Entomological determinants of malaria transmission in an epidemic prone area of District Nuh (Haryana state), India


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ABSTRACT

Background & objectives: Entomological investigations were carried out in highly malarious villages under Ujina PHC of District Nuh (Haryana state) which is an epidemic prone area in northwestern region of India. The study was aimed to have an in-depth understanding of the entomological parameters influencing malaria transmission in the study area.

Methods: The seasonal prevalence and biological attributes of vector mosquitoes were investigated during 2015 and 2016. Indoor resting vector mosquitoes were collected from human dwellings/cattle sheds and morphologically identified. Anopheles culicifacies were categorized to sibling species by species-specific inversions in polytene chromosomes and An. stephensi to ecological races on the basis of ridge number on egg float. The blood meal source analysis and incrimination studies of vectors were done by counter-current immunoelectrophoresis and enzyme-linked immunosorbent assay, respectively. Insecticide susceptibility test on vectors was performed as per WHO guidelines.

Results: Seasonal abundance of An. culicifacies and An. stephensi in the study area showed variation; the peak densities of both the vectors were observed during monsoon months which correlated well with the average monthly rainfall data. Though both vectors were found to be primarily zoophagic, the human blood index of An. culicifacies (HBI = 0.17) was significantly higher than that of An. stephensi (HBI= 0.02). Analysis of sibling species composition of An. culicifacies population showed that it comprised almost of sibling species A (>98%) which is an established malaria vector. Anopheles culicifacies was incriminated for Plasmodium vivax and P. falciparum circumsporozoite (CS) antigen during monsoon months in 2015 and 2016. Assessment of insecticide susceptibility status of malaria vectors against 0.5% deltamethrin revealed that An. culicifacies is more susceptible (95% mortality) than An. stephensi (85% mortality).

Interpretation & conclusion: The results suggest that An. culicifacies (species A) is playing a major role in malaria transmission in the study area and is almost susceptible to deltamethrin. Timely two rounds of indoor residual spray of synthetic pyrethroid with proper dosage and good coverage would be helpful in reducing vector population and consequently the malaria incidence. In addition, personal protection measures by the community would supplement the major intervention tool (IRS) in decreasing the man-vector contact.

Key words Anopheles culicifacies; An. stephensi; biological attributes; malaria vectors; malaria transmission; Nuh district

INTRODUCTION

The State of Haryana in northern India comprises of 21 districts and District Mewat1 was carved out as the 20th district of Haryana from erstwhile Gurgaon and Faridabad districts in April 2005, which has now been renamed as District Nuh in 20161. Since the major outbreak of malaria in Mewat region in 1996, several studies have been carried out enumerating various factors associated with malaria prevalence and persistence in the traditionally known epidemic belt of the northwestern plains of India. These included studies related to malaria vectors and parasite prevalence2–3; geographic information systems (GIS) and remote sensing (RS) based surveys defining malaria paradigms and delineating high risk areas4–5, and trends of malaria incidence and infection over the years6–7. Despite this, malaria still continues to be persistent in the region mainly because it lags far behind on almost all aspects of development as compared to the rest of Haryana and much remains to be achieved in terms of public health.

District Nuh is endemic for malaria and has three community health centres (CHCs), namely Nuh, Firozpur Jhirka and Punhana. Among these, Nuh CHC is highly malarious and contributes bulk of malaria cases reported from the district6. CHC Nuh comprises of five primary health centres (PHC), viz Nuh, Ujina, Ghasera, Taruru and Mohammadpur Aahir (Figs. 1 a–c). As per epidemiological data available with the Chief Medical Officer (Nuh
CHC); PHC Ujina has reported highest malaria cases from 2010–15 followed by PHC Nuh. Though An. culicifacies and An. stephensi have been reported as malaria vectors in this region, an in-depth study of their seasonal prevalence and biological characteristics is lacking. Therefore, a systematic longitudinal study was carried out to investigate the environment-dependent entomological factors that influence malaria transmission dynamics in the highly malarious villages under PHC Ujina. The findings of this study are presented in this communication.

MATERIAL & METHODS

Study area

District Nuh in Haryana state lies between 26° and 30° N latitude and 76° and 78° E longitude, and falls under the sub-tropical and semi-arid climatic zone. Though this district largely comprises of plains, there are patches of upland due to hills and hillock of the Aravali mountain. Nuh district is predominated by Muslims (Meos) whose main occupation is agriculture while animal husbandry is the secondary source of income.

Four highly malarious villages namely, Jaisinghpur, Karamchandpur, Chilawali and Gundbas falling under Ujina PHC were selected for this study on the basis of high annual parasite incidence (API) which ranged between 14.55 to 60.39 during 2010–14. These four villages in plain area are located within a radius of 3–5 km from each other and mainly have Pucca houses with adjacent cattlesheds. There are perennial ponds, and low-lying areas with seepage water due to high water table in the vicinity of these villages and majority of the houses have cemented tanks to store piped water supply.

Entomological investigations

Mosquito collection and processing: Indoor resting mosquitoes were collected from human dwellings and cattlesheds during early morning (0600 to 0800 hrs) in each village using a suction tube and torch light. The collections were made almost every month during 2015 and during the transmission season (July–September) in 2016. The anophelines collected were morphologically identified to species following standard keys. The average monthly man hour densities of vectors An. culicifacies and An. stephensi were calculated using the formula: Number of mosquitoes of each vector species collected × 60/Total collection time in minutes.

The dead and alive mosquitoes were brought to the laboratory for further processing to study various entomological parameters.

Anopheles culicifacies sibling species identification

In case of An. culicifacies, the blood-fed females were kept under ambient conditions in cloth cages and allowed to reach half gravid stage. From each individual half gravid female, ovaries were removed and preserved in modified Carnoy’s fixative (glacial acetic acid: methanol—1: 3). The preserved ovaries were processed for making polytene chromosome plates following method of Green and Hunt. Anopheles culicifacies were identified to sibling species using diagnostic paracentric inversions in polytene chromosomes.

Identification of An. stephensi for ecological races

Single tubing of field collected An. stephensi females was carried out in insectary (maintained at 28 ± 2°C and...
RH 60–70% in order to obtain F₁ progeny. Around 20–25 eggs of individual females were examined in binocular (Zeiss Axioplan, West Germany) for ridge number on the egg float. The individual single female was categorized as type form, intermediate and variety mysorensis as per the grading described by Subbarao et al.¹³.

**Blood meal source identification of vector species**

Anopheles culicifacies and An. stephensi collected from study villages were analysed for host feeding pattern. Midgut blood smears from fully-fed and half gravid females were subjected to blood meal source identification using human and bovine antisera by counter-current immunoelectrophoresis following the method described by Bray et al.¹⁴. The human blood index (HBI) was calculated for each vector species.

**Vector incrimination**

From the field collected vector species, the head and thorax of individual An. culicifacies and An. stephensi were processed for detection of circumsporozoite (CS) antigen of malaria parasite species prevalent in the area. Homogenate of head and thorax of individual mosquito in grinding buffer was tested for the presence of CS proteins using P. vivax (210 and 247 variants) and P. falciparum-specific monoclonal antibodies by enzyme-linked immunosorbent assay (ELISA)¹⁵-¹⁶.

**Insecticide susceptibility status of vectors**

The susceptibility status of An. culicifacies and An. stephensi was determined against deltamethrin which is presently used as indoor residual spray (IRS) in District Nuh. Susceptibility test was performed following the standard procedure. Blood-fed An. culicifacies and An. stephensi were exposed to 0.05% deltamethrin-impregnated papers for 1 h along with parallel controls. Mortality was recorded after 24 h holding period. The percent corrected mortality was calculated and the mosquitoes of each vector species were categorized as resistant, susceptible and under ‘verification required category’ following the guidelines of WHO.¹⁸

In addition, the average monthly rainfall data were obtained from Sadar Kanungo Department, District Nuh for the year 2015 for correlating with the seasonal variation in abundance of the malaria vectors.

**RESULTS**

Since perennial ponds and low-lying areas with seepage water were present in the vicinity of study villages and the inhabitants used cemented water storage tanks/wells (Figs. 2 a–e), both An. culicifacies and An. stephensi, the primary vectors of malaria were prevalent in the villages almost all through the year, though their abundance varied with season. The average man hour density

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**Figs. 2 (a–e):** Breeding habitats of vector anophelines in study area—(a) Perennial pond; (b) Low-lying area with seepage water; and (c–e) Cemented water storage tanks.
of primary malaria vectors in different months/seasons is shown in Fig. 3. Extremely low densities were observed in winter months (December–January) and a build-up of population of both vector species was noticed during spring/early summer, which was more pronounced in case of *An. stephensi*, probably due to favourable temperature of the water in cemented storage tanks (the preferred breeding habitat of *An. stephensi*) and a short spell of rains during that period. The peak densities of both vectors were observed during monsoon months (July–September) with average man hour density ranging from 30 to >70 which later declined in post-monsoon months (October–November). The seasonal variation in the vector density correlated well with average monthly rainfall data (Fig. 3). Besides *An. culicifacies* and *An. stephensi*, the other anophelines, viz. *An. subpictus*, *An. aconitus* and *An. annularis* were also prevalent in the study villages.

For determining *An. culicifacies* sibling species composition in study villages, a total of 264 females were examined during 2015–16. It revealed that species A was predominant in the study area, comprising 98.48% of total samples identified and was polymorphic for $i^1$ inversion (Fig. 4). However, the relative proportion of sibling species B and C was very low, comprising only 1.14 and 0.40% of the total identified samples, respectively. To know the ecological variants of *An. stephensi*, 50 isofemale lines were examined for ridge number on the egg float. The egg ridge number observed in single female culture ranged from 15–20 and the mode number of ridges was 16–17 which indicated that *An. stephensi* ‘type form’ was mainly prevalent in study area.

Regarding resting behaviour of *An. culicifacies* and *An. stephensi*, the preferred resting sites of both the species were cattle sheds. Out of 1410 *An. culicifacies* collected during study period 1311 (92.98%) were from cattle sheds and only 99 (7.02%) were found resting in human dwellings. Similarly, out of 1918 *An. stephensi* collected, 1806 (94.16%) were from cattle sheds and only 112 (5.84%) were found resting in human dwellings.

Feeding preference of vector species was also analysed in the study. Results of blood meal source identification revealed both *An. culicifacies* and *An. stephensi* to be primarily zoophagic. The overall HBI of *An. culicifacies* was 0.164 and the proportion of mixed positive samples was high (Table 1). In case of *An. stephensi*, the HBI was comparatively much lower (0.019) and only a few mosquitoes were found exclusively feeding on human blood.

Vector incrimination studies revealed two specimens of *An. culicifacies*, out of 742 tested, harbouring *P. vivax* and *P. falciparum* sporozoites in the year 2015 and 2016, respectively. However, none of *An. stephensi* tested was found positive for CS antigen of *Pv* and *Pf* parasites (Table 2). The sporozoite positive samples of *An. culicifacies* were retested to confirm the results.

![Fig. 3: Average man hour density of *An. culicifacies* and *An. stephensi* in study villages of PHC Ujina, CHC Nuh and average monthly rainfall in 2015.](image)

**Fig. 4:** Sibling species composition of *An. culicifacies* complex in study villages under Ujina PHC, District Nuh* (n=264). *Pooled data of all study villages (2015–2016).**
Results of susceptibility status of *An. culicifacies* and *An. stephensi* against 0.05% deltamethrin in study villages are summarized in Table 3. The percent mortality in *An. culicifacies* was 95.4, while in *An. stephensi* it was comparatively lower (85%). These observations indicate that *An. culicifacies* is more susceptible to deltamethrin as compared to *An. stephensi*.

**DISCUSSION**

Malaria transmission is complex and dynamic process and various factors, viz environmental, entomological, parasitological and socioeconomic conditions of the community influence the transmission pattern. These factors are strongly inter-linked and even minor change in one of the factors has its repercussion on others. Among entomological determinants of malaria, the prevalence and abundance (density) of vector species; the proportion of vector population biting humans (particularly in case of primarily zoophagic vectors) and longevity of vectors so as to enable them to support sporogonic development of malaria parasites are crucial in disease transmission. Seasonal variation in the prevalence of vector mosquito species and its biological attributes are very important in shaping malaria transmission pattern, especially in epidemic prone areas like Mewat. Malaria transmission in Nuh is essentially seasonal and of the three CHCs in the district, highest malaria cases are reported from Nuh CHC. An upsurge in malaria cases was reported in this area during 2011 and 2012. A hospital-based longitudinal study carried out by Naz et al. in Mewat has shown that almost 98% of the malaria cases were recorded between July and November and majority of the cases (80%) were reported during October and November. In such areas a spurt in malaria incidence is reported if vector control measures are not taken timely. For appropriate vector control measures adequate information on seasonal prevalence and biological attributes of vector species are essential.

In highly malarious study villages under Ujina PHC, longitudinal study of malaria vectors revealed seasonal variation in their abundance and peak densities of *An. culicifacies* and *An. stephensi* were observed during monsoon months (July–September) which correlated well with good rainfall during this period. The rainfall not only creates numerous fresh water pools, puddles, temporary ponds etc conducive for vector breeding, but also aids in favourable temperature and humidity conditions that help in enhancing the longevity of vectors.

Analysis of *An. culicifacies* sibling species composition in study villages revealed predominance of species A (>98%) which is an established malaria vector in malaria endemic regions of India. Additionally, *An. stephensi* population comprised of Type form, which is also an efficient malaria vector.

Though, both the vector species were primarily zoophagic, the HBI in case of *An. culicifacies* was comparatively much higher than that of *An. stephensi*. Moreover, *An. culicifacies* was detected with plasmodial sporozoites for two consecutive years (2015–16). These observations suggest that *An. culicifacies* is playing a major role in malaria transmission in the study area. *Anopheles culicifacies* plays a significant role in malaria transmission in many other parts of India mainly due to high densities.
Though there were indications of tolerance/resistance in malaria vectors against deltamethrin, which is presently used for IRS in the study area. Nevertheless, timely spray of two rounds of this insecticide with proper dosage and good coverage can significantly bring down the vector population and that will have an epidemiological impact, leading to substantial reduction in malaria incidence. There are such reports where IRS operation undertaken with proper dosage and good coverage, significantly brought down the malaria incidence in areas with prevalence of insecticide resistant An. culicifacies. From the present study, it is evident that topography and rainfall greatly influence the abundance of malaria vectors. The potential environmental and entomological risk factors include long spell of monsoon season with intermittent rainfall, water accumulation in form of ponds, rain and seepage water collections, cemented tanks (for water storage) and prevalence of established malaria vectors like An. culicifacies (species A) and An. stephensi (Type form). In such a situation, microstratification of problematic areas, based on epidemiological data to identify the hot spots in epidemic prone area, and concerted vector control measures in such areas on priority basis assume greater importance to prevent an upsurge in vector population and consequently the malaria incidence. Periodic vector surveillance, timely procurement of insecticide for two rounds of IRS with proper dosage and good coverage, and arranging man power for execution and supervision of spray operations are essential components of vector control measures. Prior information to villagers regarding spray schedule and seeking their cooperation contribute to the success of IRS operation. In addition, the communities may be sensitized through IEC activities for personal protection measures like use of repellents, coils, vaporizers and bednets which will supplement the major control measures. Prior information to villagers regarding spray schedule and seeking their cooperation contribute to the success of IRS operation. In addition, the communities may be sensitized through IEC activities for personal protection measures like use of repellents, coils, vaporizers and bednets which will supplement the major intervention tool in decreasing the man vector contact. Though vector control is an integral component of malaria containment programme, the importance of early diagnosis and prompt proper treatment of malaria infected individuals cannot be underrated.

Conflict of interest
The authors declare that they have no competing interests.

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REFERENCES

16. Wirtz RA, Duncan JF, NJeeleanjki EK, Schneider I, Brown AE,


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