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Review Articles

Culex gelidus: An emerging mosquito vector with potential to transmit multiple virus infections

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ABSTRACT

Culex gelidus Theobald has emerged as a major vector of Japanese encephalitis virus (JEV) in India, Southeast Asian countries and Australia. The species has expanded its geographic distribution from the Indian subcontinent to Japan, China, other Southeast Asian countries, Island nations in Australasian region and Australia. In recent years, a sudden increase in its population especially in the urban and sub-urban areas has been observed in several countries, thus, becoming a dominant mosquito species. The virus has been repeatedly isolated from from different geographical locations making it one of the most important vectors of JEV. Apart from JEV, other viruses of public health importance, *viz*. Getah, Ross River (RRV), Sindbis and Tembusu have been isolated from the mosquito. Experimental studies have shown that the mosquito *Cx. qelidus* is highly competent to transmit West Nile, Kunjin and Murray valley encephalitis viruses with infection and transmission rates of >80 and >50%, respectively for each virus. The species is also found competent to transmit RRV, but at a lower rate. Experimental studies have shown that the species is susceptible to chikungunya, Chandipura and Chittoor (Batai) viruses. Development of resistance to DDT and malathion has also been detected in the species recently. The invasive nature, ability to breed both in fresh and dirty waters, development of resistance to insecticides, high anthropophily and its potential to transmit important human viruses pose an increased threat of viral encephalitis in India and Oriental region especially in the light of explosive increase in its population.

Key words *Culex gelidus*; insecticide resistance; Japanese encephalitis; Ross River virus; vector competence; West Nile virus

INTRODUCTION

Culex gelidus Theobald (Diptera: Culicidae) has emerged into dominance as a major mosquito vector with potential to transmit a number of viruses of public health importance. Native to Southeast Asia, the mosquito has expanded its geographical range from India to Australia establishing itself in almost all the Southeast Asian countries, China, Japan, Australia, New Guinea, *etc*[1–3]. The mosquito is implicated as one of the important vectors of Japanese encephalitis virus (JEV) as evidenced by repeated natural isolations and its ability to transmit the virus experimentally[4–12]. The species has been attributed the status of primary vector of JEV in Malaysia and Australia while in several other countries it is emerging as the secondary vector [4–5, 13].

Culex gelidus is a highly invasive mosquito, a voracious biter of humans and breeds profusely in rice fields/ ground pools *etc*[3,14]. Growing need for rice cultivation has a directly proportional effect in the rapid increase of the mosquito population due to availability of breeding habitats in south India and Southeast Asian countries, and could be attributed as one of the explanations for the rapid geographical expansion and establishment[3]. Another advantage could be its ability to breed in fresh as well as dirty waters with high concentration of organic matter, *i.e.* marshes and waste water canals, *etc*[1, 15]. Though, the species shows a preference to larger animals such as cattle and pigs, they exhibit increased human biting behaviour in the absence of the former[3] and feeds throughout the night with maximum feeding occurring between 0300 and 0600 hrs[3–4, 16–17]. They also feed readily on other animals such as goats, deers, chickens and wild birds[3–4]. The availability of a variety of breeding habitats, its ability to breed in both fresh and dirty waters and a broad host range made the species highly successful in establishing itself in new niches.

Apart from JEV, natural isolation of viruses of public health importance, *viz.* Ross River (RRV), Getah (GETV), Sindbis (SINV) and Tembusu (Bunyamwera) were also been reported from the mosquito from different geographical locations[18–21]. Experimental studies in Australia have demonstrated high vector competence to New York strain (1999) of West Nile virus (WNV), Kunjin virus (KUNV) and Murray valley encephalitis virus (MVEV)[12, 22]. Its invasive nature, voracious biting on humans and its potential to transmit different arboviruses has made this species as one of the most important mosquito species of public health importance. Recent studies in India have shown an exponential growth in the mosquito population especially in the southern states. In this review, the author discusses the expanding geographic distribution of the mosquito, the sudden spurt in its population and its association with viruses of public health importance.

Global distribution

The global distribution of *Cx. gelidus* is given in Fig. 1. Though, the mosquito shows distribution in the entire Oriental region, Papua New Guinea, Islands in the Australasian region and tropical Australia, the review is restricted to the status of the mosquito in those countries where the mosquito is either dominant or it plays an important role in the transmission of JEV. An overview of the present status of *Cx. gelidus* in these countries is briefed below.

Malaysia: In Malaysia, *Cx. gelidus* is widely prevalent and is considered as the primary vector of JEV due to repeated isolations from the field collected mosquitoes since 1954[3–4, 13, 23–25]. In JEV endemic Sarawak, the virus was almost exclusively isolated from *Cx. gelidus* and *Cx. tritaeniorhynchus* mosquitoes[5]. It is considered as the primary vector of JEV not only because of the higher yield of JEV isolates but also for its role in maintenance of the virus in a pig-mosquito cycle[3,25]. The species is abundant in JEV endemic areas of Malaysia, *i.e.* Sarawak, Selangor, Penang, Kampong Tijirak, *etc.* and plays an important role in the maintenance and transmission of JEV[3, 5, 9, 26–27]. Virological investigations carried out in the mosquitoes collected from different villages of Sarawak during 1962–64 and 1968–70 led to the isolation of GETV, SINV, Tembusu viruses in addition to JEV[18–19, 25].

Thailand: Culex gelidus constituted the major population of the mosquito fauna along with *Cx. tritaeniorhynchus* constituting 71–96% of the total catch in Bangkok during 1986–87[28]. The investigators also observed that though the former yielded more number of JEV isolates, the minimum infection rates (MIR) between the two species were found comparable. In northern Thailand also, *Cx. gelidus* was found to be the dominant species as observed during mosquito collections made using black light, truck, and pig-baited traps[29–30]. Similarly, studies conducted in Pathum Thani province, central Thailand also demonstrated the high population density of the species[31]. The studies also reported that the species was found predominant during November though rest of the period was dominated by *Cx. tritaeniorhynchus*. Changbunjong *et al*[32] recently rated *Cx. gelidus* as one of the five most abundant mosquito species in Thailand as per the data collected during 2009–10.

Vietnam: Culex gelidus is one of the most dominant mosquito species prevalent in Vietnam. Lindahl *et al*[33] reported the prevalence of this mosquito to be 24% of the total collections in households irrespective of the presence of pig holdings in Can Tho City, Vietnam. How-

Fig. 1: Global distribution of *Culex gelidus* Theobald.

ever, the investigators observed higher density of the species in houses close to pig holdings, a characteristic which was also observed in Sarawak, Malaysia[25]. They also observed highest proportion of fully engorged females (37%) encountered during collections in comparison to *Cx. tritaeniorhynchus* (35%), *Cx. quinquefasciatus* (5%) and other JEV vectors. Species composition studies carried out in Cat Que village in Hatay province also showed *Cx. gelidus* as the most dominant species in outdoor collections, while indoor collections were dominated by *Cx. quinquefasciatus* and *Cx. vishnui* spp[34]. They also observed that the domination of the species was higher between 2300 and 0900 hrs in comparison to that collected between 1900 and 2300 hrs. Another observation made was that the species preferred cows rather than pigs in the study area demonstrating that proximity towards hosts from the breeding sites was more important than host preference[34].

Indonesia: In Indonesia, the major vectors of JEV are *Cx. tritaeniorhynchus* and *Cx. gelidus* are found abundant during rainfall[35]. Presence of *Cx. gelidus* has been reported from Java, Bali and central Sulawesi. During virological investigations carried out in mosquitoes, JEV has been isolated from *Cx. gelidus* collected from different places in Jakarta[6–7]. In Bali and Sulawesi, though the mosquito was prevalent, JEV could not be detected[36]. *Cx. gelidus* was detected even in open temporary ground pools, formed due to impressions made by heavy machines such as bulldozers in addition to rice fields and other breeding habitats.

Sri Lanka: High density of *Cx. gelidus* has been reported in the filariasis endemic areas of Sri Lanka[37]. The mosquito is also indicted as a probable vector of Bancroftian and Malayan filariasis[1]. Extensive surveys for the vectors of JEV in Sri Lanka have shown predominance of the mosquito in the coconut husk pits in Panadurra (50.9%), Allutgama (77.7%) and Talalla (75.2%) districts. Surveys carried out in Polhena and Matara districts have also shown higher density (72.2%) of the mosquito in coconut husk pits. Prevalence of *Cx. gelidus* was also reported from the North Central province and is incriminated as one of the major vectors of JEV in Sri Lanka, based on virus isolation and ELISA based antigen detection in wild caught mosquitoes[21, 38]. *Culex gelidus* was also detected in Mahaveli irrigation project area during a study conducted on ecosystem changes before and after the project came to existence and mosquito breeding pattern in 1988–89[39].

Australia: In Australia, *Cx. gelidus* was first recorded in May 1999 from Brisbane and Mackay in Queensland followed by detection in Melaleuca swamps in Cairns, Queensland and Alice springs in Northern Territory in 2000[2, 20, 40]. Subsequently, the mosquito was detected in Badu Island in the Torres strait during entomological investigations carried out to detect JEV activity in mosquitoes after the JEV outbreak in 2000[10, 41]. JEV was isolated from *Cx. gelidus* mosquitoes collected from a newly established piggery in Badu Island, Torres Strait for the first time in Australian region[10]. Presence of the species was also recorded from western Australia[10].

Indian scenario

In India, the presence of *Cx. gelidus* has been reported from Maharashtra, Goa[17], Rajasthan[42], Karnataka[43–45], Kerala[45], Tamil Nadu[46], Andhra Pradesh[47–48], Uttar Pradesh[49], West Bengal[50] and Assam[51]. Though the mosquito was present in many states of India, its relative abundance was considerably negligible. Extensive studies carried in Mysore district of Karnataka state could record only a negligible percentage (0.02%) of the total mosquito population[52–53]. In Mysore, breeding of the species was seen only in ground pools and not in paddy fields. In Mandya and Kolar districts, JEV endemic areas in Karnataka, *Cx. gelidus* population was found to be <10% of the total mosquitoes collected during 1983–88[44]. However, during the last two decades, a spurt in the population of *Cx. gelidus* has been observed in the southern states of Indian Peninsula, *viz*. Andhra Pradesh, Tamil Nadu and Kerala. Arunachalam *et al*[47] reported the population of *Cx. gelidus* to approx 50% of the total mosquito population in peri-urban areas of Kurnool district of Andhra Pradesh during a four year study. They however found that in village settings, the percentage of the mosquito was negligible and *Cx. tritaeniorhynchus* was the predominant species. This has been substantiated by subsequent studies carried out by Murthy *et al*[54] as they observed predominantly high percentage (68.05%) of *Cx. gelidus* in the urban areas of Kurnool district. In rural areas, they also observed high prevalence of *Cx. tritaeniorhynchus* (57.51%) in comparison to the former. Earlier studies by Gajanana *et al*[46] in South Arcot district of Tamil Nadu have also reported high density of *Cx. gelidus* as *Cx. tritaeniorhynchus, Cx. vishnui, Cx. gelidus* and *Cx. fuscocephala* constituted 93.6% of the total mosquito population during 1991–94. Similarly, Alappuzha district; Kerala has also shown a tremendous increase in the population of *Cx. gelidus* during the last few years. In a year-long study conducted during 2012–13, 57.9% of the total mosquito collection was constituted by *Cx. gelidus* whereas earlier studies conducted in 2009 have recorded only 17% of the total population in the same area (NIV Annual Report 2009–10; and 2012–13). Surprisingly, the *Cx. tritaeniorhynchus* population of Alappuzha has shown an inversely proportional growth, *i.e.* from 67 to <22% during the same period. The recent trend in south India points to a gradual replacement of *Cx. tritaeniorhynchus* mosquitoes by *Cx. gelidus* at least in the urban and semi-urban areas. What made the sudden surge in *Cx. gelidus* population and relative decrease in *Cx. tritaeniorhynchus* population in Alappuzha is not clear. The availability of ample breeding habitats, *i.e.* rice fields and water bodies such as ponds, ditches and canals and the presence of a large number of cattle in the area could have probably attributed to the sudden increase in *Cx. gelidus* populations. However, more systematic studies are needed to understand the decrease in *Cx. tritaeniorhynchus* population as both share identical breeding habitats and hosts. Changes in environment and pollution of water bodies might have given an advantage to *Cx. gelidus* over *Cx. tritaeniorhynchus* as the former is known to breed in dirty waters with high concentration of organic matter[1, 15].

Increase in the population of *Cx. gelidus* in India pose a major threat to public health in the JEV endemic areas especially in Uttar Pradesh (UP), Bihar and Assam. In UP and Bihar, however, the population is negligible and the major JEV vectors are *Cx. vishnui* group, mainly *Cx. tritaeniorhynchus*. However, in Assam, it has been observed that *Cx. gelidus* is widely distributed in large numbers though the major JEV vector belongs to the *Cx. vishnui* group[51]. Earlier studies in Tamil Nadu and Thailand have shown comparable MIR with *Cx. tritaeniorhynchus* mosquitoes despite the less number of isolates from *Cx. gelidus*[28]. In the background of the higher densities of the mosquito in the JEV endemic areas and its efficiency to transmit JEV, a surveillance mechanism needs to be implemented to check the population control of the mosquito.

Vector potential

Culex gelidus has established itself as one of the important vectors of JEV in Southeast Asia. Recent studies have shown that the mosquito has expanded not only its geographic distribution but also improved its vector potential to transmit JEV in many countries[3, 10]. In Malaysia, it is the primary vector for JEV and is important in maintaining JEV in a pig-mosquito cycle[25]. In other Southeast Asian countries also, the mosquito is emerging into dominance as well as playing an important role in the transmission of JEV. In India, the mosquito acts as secondary vector after *Cx. tritaeniorhynchus* for JEV in suburban areas[47, 54]. In Australia too, *Cx. gelidus* is the only mosquito which yielded JEV isolation during an outbreak in 2000[10]. Infection and transmission potential of the mosquito to JEV was >90 and >50%, respectively after an incubation period of 13 days in experimentally infected mosquitoes[11]. Apart from JEV, several other viruses of public health importance, *viz*. RRV, GETV, SINV, Tembusu, *etc* were also isolated from field collected mosquitoes. The natural isolations and its ability to transmit important flaviviruses make it as an important mosquito species to be put under constant surveillance. Natural isolation of JEV from the mosquito obtained so far is given in Table 1.

Natural isolations of other arboviruses of public health importance

Viral investigations in *Cx. gelidus* mosquitoes yielded several viruses of public health importance belonging to the family *Togaviridae* and *Bunyaviridae* apart from JEV. GETV (Family Togaviridae), an important viral pathogen of horses has been first isolated from the mosquito in 1955 from Malaysia[18]. The virus has been subsequently isolated from *Cx. gelidus* collected from Sri Lanka and Malaysia on several occasions[21]. In Malaysia, GETV is

Table 1. Natural isolations of JEV from *Cx. gelidus* mosquitoes

Year(s) of mosquito collection	Place of isolation	Reference
2002–06	Kurnool district, Andhra Pradesh, India	Arunachalam *et al*[47]
2000	Badu Island, Torres strait, Australia	van den Hurk *et al*[10]
1992–93	Sepang district, Selangor, Malaysia	Vythilingam *et al*[9]
1991–94	South Arcot district, Tamil Nadu	Gajanana *et al*[46]
1987–88	Different ecologic areas of Sri Lanka	Peiris *et al*[21]
1986–87	Bangkok, Thailand	Gingrich *et al*[28]
1985–87	Kolar and Mandya districts, Karnataka, India	Mourya *et al*[43]
1983–87	Yunnan Province, China	Zhang[55]
1978–80	Kapuk, Indonesia	Olson *et al*[7]
1972–74	Jakarta, West Java, Indonesia	Dirk *et al*[6]
1972–74	Malaysia	Simpson *et al*[25]
1960–70	Malaysia	Heathcote[5]
1954–60	Malaysia	Gould *et al*[4]

Table 2. Natural isolations of other arboviruses from *Cx. gelidus*

Arbovirus isolated	No. of isolates	Place of isolation	Reference
Getah	Several	Malaysia	Berge[18]
Getah	1	Sarawak, Malaysia	Simpson *et al*[13]
Getah	1	Sri Lanka	Peiris *et al*[21]
Ross River	1	Cairns, Australia	Harley *et al*[20]
Sindbis	2	Sarawak, Malaysia	Platt *et al*[19]
Tembusu	2	Sarawak, Malaysia	Platt *et al*[19]

maintained in a cycle involving *Cx. tritaeniorhynchus*, *Cx. gelidus* and pigs[13]. In addition to GETV, several other viruses of public health importance belonging to family *Togaviridae* and *Bunyaviridae* were isolated from the mosquito (Table 2).

Susceptibility to other arboviruses

The mosquitoes showed a wide-spectrum susceptibility to arboviruses which included members of the family *Togaviridae, Flaviviridae, Rhabdoviridae* and *Bunyaviridae*. The natural isolations of JEV, RRV, GETV and Tembusu virus has already been documented earlier suggesting their susceptibility to these viruses. In addition, the mosquitoes were also found susceptible to WNV (New York 1999), MVEV and KUNV as demonstrated by experimental studies[12]. Barmah Forest virus, a member of the family *Togaviridae*, was however, found to be refractive[12]. Recent experimental studies carried out by the author have shown that the mosquitoes are susceptible to Chikungunya virus (CHIKV), WNV (Eg101), Chandipura virus (CHPV) and Chittoor (CHITV, Batai group) virus (Sudeep unpublished data).

Experimental transmission of arboviruses by Cx. gelidus

Vector competence studies carried out in Australian strain of *Cx. gelidus* (Brisbane and Sidney) with the New York (1999) strain of WNV demonstrated high transmission potential by the mosquito[22]. The infection and transmission rates were >80 and >50%, respectively. In another study, Johnson *et al*[12] reported high transmission potential of the mosquito to JEV, KUNV and MVEV with transmission rates of 96, 95 and 41%, respectively. Though the mosquitoes transmitted RRV, transmission rate was only 25%. Recently, Sudeep (Unpublished data) demonstrated efficient horizontal transmission of WNV (Eg101) by orally infected mosquitoes to infant mice. Growth kinetic studies with the same virus have shown a 3 $\log_{10}$ $TCID_{50}$/ml increase in virus titre on the Day 8 post-infection (PI) and maintained the titre up to Day 14 PI.

Insecticide susceptibility

Culex gelidus is found to be susceptible to a number of natural and synthetic insecticides. However, recent studies have shown the development of resistance to DDT in certain populations of the species. It needs more verification as the available data are not sufficient to come to any conclusion. The available data are briefly described below.

Susceptibility to natural products: Several herbal extracts have shown adulticidal and larvicidal properties against the species. Extracts of *Zingiber zerumbet*, *Dolichos biflorus*, and *Aristolochia indica*, have shown larvicidal and adulticidal as well as repellant properties against *Cx. gelidus*[56]. Essential oil derived from *Zanthoxylum piperitum* and extract of *Apium graveolens* exhibited repellent activity comparable to DEET against the species[57–58]. Extracts of *Z. limonella*, *Syzygium aromaticum* and *Kaempferia galangal* have also showed repellent action against the species[59–60]. A number of marine actinobacteria has also been found to have larvicidal, ovicidal and repellent properties against the species[61].

Synthesized silver and nickel nanoparticles have exhibited larvicidal properties against larvae of *Cx. gelidus* mosquitoes. Silver nanoparticles synthesized using bark aqueous extract of *Ficus racemosa* have been found to be highly larvicidal against *Cx. quinquefasciatus* and *Cx. gelidus*[62]. Similarly, synthesized nickel nanoparticles also found to be highly larvicidal against immature stages of a number of hard ticks and mosquitoes[63].

Synthetic insecticides: Culex gelidus has been found highly susceptible to a majority of synthetic insecticides due its breeding in habitats that are less prone to insecticides[51, 64–66]. Temephos and fenthion have been found to be the most effective against the species as 100% mortality could be achieved with 0.005 ppm while other organophosphorous compounds, *viz.* fenitrothion and malathion required 0.125 ppm to achieve 100% mortality[64]. Studies conducted in Sri Lanka during the 1980s and 90s also substantiated the high susceptibility of the species to different insecticides. Karunaratne and Hemingway[65] during a comparative study to determine the susceptibility levels of different species of mosquitoes have found that *Cx. gelidus* is highly susceptible to malathion and propoxur. They also observed that *Cx. tritaeniorhynchus* had developed resistance to both the compounds and were 100 and 10 times more resistant than *Cx. gelidus*. This could be due to selection pressure as the former is exposed to these insecticides in irrigated paddy fields while the latter breeds in habitats which are less prone to insecticide application. In an earlier study, Kulkarni *et al*[66] have also reported the susceptibility of

Cx. gelidus to different insecticides such as malathion, dieldrin, fenitrothion and propoxur. Recent studies carried out in Assam, India, Dhiman *et al*[51] have shown the development of resistance in *Cx. gelidus* to DDT as they observed only 81 mortality in the treated population. The knockdown time was also found exceptionally high in the species in comparison to *Cx. vishnui* group and *Mansonia* species. However, the species remained susceptible to deltamethrin (100%). Development of resistance to DDT by *Cx. gelidus* has already been reported in Phnom Penh, Kampuchea[67].

Among synthetic insect repellents, DEET and ethylbutylacetylaminopropionate (IR3535) provided repellency for at least 5 h against *Cx. gelidus* after application. Both the products gave protection for > 9 h after topical application in field and laboratory conditions against several species of mosquitoes[68–69]. Soap formulations containing DEET and permethrin also found to give protection against the species in landing and biting for at least 4 h after application[70].

CONCLUSION

Culex gelidus has emerged as an important arboviral vector in Southeast Asian countries and Australia. It has been implicated as an important vector of JEV in Asia due to repeated natural isolations and experimental studies. The mosquito is highly invasive, establishes rapidly finding new niches and makes its dominance in the mosquito populations. In several countries, the mosquito has become predominant in a very short span. Apart from JEV, natural isolations of RRV, GETV, SINV and Tembusu have been reported from the mosquito. The mosquitoes are found competent to transmit highly pathogenic viruses such as WNV, KUNV, and MVEV experimentally with high rates of infection and transmission. They are also found susceptible to CHIKV, CHPV and CHITV experimentally and replicated to very high titers. Recent studies have shown the development of resistance to DDT and malathion, which may pose issues in the management of the mosquito. The invasive nature, ability to breed in fresh and dirty waters, development of resistance to insecticides, high anthropophily and its competence to transmit important human viruses, especially encephalitis causing viruses, makes this mosquito a potential threat for mankind especially in the Oriental region.

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