulting in changed malaria transmission dynamics. Vector incrimination using ELISA and its confirmation by PCR is done to know the malaria transmission potential of Anopheles mosquito. Only 2.62 % mosquitoes collected were Anopheles spp. along with *Culex quinquefasciatus* and *Aedes* spp. 1.17% anopheles mosquitoes were found infected with Plasmodium parasite as detected by ELISA of which 0.58% were confirmed by PCR. Reportedly *An. culicifacies* acting as vector of malaria since 1976. It is still doing its part in transmission while urban vector *An. stephensi* which appeared around 2005 is slowly becoming a major vector for malaria transmission in rural as well as urban along with peri-urban.

Keywords: Epidemiology, Urbanization, Primary Health Centre (PHC), Vector

1. Introduction
Urbanization in India is occurring since 1950s with the industrial revolution. Population living in urban areas was 28.53% in 2001 which increased to more than 31% in 2011 [1]. Urbanization has caused ecological changes which have influenced vector borne diseases by altering breeding places of mosquitoes causing change in their population structure and distribution. Malaria, most unstable vector borne diseases of tropical environment caused by Anopheles mosquito reportedly responsible for around one million cases and thousands of deaths in 2010 [2-4]. With increasing population and urbanization breeding pattern of Anopheles mosquito is influenced causing change in malaria transmission [5]. Normally Anopheles does not breed in polluted water but some have adapted to contaminated water for breeding [6]. Thus to understand malaria transmission there is a need to study malaria vectors and their bionomics. In India about 58 species of Anopheles are reported of which 6, the primary vectors of malaria are i.e. *Anopheles culicifacies, An. stephensi, An. dirus, An. fluviatilis, An. sundaicus* and *An. minimus*, 4 are secondary vectors *An. annularis, An. varuna, An. jeyporiensis* and *An. philippinensis*. among anopheles vectors, *An. culicifacies*, alone is responsible for 70-80 % of malaria cases in India [7]. Ghaziabad, a district of Uttar Pradesh is included under National Capital Region and is witnessing fast growing urban population from around 1.5 lakhs in 1951 to more than 31 lakhs in 2011 (Census 2011) with fast-paced development. Similar change in surrounding rural villages like lifestyle, livelihood and waste generation. New urban settlements have been created by filling wetlands and rivers in Ghaziabad city [8]. Ghaziabad once endemic to malaria had around 20,000 malaria cases in 1977 with *An. culicifacies* as the principal malaria vector (Ghaziabad PHC).There is significant variation in malaria transmission in different areas of the same district of Ghaziabad with old city, new urban areas, developing rural areas and urban areas proximal to Delhi. Increasing population and urbanization has caused ecological changes which are

expected to change malaria scenario in Ghaziabad. For malaria intensity assessment it is important to determine human-vector contact. For determining dynamics of malaria transmission in Ghaziabad serological and molecular-based analysis will be done using ELISA to find out extent of infection and further confirmation of positive samples by PCR [9]. Thus this study on the potential of Anopheles mosquitoes in Ghaziabad for malaria transmission by studying serological prevalence of malaria will help in knowing malaria transmission dynamics.

2. Materials and methods

2.1 Study area

Ghaziabad District lies at the latitude 28°40'north and longitude 77°25'east. It is situated in the northeastern part of National Capital Region. Rapidly urbanizing Ghaziabad city tagged as the 2nd fastest growing city in world [10]. It is in the middle of the Ganga-Yamuna Doab (land between two rivers) having Meerut on North, Bulandshahar & Noida on South, Delhi on West and Hapur on East side. It is well connected with important cities via three national highways NH-58, NH-91 & NH-24. The main railway line and two branches of northern railway pass through the City Ghaziabad. This city falls under severe seismic zone (zone IV) by seismic zoning map (India environmental portal report). Hindon River, the important tributary of Yamuna River passes through the center of the city. Annual precipitation of Ghaziabad is 732 mm limited from June to September and temperature ranging from 7°C to 46°C. Ghaziabad has malaria endemicity with *An. culicifacies* reportedly a primary malaria vector till 2004 [11] and after 2006 *An. culicifacies* and *An. stephensi* as principal malaria vector along with other disease-causing mosquitoes *Ae. aegypti* and *Cx. quinquefasciatus* [12].

Our study comprises urban, rural and peri-urban parts among 5 PHCs of Ghaziabad district as follows:

Table 1: Areas selected for mosquito collection in Ghaziabad.

Type	PHC	Villages	Latitude	Longitude
Rural	Bhojpur	Manaki	28.796224	77.570729
		Kalchhina	28.766509	77.579055
	Muradnagar	Bhanaira	28.857132	77.484298
		Didauli	28.818787	77.514596
	Loni	Pachayara	28.800211	77.211614
		Harampur	28.772453	77.237706
	Razapur	Bhadoli	28.770723	77.429967
		Mathurapur	28.748526	77.410741
Peri-urban	Ghaziabad	Duhai	28.741637	77.480184
		Sadarpur	28.704469	77.486547
Urban	Ghaziabad	Ghaziabad city	28.669156	77.453758

Fig 1: Ghaziabad Map showing PHCs and study sites

2.2 Data Collection

Malaria data collected from all the available sources like Primary Health Centers, sites [13] and books [14-16] and from health data of NVBDCP (National Vector Borne Disease Control Programme) and NIMR (National Institute of Malaria Research).

2.3 Mosquito sample collection

Mosquitoes were collected from Ghaziabad in Pre-monsoon (April- June), Monsoon (July-September) and Post-monsoon (October-February) seasons from April 2014 to January 2016 using following methods:

2.3.1 The Hand catch method

The indoor resting adults were collected by using suction tube and torch between 6 am to 11 am in the morning [17] and collected mosquitoes were transferred to test tubes in batches of 8-10. Prior to collection consent was obtained from each house. 5 houses were selected based on the proximity to breeding sites i.e. the houses within 2 km of breeding sites. The collections were done from human dwelling, cattle sheds, and mixed dwellings

2.3.2 The Space spray method

Pyrethrum spray/Aerosol was carried out in few suitable human dwellings. It was done after closing all entry and exit points of the room and covering entire floor space with white bed sheets and sprayed with pyrethrum. After waiting for 10-15 minutes, mosquitoes were collected from the bed sheets with the help of a pair of fine forceps and transferred to the

labeled Petri-dishes. The collected mosquitoes were stored after drying in silica gel.

2.3.3 Outdoor larva collection

Was done from all the expected breeding sites of anopheles mosquitoes like pools, ponds, rivers, agricultural fields, pits, tanks, irrigation channels, drains, rainwater collections, containers, sintex, flower pots etc. For this standard WHO Dipper of 500 ml volume was used for sampling of larva. The immatures in all instars were then allowed to emerge in insectary. The emerged mosquitoes and collected adult mosquitoes were then identified to species level using keys [18-21].

2.3.4 Temperature and relative humidity were recorded using thermo-hydrograph in each selected village and town of all PHCs.

2.4 Detection of parasite by ELISA

For ELISA test primary vectors *An. culicifacies* and *An. stephensi* were processed individually while secondary vector *An. annularis* and non-vector *An. subpictus* were processed in pools of 5-10 following Protocol of Wirtz *et al* by macerating head and thorax of anopheles mosquito in 100 µl of grinding buffer using pestles. Pestles were rinsed with 150 µl of grinding solution. Pestles were cleaned in between to prevent cross-contamination. Micro titration plates in replicas of 2 variants of circumsporozoite protein of *Plasmodium vivax*; VK 247, VK 210 [5] and *Plasmodium falciparum*; Pf were incubated with 50 µl of monoclonal antibody solution in each well making this as the first antibody layer. Well were then filled with 200µl of blocking buffer and incubated again for 1hr at RT. After aspiration, the mosquito triturate, positive control and negative control acting as antigen were loaded and incubated again at RT for 2 hrs. Washing of wells was done two times with PBS-Tween and then peroxidase conjugate solution was added. Finally, 100µl of freshly prepared substrate solution prepared from citric acid, OPD (o-phenylenediamine dihydrochloride) and H_2O_2 (Hydrogen peroxide) was added to each well and incubated in dark for 30 min. The plates were then read visually at 405 nm.

2.5 PCR confirmation of Parasites

DNA was extracted from ELISA lysate stored at -20°C following modified phenol-chloroform method. The target DNA was amplified using Multiplex PCR using veriti ™ 96 well thermal cycler as described Cuhna *et al*. The amplification reaction for the Multiplex PCR is as follows: 96°C for 10 min; 30 cycles of 95°C for 1 min, 55°C for 2 min; and 60°C for 30 min. Positive controls were DNA extracted from *Plasmodium vivax* and *P. falciparum* prepared from culture. Vials without DNA served as negative controls. The PCR products were fractionated on 1.5 % agarose gel and stained with ethidium bromide and photo documented.

Table 2: Primers for PCR Confirmation

Plasmodium sp.	Primers [22]	Base pair
Plasmodium falciparum	5'CCTGCATTAACATCATTATATGGTACATCT3')	273
	(5'GATTAACATTCTTGATGAAGTAATGATAATACCTT3')	
Plasmodium vivax	(5'AAGTGTTGTATGGGCTCATCATATG3')	290
	(5'CAAAATGGAAATGAGCGATTACAT3')	

2.6 Entomological data analysis

Entomological analysis will be done using following formulas [23].

A. Man Biting Rate

The Man Biting Rate of Anopheles mosquitoes was calculated using formula

$$\textbf{MBR} = \frac{\text{No. of Vector species caught}}{\text{No. of occupants in rooms sampled}}$$

B. Sporozoite Rate

The sporozoite rate is calculated from these results using formula

$$\textbf{SR} = \frac{\text{No. of Vector species found infected}}{\text{Total No. of Vector species examined}}$$

C. Entomological Inoculation Rate

EIR is calculated from sporozoite rate and Man biting rate using formula.

Entomological Inoculation Rate (EIR) = Sporozoite Rate X Man Biting Rate

3 Results

3.1 Malaria incidence in Ghaziabad

3.1.1 District wise

Data from 1977 to 2015 was taken from books [14-16], District Malaria Officer, Ghaziabad and from NVBDCP report. According to this data, malaria cases were highest in 1970s where population was very low (figure 2). Plasmodium vivax remain major contributor to the malaria cases (80-90%). Overall malaria shows decreasing trend in last 38 years with dominating Plasmodium vivax over P. falciparum. No mortality was reported in the given period.

3.1.2 PHC wise

Annual parasite incidence (API) was analyzed from 2011 to 2015 in PHCs of Ghaziabad district (figure 3). API shows a different trend in each PHC. Loni and Ghaziabad PHC has similar trend in which malaria cases increased in 2012 and again started decreasing. Bhojpur and Muradnagar has decreased trend of malaria from 2011 onwards. Ghaziabad is an urban area while Loni is proximal to Delhi region. Thus both suspected of this trend temporarily due to increasing urbanization and changing malaria vector and hence malaria transmission.

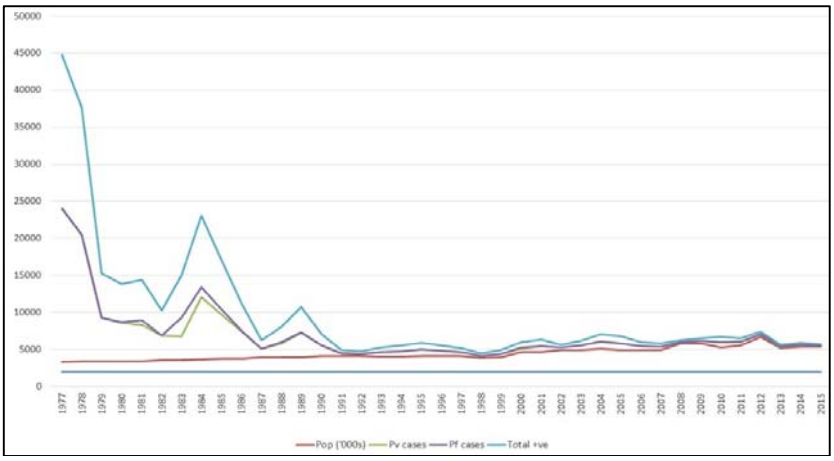

Fig 2: Trend of malaria in Ghaziabad district.

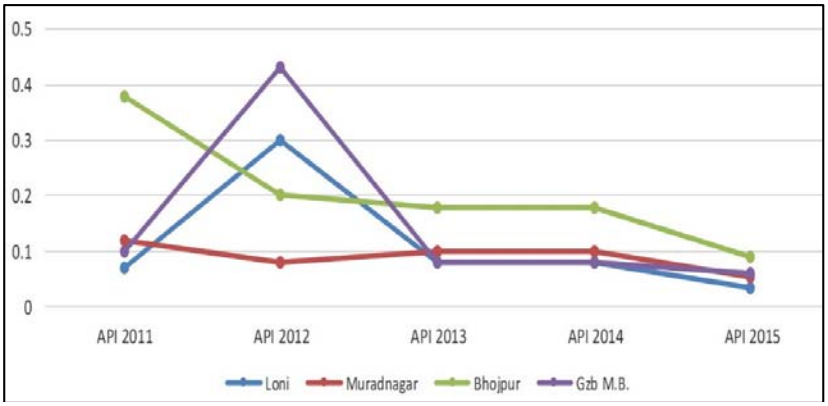

Fig 3: Incidence of malaria in PHCs of Ghaziabad in last 5 years

3.2 MHD in field survey

Out of total 36,170 mosquitoes were collected, of which 949 mosquitoes were Anopheles mosquito i.e. 2.62 %. Other mosquito species collected were *Culex quinquefasciatus* and *Aedes aegypti***.** The detailed MHD of Anophelines collected from April 2014 to January 2016 is given in table 3. Among Anopheles species, *An. subpictus* was the most dominating followed by *An. annularis* (Secondary vector). Among primary vectors *An. culicifacies* was dominant followed by *An. stephensi* (Urban Malaria Vector).

Table 3: Man hour density of Anophelines in our field survey.

Species	2014				2015			
	Rural	**Periurban**	**Urban**	**Total**	**Rural**	**Periurban**	**Urban**	**Total**
An. culicifacies	39(33.91)	15(13.04)	1(0.86)	55(5.7)	45(39.13)	11(9.56)	4(3.47)	60(6.3)
An. stephensi	12(6.74)	6(3.4)	3(1.7)	21(2.2)	118(66.3)	28(15.73)	11(6.2)	157(16.5)
An. annularis	77(42.3)	4(2.2)	0(0)	81(8.5)	98(53.8)	3(1.6)	0(0)	101(10.6)
An. subpictus	124(26.5)	59(12.6)	25(5.3)	208(22)	209(44.65)	32(6.8)	19(4.01)	260(27.4)
An. pulcherrimus	0	0	0	0	3 (3.2)	0	0	3(0.3)
An. nigerrimus	0	0	0	0	0	3(3.2)	0	3(0.3)
Total	949							

3.3 Detection of Plasmodium Parasite

For detection of infection with *Plasmodium* spp., 680 female Anopheles mosquitoes were analyzed by ELISA. The results for ELISA are given in table 4. Of the analyzed specimens, 74 were *An. culicifacies*: 66 were *An. stephensi:* 399 were *An. subpictus* and 141 were *An. annularis* in which an infection rate of 1.17% (8/680) was found in Ghaziabad district. Of these *An. culicifacies* shows infection of 4.05 %(3/74) with Plasmodium vivax; one in Pachayara (Loni), one in Bhadoli village (Razapur) and one in Kavi Nagar slums. *An. stephensi* shows infection of 4.5 %(3/66) from both Plasmodium vivax and Plasmodium falciparum: one from Pachayara (Loni) and two from Bhadoli village (Razapur). Nonvector *An. subpictus* also shows infectivity of 0.5% (2/399) with Plasmodium vivax in Bhanera village (Muradnagar) and Manaki village (Bhojpur). Among malaria parasites, only 6.25% was *Plasmodium falciparum* while rest of the parasite was *Plasmodium vivax*.

Table 4: Detection of *Plasmodium parasite* in Ghaziabad mosquito samples using ELISA

S.no.	Anopheles species	Samples processed	Number of ELISA positive	Type of positive	AREA (PHC)	%
1.	*Anopheles culicifacies*	74	3	PV210(1), PV247(1) PV210+PV247(1)	Pachayra (Loni)+ Bhadoli (Razapur)+ Kavi Nagar (Ghaziabad M.B)	4.05
2.	*Anopheles stephensi*	66	3	Pf+PV 247(1) PV210(2)	Pachayra (Loni) Bhadoli (Razapur)	4.5
3.	*Anopheles subpictus*	399	2	PV247(2)	Bhanera (Muradnagar) Manaki (Bhojpur)	0.5
4.	*Anopheles annularis*	141	0	NA		
5.	*Total*	680	8			1.17

3.4 PCR confirmation of Malaria parasites

The mosquitoes identified positive by ELISA were confirmed using PCR. Elisa Lysate of all samples was used for examination i.e. malaria vectors, non-vectors, and secondary vectors. This was done as ELISA has been reported of false positivity or sometimes non-detection of positives. *An. subpictus* have been found positive (reported vector of malaria recently) [24, 25] by ELISA hence examination of all positive samples is essential. Out of 8 ELISA positive samples, 4 were confirmed to be positive by Multiplex PCR. The Anopheles found positive for Plasmodium vivax of 293 bp DNA. Of these 4 samples; 2 were *An. culicifacies* (Rural malaria vector) one *An. stephensi (*Urban malaria vector*)* and one *An. subpictus*.

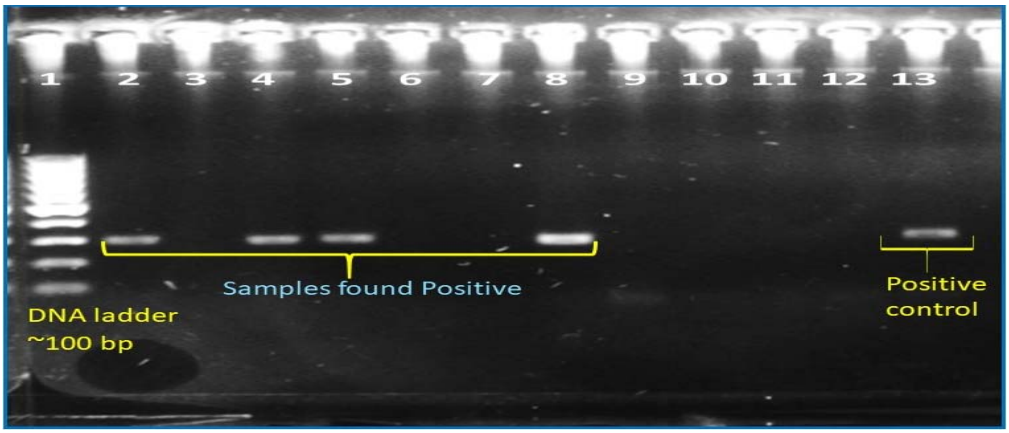

Fig 4: Detection of sporozoites in *Anopheles Mosquitoes by* Multiplex PCR.

Lane 1:100bp ladder; Lanes 2-11, test samples; Lane 12, negative control; Lane 13: Positive Control, Plasmodium vivax; Lane 2,4,5,8, P. *vivax* positive sample showing an amplification of 293 bp.

3.5 Entomological Analysis

3.5.1 Man Biting Rate (MBR)

As observed in table 5, Maximum Man biting rate was found for the areas selected for total catch collection. The sites were selected based on high vector density and suitable room for collection. Manaki (Bhojpur) has highest biting rate of 6.16 followed by sadarpur. Maximum MBR for *An.culicifacies* was 1.0 for Kavi Nagar of Urban Ghaziabad followed by Manaki Village (Bhojpur) having MBR of 0.83. *An.stephensi* has maximum biting rate of 2.0 in Kavi Nagar (Urban) followed by Mathurapur with biting rate of 1.0. Other Anophelines shows maximum biting rate in Sadarpur of Peri-urban Ghaziabad.

Table 5: Man biting rate of Ghaziabad in Monsoon season

PHC	Type	Village/ Colony	No. of rooms visited	Total No. of occupants	No. of fed *An. culicifacies*	MBR/ person	No. of fed *An. stephensi*	MBR/ person	No. of other fed anophelines	MBR/ person	Overall MBR/ Person
Bhojpur	Rural	Manaki (2014-15)	1	6	5	0.83	1	0.16	31	5.16	6.16
	Rural	Kalchhina (2014-15)	1	3	2	0.66	2	0.66	14	4.66	6
Razapur	Rural	Mathurapur (2015-16)	1	5	4	0.8	5	1.0	9	1.8	3.6
Muradnagar	Rural	Bhanaira (2014-15)	1	5	1	0.2	0	0	18	3.6	3.8
Razapur	Rural	Bhadoli (2015-16)	1	2	1	0.5	0	0	6	3.0	3.5
Loni	Rural	Pachayara (2014-15)	1	4	2	0.5	1	0.25	8	2.0	2.75
Total											4.3
Ghaziabad	Urban	Kavi nagar	1	3	3	1.0	6	2.0	7	2.33	5.3

city		(2015-16)									
Ghaziabad	Peri-urban	Sadarpur (2014-15)	1	4	0	0	0	0	24	6.0	6

3.5.2 Sporozoite rate (SR %)

Sporozoite rate was calculated in each village and town of rural, urban and peri-urban Ghaziabad as shown in table 7. Pachayara village of Loni shows the highest sporozoite rate(8.3%) followed by Kavi Nagar,Urban(5.2), Bhadoli village of Razapur (3.4 %), Manaki village of Bhojpur(1.75%) and Bhanaira village of Muradnagar(0.4%). Rural Ghaziabad, therefore, contributing 1.07 % to Ghaziabad district in malaria transmission. Urban Ghaziabad (Kavi Nagar slums) although has low density of *An. culicifacies* yet has sporozoite rate of 5.2 %. Urban Ghaziabad has higher sporozoite rate compared to rural while no infective mosquito was found in peri-urban Ghaziabad. *An. culicifacies* and *An. stephensi* are both incriminated as vector of malaria in Ghaziabad with equal potential for malaria transmission. *An. subpictus*, a non-vector also found positive for *Plasmodium* in this study as well as in earlier studies [24, 25].

Table 7: Sporozoite rate of Anophelines

Village/ Town	*An. culicifacies*		*An. stephensi*		*An. subpictus*		*An. annularis*		SR%
	Number examined	Number positive	Number examined	Number positive	Number examined	Number positive	Number examined	Number positive	
Bhanaira Rural	12	0	3	0	106	1	132	0	0.4
Didauli Rural	18	0	1	0	60	0	0	0	0
Bhadoli Rural	10	1	8	2	39	0	1	0	3.4
Mathurapur Rural	5	0	12	0	42	0	6	0	0
Manaki Rural	11	0	2	0	43	1	1	0	1.75
Kalchhina Rural	3	0	4	0	15	0	1	0	0
Harampur Rural	0	0	1	0	0	0	0	0	0
Pachayra Rural	10	1	2	1	10	0	0	0	8.3
Total Rural	69	2	33	3	315	2	141	0	1.07
Peri-urban (Duhai+Sadarpur)	0	0	0	0	75	0	0	0	0
Urban (all paradigms)	5	1	33		9	0	0	0	5.2
Total	74	3	66	3	399	2	141	0	1.17

3.5.3 Entomological inoculation rate (EIR)

EIR is an important parameter to find out the malaria transmission status of an area. Thus each type of Ghaziabad was studied for its EIR. Highest EIR was found to be of urban Ghaziabad i.e. 27.56 which states it to be at high risk of malaria. Rural area has lower EIR 4.6 but still at risk of malaria epidemic. Pachayra (Loni) has high EIR of 22.825 hence at high risk of malaria.

Table 8: Entomological Inoculation Rate of village/city

Type	MBR	SR%	EIR
Rural	4.3	1.07	4.601
Manaki	6.16	1.75	10.78
Mathurapur	3.6	0	0
Bhanaira	3.8	0.4	1.52
Bhadoli	3.5	0	0
Pachayra	2.75	8.3	22.825
Urban	5.3	5.2	27.56
Peri-urban	6	0	0

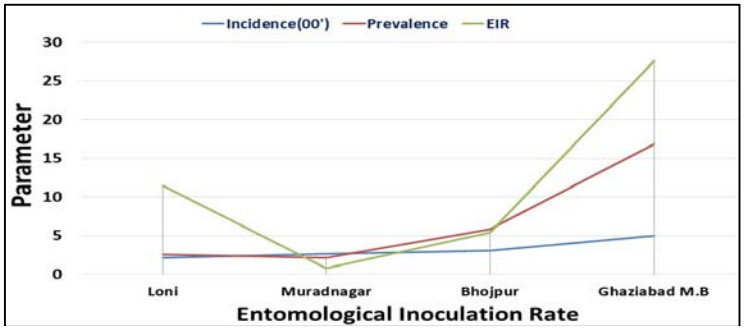

Fig 5: Relationship of Annual EIR with Incidence and Prevalence in PHCs of Ghaziabad.

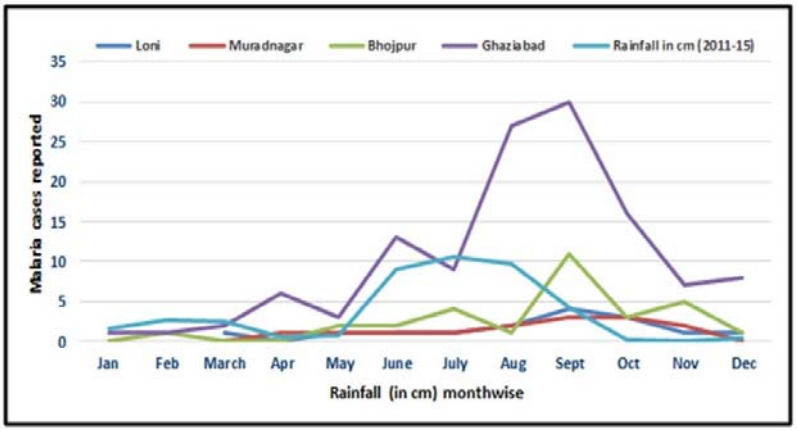

Fig 6: Rainfall pattern and its Relation with morbidity in Ghaziabad

4 Discussion

Ghaziabad used to be a rural district which has witnessed rapid development in last 20 years due to migration from Delhi and all over India. This increasing population and urbanization has caused various ecological changes in Ghaziabad. Earlier Ghaziabad had unstable rural malaria with spurts of cases seasonally (EIR=0-27.56). But with increasing urbanization due to change in breeding pattern of vector of malaria change in malaria transmission is found. A study was conducted for 5 years (2011-2015) to assess risk of malaria in 3 PHCs i.e. Loni, Muradnagar and Bhojpur, Ghaziabad district from rural whereas the Ghaziabad PHC was taken as suburban and urban.

Malaria incidence and the prevalence were found maximum for Ghaziabad PHC to the rural PHCs. Month wise data showed transmission from June to October i.e. during monsoon season only. This may be due to the construction boom leading to presence of breeding sites for urban malaria vector *An. stephensi* that has caused more cases in urban Ghaziabad. Rural Ghaziabad had seasonal breeding sites like pools of water in which *An. culicifacies* bred and caused seasonal malaria in whole Ghaziabad district.

In our study, density of collected mosquitoes during July to October i.e. rainy season was relatively higher. Figure 6 also shows peaks during rainy season and post rains for rural Ghaziabad i.e. from August to October showing maximum cases. While for Ghaziabad PHC peaks of breeding were present irrespective of monsoon, although highest in rainy season showing presence of malaria. Thus rains showed no significant impact on malaria transmission in urban and peri-urban Ghaziabad.

The infection status of different Anopheles mosquitoes in Ghaziabad showed prevalence of two vectors i.e. *An. culicifacies* and *An. stephensi*. *Plasmodium vivax* is contributing majority of malaria cases. Maximum EIR (Entomological Inoculation Rate) was found in urban Ghaziabad i.e. 27.56 further clarifying the fact that urbanization has changed malaria scenario from rural to urban vector responsible for majority of malaria transmission. A non-vector *An. subpictus* is found positive for *Plasmodium vivax* infection due to unknown reasons. Further study is needed for its confirmation. *An. subpictus* is breeding in rural, urban and peri-urban in habitats like pools, polluted water, muddy water at construction sites etc. i.e. this species is omnipresent with maximum density. Currently, both primary vectors *An. culicifacies* and *An. stephensi* are breeding in urban as well as rural while peri-urban has breeding of mainly *Aedes aegypti* and *Culex quinquefasciatus*. This may be due to lifestyle changes in rural Ghaziabad storing practices such as cemented tanks, curing tanks, ground tanks and tube-wells which have become breeding places of *An. stephensi*. Earlier breeding sites for *An. culicifacies* used to be rivers, canals, ponds which have become polluted completely thus no breeding is occurring in these sites. Pits in rural as well as urban construction sites has become temporary breeding sites during rainy season for *An. culicifacies.* Peri-urban has either no provision of drainage or improper drainage which results in water stagnation and became suitable breeding site for *Culex quinquefasciatus*. *Aedes aegypti* has breeding sites available everywhere from drums, flowerpots, and tires in urban to coolers and plastic tanks in rural, peri-urban and urban. *An.stephensi* was mainly collected from the rural areas like Mathurapur, Panchayra, Bhanera villages; and Kavi Nagar slums and Viklang colony in urban but in low density. Above results clearly indicates impact of urbanization on breeding and *Plasmodium* infection of Anopheles Spp. Increase in population, urban area, and urban lifestyle all have influence on breeding and habitats of malaria vector which in turn has changed pattern of malaria transmission. Thus malaria transmission has decreased in Ghaziabad in last few years due to decrease in breeding sites of rural vector and increase in breeding sites of urban vector. Ghaziabad district is at high risk of malaria in both rural and urban and expected to transmit malaria in peri-urban also in the near future. *Aedes* which is already breeding in high number is cause of dengue in Ghaziabad that had appeared in 2004 (Ghaziabad PHC) and increasing at rapid rate. Other diseases like filaria and chikungunya may also appear if condition prevails.

5 Conclusion

Malaria has shown a decreasing trend in Ghaziabad in both at district as well as at PHC level. This might be due to decreasing breeding sites of *An. culicifacies* which breeds outdoors profusely and is capable of high transmission during the post-monsoon season. In addition to this urbanization has reduced the available breeding sites increased the population density in both urban and rural providing more habitat indoors than outside as a result of which container breeder *Aedes aegypti* is commonly found in the area and dengue cases are being reported every year now. Thus the transmission of

malaria which is mainly due to *An. culicifacies* in last 38 years and still contributing majorly is decreasing very rapid rate. This species appears mainly in rainy season when its breeding sites are present. Another vector of malaria *An. stephensi* is increasing at a rapid rate due to availability of its man-made breeding sites and has started contributing to the persistent malaria and is suspected to establish as a major vector of the region in years to come.

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Data statement
All the data generated is presented here except epidemiological data (district wise and PHC wise)

Ethics in publishing
All entomological surveys and collections conducted on private lands or in private residential areas were done with the owners'/residents' permission, consent and presence. These studies did not involve endangered or protected species.

Declaration in interest
Authors show no competing interest

Mission Declaration and verification
This work has not been published previously and is not under consideration for publication elsewhere.

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