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BIONOMICS OF *CULEX EPIDESMUS* ASSOCIATED WITH JAPANESE ENCEPHALITIS VIRUS IN INDIA

P. C. KANOJIA

National Institute of Virology, 20-A, Dr. Ambedkar Road, PO Box 11, Pune-411001, India

ABSTRACT. Various biological characteristics, including seasonal abundance, diurnal resting habits, feeding behavior, larval habitats, and oviposition, of *Culex epidesmus* were studied in Gorakhpur District, Uttar Pradesh, India, from 1990 to 1999. Prevalence of adults was recorded between June and October, with a peak in August. The bushy undergrowth of mango and teakwood plantations served as diurnal resting habitat. Blood-meal analysis of wild-caught females showed that the most of them had fed on bovines. Temporary pools of rainwater and paddy fields containing freshwater and filamentous green algae were found to be suitable larval habitats. Gravid females laid eggs and immature stages grew well in the presence of green algae. Freshly laid egg rafts were found to be boat shaped and individual eggs were a white-cream color. The number of eggs per raft deposited by individual *Cx. epidesmus* varied from 118 to 288, with an average of 199 eggs per specimen.

KEY WORDS *Culex epidesmus,* adult population, larval habitats, oviposition, Japanese encephalitis virus, Gorakhpur District

INTRODUCTION

Culex epidesmus (Theobald) belongs to the *Cx. bitaeniorhynchus* subgroup of mosquitoes. Several species of this group are widely distributed in southeast and south Asian countries (Reuben 1994), but *Cx. epidesmus* is mainly confined to the Indian subcontinent, particularly India, Bangladesh, Nepal, and Pakistan. The record from Sri Lanka is very doubtful because it has never been confirmed by subsequent studies (Sirivanakarn 1976). One isolate of Japanese encephalitis virus (JE) has been obtained from this species in the Bankura District of West Bengal, India (Banerjee et al. 1979).

This species has often been mentioned in the literature because of its association with JE (Burke and Leake 1988, Reuben et al. 1994). However, the majority of published work has dealt only with the prevalence of adult populations (Dhanda and Kaul 1980). In view of this, an attempt was made to study the bionomics of this species, including adult biology and breeding habitats, during a long-term entomological surveillance of JE in Gorakhpur District, Uttar Pradesh, India, from 1990 to 1999.

MATERIALS AND METHODS

Study area: Gorakhpur District (26°16′ to 27°30′N, 83°6′ to 86°56′E) is situated in the northeastern corner of Uttar Pradesh State and lies immediately south of the Himalayan slopes. The southwest monsoon usually commences in the 2nd week of June and lasts to the end of September, with maximum precipitation in August. The area receives total annual rainfall varying from 807 to 1,564 mm. Relative humidity ranges from 72 to 85%. May is the hottest month of the year, with a maximum temperature of 43°C, whereas January is the coolest month of the year, with a minimum temperature down to 4.1°C. Because of inadequate natural drainage, flooding and waterlogging occur during the rainy season in the area.

Mosquito collections: Weekly visits were made to 3 villages where JE was known to be present (Bhathat, Naharpur, and Kusmi) to collect adult specimens from resting sites such as cattle sheds and in and around pig enclosures with the help of mouth aspirators and flashlights at dusk. During the day, adults were collected from indoor and outdoor resting places. Immature stages were sampled from natural breeding habitats with dippers and pipettes. These specimens were transferred to white enamel trays containing water and green algae obtained from the same breeding sites for rearing to adults. Adult specimens were identified with the help of keys provided by Barraud (1934) and Sirivanakarn (1976). Field-caught fully engorged females were held in the laboratory to record the egg-laying pattern. Identification of host blood meals from fully fed field-caught *Cx. epidesmus* was carried out by the double immunodiffusion technique, which is a modified version of the agarose gel diffusion technique published by Rao (1984).

RESULTS

Adult abundance

During the present study, 7,276 female *Cx. epidesmus* were collected in 3,871 man hours at dusk. Monthly distribution of adult *Cx. epidesmus* encountered in different years is presented in Table 1. Adult populations appeared in June at a very low density (n = 15; 0.04 per man hour density [PMD]). Adult populations showed an upward trend in July (n = 1447; 4.0 PMD) and reached a peak in August (n = 4,327; 13.0 PMD). Thereafter, population density declined in September (n = 1,319; 4.0 PMD) and was greatly reduced in October (n = 164; 1.0 PMD). No adult *Cx. epidesmus* were found from January to May and November to

Table 1. Monthly incidence of female *Culex epidesmus* collected in different years in Gorakhpur District.[1]

	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1990	—	—	—	—	—	1/N	212/3.4	807/15.0	100/2.1	9/0.2	—	—
1991	—	—	—	—	—	5/0.1	201/3.4	193/4.0	139/2.9	31/0.6	—	—
1992	—	—	—	—	—	2/N	119/2.4	1,266/26.4	141/2.4	19/0.5	—	—
1993	—	—	—	—	—	1/N	153/3.7	559/17.0	364/7.0	7/0.2	—	—
1994	—	—	—	—	—	1/N	5/0.4	132/4.0	108/5.7	24/1.0	—	—
1995	—	—	—	—	—	2/0.1	233/6.3	149/4.1	129/3.8	7/0.4	—	—
1996	—	—	—	—	—	1/N	134/5.4	244/8.7	133/4.2	21/0.7	—	—
1997	—	—	—	—	—	—	7/0.4	171/14.3	65/3.0	4/0.2	—	—
1998	—	—	—	—	—	—	17/0.7	127/7.5	79/3.0	27/1.5	4/0.2	—
1999	—	—	—	—	—	2/N	366/10.8	679/20.6	61/5.1	15/1.0	—	—
Total	—	—	—	—	—	15/N	1,447/4.0	4,327/13.0	1,319/4.0	164/1.0	4/N	

[1] Mosquitoes were collected by aspiration at dusk from 1915 to 2015 h. The values given are total number of mosquitoes/total per man hour density. N, < 0.1.

December except once in 1998 when a few adult specimens were collected up to the 1st week of November.

Diurnal resting habits

Fifty-five adult *Cx. epidesmus* were collected during daytime resting collections made from 1990 to 1994. Of these, 54 specimens were captured from bushy undergrowth of mango and teakwood plantations. However, collections made in other outdoor vegetation, including sugarcane fields, which are abundantly found in the area, did not yield a single specimen. Only 1 specimen was recovered from inside a house.

Feeding behavior

Fifteen blood smears prepared from gut contents of field-caught *Cx. epidesmus* were tested against human, bovine, and pig antisera. Of these, 12 (80%) were found positive for bovine and only 1 (7%) reacted with both bovine and human antisera. Two smears did not react with any antisera.

Larval habitats

As many as 178 larval *Cx. epidesmus* were sampled from a variety of breeding places, including paddy fields, swamps, perennial ponds, and temporary rainwater pools. Larval populations were 1st observed in July and continued to be observed until October, with the highest numbers in August. No larvae were found from November to June except once in 1998 when a single larva was obtained in November. Distribution of larvae by habitat was as follows: 116 (65.2%) in temporary rainwater pools, 34 (19.1%) in paddy fields, 22 (12.4%) in swamps, and 6 (3.4%) in perennial ponds. Most of the breeding sites were fully or partially exposed to sunlight and had abundant vegetation, particularly filamentous green algae with some scum on the water surface. These breeding sites usually had clear water and a muddy bottom. At the beginning of the rainy season, temporary rainwater pools and paddy fields formed good breeding sites. With the end of the rains, receding water levels exposed aquatic vegetation, including filamentous green algae. This adversely affected breeding of *Cx. epidesmus*.

Oviposition

Thirty-three fully engorged, field-caught females were held in a separate glass jar containing water and green algae to record the egg-laying pattern. Of these, 25 females laid egg rafts on algae mats, whereas 8 females did not lay any eggs. Freshly laid egg rafts were found to be boat shaped and individual eggs were a white-cream color. The number of eggs deposited by individual *Cx. epidesmus* varied widely. Thirteen females deposited between 118 and 200 eggs, whereas 12 females deposited from 202 to 288 eggs, with an average of 199 eggs per female.

Discussion

Little information is available on the occurrence of *Cx. epidesmus* in India. Barraud (1934) reported that this species is rare but widely spread from Punjab to Assam. Studies carried out in the Bankura District of West Bengal reported occurrence of adult populations from July to November (Mahadev et al. 1978). The prevalence of this species was also recorded in Dhanbad District of Bihar State from August to September (Kaul et al. 1982). The present study confirms these observations and shows a clearly defined monthly incidence in the adult population, with 1st appearance in June, a peak in August, and declining populations through September. Adults were rarely collected in November. In the Gorakhpur region, the monsoon begins in mid-June and remains active until September, with maximum precipitation in August. During the monsoon period, water bodies are formed because of waterlogging and these become colonized by aquatic plants, particularly filamentous green algae. These conditions support the breeding of *Cx. epidesmus*.

The high population density in August may be explained by the availability of excessive breeding places, and the decline in populations through September can be attributed to the loss of eggs and immature stages because of water overflowing from the breeding places.

In earlier studies, diurnal resting sites of *Cx. epidesmus* were not observed. But during the present study, *Cx. epidesmus* were found resting in bushy undergrowth of mango and teakwood plantations. Collections made in open sugarcane fields, which included other vegetation, did not yield a single specimen in spite of the efforts made, suggesting that *Cx. epidesmus* prefers more covered ground vegetation as diurnal harborages rather than exposed areas.

Information available on the feeding habits of *Cx. epidesmus* is scanty. Blood-meal analyses of *Cx. epidesmus* collected from Sundargargh District, Orissa, were found positive for bovines (Dash et al. 2001). The present study confirmed that *Cx. epidesmus* predominantly feeds on bovine hosts. In contrast, 2 closely associated species, *Culex bitaeniorhynchus* Giles and *Culex infula* Theobald, have wide host ranges. *Culex bitaeniorhynchus* is considered to be aviphilic, although these mosquitoes occasionally are found positive for cattle, pigs, dogs, and humans (Christopher and Ruben 1971, Reuben 1971). *Culex infula* feeds mainly on cattle but also shows a low proportion of human and bird blood meals (Reuben et al. 1992).

The breeding habitats of *Cx. epidesmus* were not well known. In earlier work, larvae were collected from uncultivated rice fields in Sri Lanka, but subsequent visits made to the same localities failed to recover any specimens (Sirivanakarn 1976). But in the present study, breeding habitats of *Cx. epidesmus* were identified. This species was found breeding in temporary rainwater pools, paddy fields, swamps, and perennial ponds containing with green algae. This suggests that females prefer to lay eggs in water containing green algae and larvae depend upon algae as food. These field observations were substantiated when field-caught females were found laying eggs on algae mats, and larvae hatched from eggs grew well in the presence of green algae in captivity. The other members of the *Cx. bitaeniorhynchus* subgroup, *Cx. bitaeniorhynchus* and *Cx. infula,* also have shown an association with green algae (Mohan 1950, Sirivanakarn 1976, Larid 1988). Thus, it seems that the breeding habitats and feeding habits of larval *Cx. epidesmus* are similar in nature to those of *Cx. bitaeniorhynchus* and *Cx. infula.*

No previously published records were available on the egg-laying patterns of species belonging to the *Cx. bitaeniorhynchus* subgroup, particularly *Cx. epidesmus, Cx. bitaeniorhynchus,* and *Cx. infula.* Mohan (1950) made a series of observations on the bionomics of *Cx. bitaeniorhynchus,* but did not record the number of eggs laid by individual mosquitoes. However, the eggs, when freshly deposited, were reported to show a light green coloration. During the present study, *Cx. epidesmus* was observed to lay eggs in boat-shaped rafts and the number of eggs laid by individual mosquitoes varied from 118 to 288, with a mean of 199.

In Gorakhpur, prevalence of adult mosquitoes showed 1 peak exclusively toward the beginning of the JE season, that is, in August. The studies carried out in Bankura District of West Bengal (JE endemic area) have also shown a high density of adult populations during August and adults were collected on pig, chicken, cattle, and human baits (Mahadev et al. 1978). In addition to this, *Cx. epidesmus* has been found to be naturally infected with JE (Banerjee et al. 1979), which indicates that this species has access to viremic hosts. Hence, it seems that *Cx. epidesmus* may be playing some role in the natural history of JE. Further experimental transmission studies are required to know its vectorial potential.

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REFERENCES CITED

Banerjee K, Mahadev PVM, Ilkal MA, Mishra AC, Dhanda V, Modi GB, Geevarghese G, Kaul HN, Shetty PS, George PJ. 1979. Isolation of Japanese encephalitis virus from mosquitoes collected in Bankura District, West Bengal, during October 1974 to December 1975. *Indian J Med Res* 69:201–205.

Barraud PJ. 1934. *The fauna of British India, including Ceylon and Burma. Diptera* Volume V. *Family Culicidae. Tribes Megarhinini and Culicini* London: Taylor and Francis.

Burke DS, Leake CJ. 1988. Japanese encephalitis. In: Monath TP, ed. *The arboviruses: epidemiology and ecology.* Volume 3. Boca Raton, FL: CRC Press. p 62–92.

Christopher S, Reuben R. 1971. Studies on the mosquitoes of North Arcot District, Madras State, India part IV. Host preference as shown by precipitin test. *J Med Entomol* 8:314–318.

Dash AP, Chhotray GP, Mohapatra N, Hazra RK. 2001. Retrospective analysis of epidemiological investigation of Japanese encephalitis outbreak occurred in Rourkela, Orissa, India. *Southeast Asian J Trop Med Public Health* 32:137–139.

Dhanda V, Kaul HN. 1980. Mosquito vectors of Japanese encephalitis virus and their bionomics in India. *Proc Indian Natl Sci Acad* 46B:759–768.

Kaul HN, Dhanpal J, Deshmukh PK, Banerjee K. 1982. A report on the mosquitoes collected during an epidem-

ic of encephalitis in Dhanbad District, Bihar. *Indian J Med Res* 76:53–58.

Larid M. 1988. *The natural history of larval mosquito habitats* London: Academic Press.

Mahadev PVM, Dhanda V, Geevarghese G, Mishra AC, Dishmukh PK, Kaul HN, Modi GB, Shetty PS, George PJ, Guttikar SN, Dhanpal J. 1978. Studies on the mosquitoes of Bankura District, West Bengal: adult populations. *Indian J Med Res* 68:248–263.

Mohan BN. 1950. Certain uncommon habits of *Culex bitaeriorhynchus* (type form). *Indian J Malariol* 4:167–173.

Rao USB. 1984. A rapid method for identification of mosquito blood meal. *Indian J Med Res* 79:836–840.

Reuben R. 1971. Studies on the mosquitoes of North Arcot District, Madras State, India, III. Host preference for pigs, birds and some small mammals. *J Med Entomol* 8:258–262.

Reuben R, Tewari SC, Hiriyan J, Akiyama J. 1994. Illustrated keys to species of *Culex* (*Culex*) associated with Japanese encephalitis in southeast Asia (Diptera: Culicidae). *Mosq Syst* 26:76–96.

Reuben R, Thenmozhi V, Philip Samuel P, Gajanana A, Mani TR. 1992. Mosquito blood feeding patterns as a factor in the epidemiology of JE in southern India. *Am J Trop Med Hyg* 46:654–663.

Sirivanakarn S. 1976. Medical entomology studies III. A revision of the subgenus *Culex* in the Oriental Region (Diptera: Culicidae). *Contrib Am Entomol Inst (Ann Arbor)* 12(2):1–272.