J. Ent. Res. Soc., 10(3): 1-9, 2008 ISSN:1302-0250

# Breeding Habitats of *Culex tritaeniorhynchus* (Diptera: Culicidae), A Japanese Encephalitis Vector, and Associated Mosquitoes in Mysore, India

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## ABSTRACT

Japanese encephalitis is endemic in at least 21 countries including India with 30,000 to 50,000 cases annually. In this regard, *Culex tritaeniorhynchus* species, the major vector of JE in Karnataka State, India was chosen for the current investigation. Information on the species composition of various mosquitoes in an area is helpful to predict epidemics. Two years long study was conducted at Mysore to document the associated culicidae mosquito species in the breeding places of *Culex tritaeniorhynchus*. The larvae of *Cx. tritaeniorhynchus* and associated species have been collected from temporary and semi permanent ground water pools. Simultaneously they have also been collected from plain rice field area from a 26 km radius outside Mysore. Third instar larvae were taken for categorization and reared until adult emergence for further confirmation of the species. A total of 19 species belonging to 4 genera (*Culex, Aedes, Anopheles* and *Armigeres*) were registered from the two habitats. Four genera with 13 species were found to breed in ground pools as against 3 genera with 14 species in paddy field habitats. *Culex bitaeniorhynchus, Cx. nigropunctatus, Cx. barraudi, Cx. hutchinsoni, An. culicifacies* and *An. stephensi* were seen only in paddy field habitat, whereas *Cx. quinquefasciatus, Cx. gelidus, Ae. albopictus* and *An. jamesii* were found only in ground pool habitats. *Culex tritaeniorhynchus, Cx. vishnui, Cx. pseudovishnui, Cx. fuscocephala, Cx. murreli, Ae vitattus, An. vagus, An. barbirostris* and *Cx. fuscanus* were recorded from both the breeding places.

*Key words*: Mosquitoes, *Culex tritaeniorhynchus*, larval habitats, Mysore, Japanese encephalitis.

## INTRODUCTION

Diptera is the most important order of insects in medical entomology and mosquitoes are belonging to the family Culicidae with about 3450 species in 37 genera. Some of the most important diseases transmitted by them are malaria, filariasis, Japanese encephalitis, yellow fever, dengue hemorrhagic fever (DHF), rift valley fever etc. (Walker, 1994). Among the mosquito transmitted viral diseases in India Japanese encephalitis occupies an important public health position.

Japanese encephalitis is a leading cause of viral encephalitis in Asia with 30,000-50,000 clinical cases reported annualy. (Gingrich *et al*., 2001). In India an estimated 378 million people are living at the risk of JE in 12 States including Karnataka State (Nagabhushana, 2000). Outbreaks of JE of varying intensity have been occurring in Kolar and Mandya districts of Karnataka state since 1977 (Mishra, 1984). Recent research shows that J.E virus has been isolated from 16 species of mosquitoes in India, the *Culex vishnui* subgroup being the major vectors consisting of *Cx. tritaeniorhynchus*, Giles. *Cx. vishnui*, Theobald and *Cx. pseudovishnui*, Colless (Samuel *et al.*, 2000). *Cx. tritaeniorhynchus* is considered as the most important vector of J.E virus in most part of the south central Asia (Gingrich *et al.*, 2001). In the present global scenario of rapidly changing ecological and environmental conditions, there is a need to monitor bionomics of disease vectors on a regular basis (Prakash *et al.,* 1997). Irrigation development and human resettlement project in the tropics often are followed by a high incidence of mosquito borne diseases such as malaria and Japanese encephalitis (Amerasinghe and Indrajith, 1994).

Different populations have certain specific characteristic features which facilitate the formation of epidemiological characteristics of vector borne-disease (Kondrashin and Kalra, 1987). Rapidly changing environment due to anthropogenic activities brings about frequent changes in vector behavior, which necessitate the regular general information on vector bionomics (Prakash *et al.,* 1998).

Biotic components include both intra specific and inter specific interactions. Intra specific interaction can be beneficial, involving co-operation or deleterious, involving competition. Competition, symbiosis, predator-prey interactions, herbivore-plant interactions and indirect interactions comprise types of interspecific interactions (Eldridge and Edmon, 2000).

JE virus is maintained in nature by a complex cycle that involves pigs as amplifying hosts, Ardeid birds as reservoirs and mosquitoes as vectors. *Culex tritaeniorhynchus* is the most common JE vector in South Central Asia associated with rice fields. It feeds readily on the amplifying host pigs and Ardeid birds, as well as humans. Larvae of *Cx. tritaeniorhynchus* generally prefer lightly shaded ground pools with low concentration of organic matter and some emergent vegetation. Also paddy fields are ideal breeding sites for this species.

The *Tritaeniorhynchus* complex comprises *Cx. tritaeniorhynchus* and infra species *summorosus* and *siamensis*. Sucharit *et al*. (1989) by studying the morphotaxonomy have shown that the *Culex tritaeniorhynchus* of different provinces of Thailand can be differentiated into three types A, B and C. Reuben *et al*. (1994) from India have provided simple illustrated keys to the species of *Culex (Culex)* associated with Japanese encephalitis in Southeast Asia. Earlier studies made by the authors on *Cx. tritaeniorhynchus* have revealed two morphological variants from the two breeding sources of Mysore, the variety from Mysore city (ground pools) as type A and the variety from outside Mysore (paddy field) as type B (Fakoorziba and Vijayan, 2003). Das *et al.* (2006) have reported that different species of Culicidae family mosquitoes can be found in various habitats. Information on the species composition of various mosquitoes in an area is important in epidemiological studies. So in this investigation associated Culicidae mosquitoes in the breeding places of two types of *Culex tritaeniorhynchus* in different habitats of an endemic area of JE was undertaken.

## MATERIAL AND METHODS

The study was carried out in Mysore city and the outskirts in Karnataka state, India. This city is situated at an altitude of 1085 MSL in a saucer shaped basin with Chamundi hill at the Southeast end. The city has an area of over 40.05 Kms with the latitudinal and longitudinal coordinates of location, 12$^{o}$ 18' N and 76$^{o}$ 42' E. The city has a salubrious climate even though it is located in the tropics and in the interior of the peninsula. This equable climate is due to its elevated position on the plateau surface and the situation in the rain shadow of the Western Ghats (hills). It has neither extremes of temperature nor high rainfall. The average annual rainfall of about 86 mm and the irrigation facility in the rice fields are quite congenial for the proliferation of mosquitoes. There are quite a few tanks, ponds, puddles, ditches, ground pools etc., present throughout the city extensions which form the major sources of mosquito breeding (Vijayan and Ningegowda, 1993). Ground pools selected for this study are fields situated near the Mysore University Campus, which get flooded during rainy season and become grassy. Paddy fields situated around 26 km radius outside Mysore (Mandya district) are cultivated at least twice a year. These areas were selected for the present study mainly because of the endemicity of JE and prevalence of *Cx. tritaeniorhynchus*, the major vector of JE. The present investigation by the author was undertaken between January 2002 and December 2003. The larvae of *Cx. tritaeniorhynchus* and associated species have been collected from temporary and semi permanent ground water pools. Simultaneously they have also been collected from plain rice field area from a 26 km radius outside Mysore.

An enamel dipper of 13 cm width and 6 cm depth with 350 ml capacity, attached to a long handle was employed for taking larval samples. In ground pools of about 10 to 12 meters square area, an average of 25 dipper samples were taken covering the whole water body. In paddy fields too varying number of dipper samples were taken covering all the sides of the field following the method of Service (1976). In all the habitats sampling was done twice every month. The larval density was calculated as the average number of immatures per dip collected from each habitat. Larval counts were made carefully and the identification of species done following standard keys (Barraud, 1934; Reuben, 1969; Sirivanakarn, 1976). Third instar larvae were taken for categorization and reared until adult emergence for further confirmation of the species. Adults were fed with 10% sucrose solution soaked in cotton. The females were blood fed with immobilized rats and the gravid ones so obtained allowed to lay egg rafts in a clean enamel bowl containing dechlorinated water. The effects of habitat and seasonal difference on the density of *Cx. tritaeniorhynchus* larvae were analysed by using one way analysis of variance (ANOVA). The Duncan Multiple Range Test (DMRT) was used for grouping and comparison of means.

## RESULT

Different Culicidae species found to breed in association with *Cx. tritaeniorhynchus* Type A and Type B were recorded for the year 2002 & 2003 (Table 1). A total of 19 species belonging to 4 genera (*Culex, Aedes, Anopheles* and *Armigeres*) were registered from the two habitats. Four genera with 13 species were found to breed in ground pools as against 3 genera with 14 species in paddy field habitats. *Culex bitaeniorhynchus, Cx. nigropunctatus, Cx. barraudi, Cx. hutchinsoni, An. culicifacies* and *An. stephensi* were seen only in paddy field habitat, whereas *Cx. quinquefasciatus, Cx. gelidus, Ae. albopictus* and *An. jamesii* were found only in ground pool habitats. *Culex tritaeniorhynchus, Cx. vishnui, Cx. pseudovishnui, Cx. fuscocephala, Cx. murreli, Ae vitattus, An. vagus, An. barbirostris* and *Cx. fuscanus* were recorded from both the breeding places. In the present investigation a total of 13 species belonging to 4 genera *ie, Culex* (7 species), *Aedes* (2 species), *Anophele*s (3 species) and *Armigeres* (1 species) were found to breed in ground pools alone. Further 14 species belonging to 3 genera *viz., Culex* (9 species), *Anopheles* (4 species) and *Aedes* (1 species) were found to breed in Paddy field habitats outside Mysore city limits (Table 1).

The mean value for larval density of *Cx. tritaeniorhynchus* type A in ground pools habitats (59.64 larvae per dip) was found to be significantly more than that of type B in paddy fields (11.09) during the study period($P< 0.01$) (Table 2). The highest larval density of *Cx. tritaeniorhynchus* was found to be during pre–monsoon season (69.95 larvae per dip) followed by monsoon (20.74) and post–monsoon (6.56) ($P<0.01$). The mean value for larval density of type A fluctuated around 18.00 per dip during pre–monsoon, 6.78 and 1.90 per dip during monsoon and post monsoon seasons respectively in 2002-03. While, type B showed this fluctuating pattern with 7.42, 1.24 and 0.61 larvae per dip in pre-monsoon, post-monsoon and monsoon seasons respectively (Fig. 1).

Table 1. Associated mosquitoes in the breeding places of *Cx. tritaeniorhynchus* types A and B during the years 2002 and 2003.

| Systematic Position | | | Species | Type A (Mysore City) | | Type B (Outside Mysore) | |
|---|---|---|---|---|---|---|---|
| | | | | 2002 | 2003 | 2002 | 2003 |
| Culicidae | Culicini | *Culex* | *Cx. fuscocephala* (a) | † | † | † | † |
| | | | *Cx. vishnui* (a) | † | † | † | † |
| | | | *Cx. pseudovishnui* (a) | † | † | † | † |
| | | | *Cx. bitaeniorhynchus* | | | † | † |
| | | | *Cx. quinquefasciatus* | † | † | | |
| | | | *Cx. fuscanus* (a) | † | † | † | † |
| | | | *Cx. murreli* (a) | † | | † | |
| | | | *Cx. nigropunctatus* | | | † | |
| | | | *Cx. barraudi* | | | † | |
| | | | *Cx. hutchinsoni* | | | | † |
| | | | *Cx. gelidus* | † | | | |
| | | *Aedes* | *Ae. vittatus* (a) | † | | † | |
| | | | *Ae. albopictus* | † | | | |
| | | *Armigeres* | *A. subalbatus* | | † | | |
| | Anophelini | *Anopheles* | *An. vagus* (a) | † | | † | † |
| | | | *An. culicifacies* | | | † | † |
| | | | *An. stephensi* | | | † | † |
| | | | *An. jamesii* | † | | | |
| | | | *An. barbirostris* (a) | † | | † | † |
| Total * | | | | 3(12) | 2(6) | 3(13) | 2(10) |

(a) Indicate the species that were observed in both the habitats.
* Total number of genera and species (given in parenthesis).

Table 2. Influence of habitat and season on the larval density of *Cx.tritaeniorhynchus* during the years 2002 and 2003.

| Variable | Groups | Mean | Minimum | Maximum | df | F |
|---|---|---|---|---|---|---|
| Area | Mysore city (ground pool) | 59.64 | 0 | 100 | 1 | 82.69* |
| | Outside Mysore (paddyfield) | 11.09 | 0 | 22 | | |
| Season | Post-monsoon | 06.56 c | 0 | 10 | 2 | 79.84* |
| | Pre-monsoon | 69.95 a | 0 | 100 | | |
| | Monsoon | 20.74 b | 0 | 35 | | |

* Differences are statistically significant by one way (ANOVA) (P value<0.01)

(a,b,c,) Indicate differences between groups in each variable by using Duncan's Multiple Range Test (DMRT) p <0.01

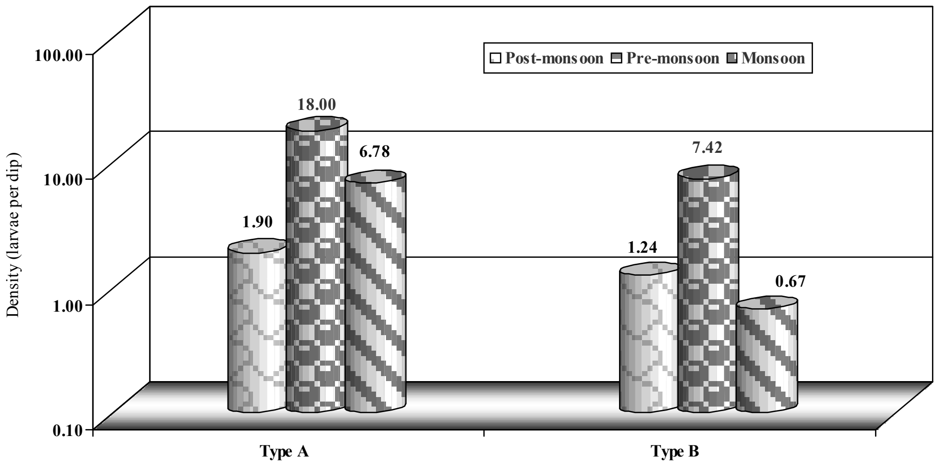


Fig. 1. Mean seasonal density of two varieties of *Culex tritaeniorhynchus* larvae during the year 2002 and 2003.

## DISCUSSION

Investigation on the species composition of various mosquitoes in an area is helpful to predict epidemics. Also studies of behavior, biology, ecology and population dynamics are necessary for vector control management. In the present investigation, an attempt was made to identify mosquitoes associated in ground pool and paddy field habitats of *Cx. tritaeniorhynchus* in Mysore City and outside. Earlier too, survey of mosquitoes was carried out in Mysore city by Ningegowda and Vijayan (1992) and have reported 24 species belonging to 5 genera *ie*, *Aedes* (8 species), *Anopheles* (3 species), *Culex* (10 species), *Mansonia* (2 species) and Armigers (1 species) from the entire Mysore city. After that, Urmila *et al.,* (1999) have reported 22 species belonging to 7 genera *viz*., *Aedes* (6 species), *Culex* (7 species), *Anopheles* (5 species), *Tripteroides* (1 species), *Toxorhynchites* (1 species), *Armigeres* (1 species) and *Ficalbia* (1 species) from different habitats of Mysore University campus. In the present study relative abundance of *Cx. quinquefasciatus* (52.02%) in the ground pool habitats was high among the *Culex* species followed by *Cx. tritaeniorhynchus* type A (26.98%), *Cx. fuscocephala* (10.43%) and *Cx. vishnui* (7.14%) during the years 2002-03. Similar picture of *Cx. quinquefasciatus* abundance followed by *Cx. tritaeniorhynchus* was also reported from Cochin, Kerala ,India (Eapen and Chandrahas, 1994); Tamilnadu, India (Pandian and Manoharan, 1994); Orissa, India (Chand *et al*., 1993); Sagar Island, India (Pramanik *et al*., 1993); Philippines (Schultz and Hayes, 1993); California (Reisen *et al*., 1990) and in Ma Cau (Easton, 1994).

Fakoorziba and Vijayan (2006) have reported that the relative abundance of *Culex* species in Mysore city during 2002 coincided with the data of the following year. However during the year 2002, abundance of *Cx. tritaeniorhynchus* type B (42.16%) was maximum among the *Culex* species followed by *Cx. fuscocephala* (22.49%),*Cx. bitaeniorhynchus* (15.92%), *Cx. vishnui* (10.71%) and *Cx. pseudovishnui* (6.64%). Further, during the year 2003, the relative abundance of *Cx. fuscocephala* (55.55%) in paddy field habitats was high among the *Culex* species followed by *Cx. tritaeniorhynchus* type B (25.16%), *Cx. pseudovishnui* (14.83%), *Cx. bitaeniorhynchus* (2.11%) and *Cx. vishnui* (1.64%). It was also revealed that during the year 2003, due to shortage of irrigation water in the rice fields, paddy cultivation has declined and agricultural activities changed. So, the relative abundance of *Culex* species showed difference between 2002 and 2003. Similar to this, Takagi *et al*., (1997) have pointed out that modification of agricultural practices could possibly reduce the abundance of *Cx. tritaeniorhynchus*. Ephantus *et al*., (2006) have also shown that irrigated rice agro-ecosystem in Africa are associated with a wide spectrum of mosquito fauna. Due to the highest larval density of *Cx. tritaeniorhynchus* during pre–monsoon season (69.95 larvae per dip), this season is very important for mosquitoes control program. The results of this investigation will also help us in Integrated Vector Management (I.V.M.) strategy for the control of J.E. in this endemic area.

## ACKNOWLEDGEMENTS

Authors are thankful to Mr. George Jacob, National Institute of Virology (NIV) field station at Bangalore, India for confirmation of species identification, ICCR India for financial assistance and Chairman, Department of Studies in Zoology, University of Mysore, Mysore, India for the facilities provided.

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*Received:* February 10, 2007 *Accepted:* August 24, 2008