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# Japanese encephalitis and the vectors involved in its transmission with special reference to north-eastern India: A review

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**Abstract**
Japanese encephalitis virus (JEV) causes Japanese encephalitis, which is a leading form of viral encephalitis in Asia, with around 50,000 cases and 10,000 deaths per year in children below 15 years of age. The JEV has shown a tendency to extend to other geographic regions. Mosquitoes acts as potent vectors in spreading the viruses to humans. A selected group/ species of mosquitoes acts as vector in the presence of suitable host and dead end host. In this review the details regarding the vector have been discussed and summarized.

**Keywords:** Japanese encephalitis Virus, encephalitis, vectors, dead end host

## 1. Introduction
During the last few decades major JE outbreaks have been reported from different parts of India predominantly in the rural areas [1]. The JE related mortality, morbidity and disability adjusted life years (DALYs) are alarming posing serious challenge to the concerted control efforts [2]. Humans are accidental, dead-end hosts as they do not develop a level of viraemia sufficient to infect mosquitoes. The infection with JEV ranges from nonspecific febrile illness to a severe meningoencephalomyelitis illness [3]. JE outbreaks are common and occur at regular intervals in different parts of India [4]. In the Northeastern States of India, the disease appears in sporadic outbreaks with its peak during the rainy season [5, 6]. Population-wise Assam is the largest State amongst the eight States in northeastern India and plays a significant role in the economy of the region [7]. Dibrugarh is one of the most affected districts of Assam and since 1978 human cases have been regularly reported from this district [8]. In India, of the 16 mosquito species detected harboring JE virus, *Culex tritaeniorhynchus* has been reported as the prominent JE vector [9, 10]. In addition, mosquitoes of *Cx. vishnui* subgroup has been recognized for many years as an important vector associated with JE transmission in India [11, 12]. Information on the population dynamics of mosquitoes, particularly vectors are necessary for implementation of control measures. In any given area common mosquito species occur during epidemics, however, the abundance of any species depends more upon the availability of preferential feeding host, breeding habitats and survival rates and has direct relevance with the disease transmission.

## 2. History
In north east (NE) region of India, the disease was first observed in 1976 in Assam and since then the disease has appeared in endemic forms or sporadic outbreaks [5].The Assam state, particularly the Upper Brahmaputra valley has been experiencing recurrent JE episodes during July to October every year [6]. Outbreaks of JE have been reported from NE region in Lakhimpur district of Assam between July-August 1989. Later, several outbreaks were reported from Assam in 3 consecutive years from August 2000-2002 [13]. Besides Assam, JE cases have been reported from NE states like Manipur and Nagaland [14]. Serological surveys in animals carried out during 1955 to 1972 showed that JE was prevalent in this region.
As the potential JE vectors of this region, viz., *Cx vishnui* group of mosquitoes is zoophilic and exophilic in nature, they abound near the cattle sheds and pigsties and therefore the people close to them seem to have a greater exposure to bites by the vector species of mosquitoes [4, 15].
JE virus has been recovered from several mosquito species (19 species) in different parts of India, and the most important vectors are *Cx. tritaeniorhynchus* Giles and *Cx. vishnui*

Theobald from which the largest number of isolations have been made [16]. In Asia, the development of irrigation systems and the expansion of rice (*Oryza sativa* L.)-growing areas have facilitated the increase of JEV vectors [17]. Although epidemics invariably are preceded by an increase in vector abundance, several other factors, including mosquito infection rate are involved [18].

## 3. Transmission of JEV by vectors

The JEV is transmitted to vertebrates by mosquitoes. Mosquito transmission was suspected during the early 1930s; in the year 1938, [19] it was reported that JEV was isolated from *Cx. tritaeniorynchus* [20]. Vector competence of *Cx. tritaeniorhynchus* was well demonstrated in laboratory studies [12]. The ecology of JEV has come from various studies carried out in Japan [21] and JEV ecology has been the subject of several reviews [22, 23, 24]. Many species of *Culex* mosquitoes can transmit JE. For Southern Asia, Eastern Asia and Southeastern Asia, the main vector of JE is *Cx. tritaeniorhynchus*. For Northern Australia, the main vector is *Cx. annulirostris*. However, various other secondary vectors maybe important. Indian studies in particular have revealed a number of secondary vectors, including *Mn. indiana*, *Cx. pseudovishnui*, *Cx. whitmorei*, *Cx. gelidus*, *Cx. epidesmus*, *Anopheles subpictus*, *A. peditaeniatus*, and *M. uniform* [25]. The natural cycle of JE virus in Asia involves water birds and *Culex* mosquitoes. However, unlike many other mosquito-borne diseases, an amplifying host is important in the epidemiology of human JE. In Asia, pigs are considered to be the most important amplifying host, providing a link to humans through their proximity to housing [26].

## 4. Mosquitoes as vector

Vector competence of *Cx. tritaeniorhynchus* was well demonstrated in laboratory studies [27]. *Cx. tritaeniorhynchus* has been incriminated as the principal vector of JE in many parts of India [28, 18]. The Minimum Infection Rate (MIR) of *Cx. tritaeniorhynchus* (0.63) was higher in Kurnool compared with MIR of *Cx. tritaeniorhynchus* (0.28) in Cuddalore, India [18]. JEV infection in *Cx. gelidus* was observed only from peri-urban areas. JEV isolations also have been made from *Cx. gelidus* in India [29, 30, 18]. MIR of *Cx. gelidus* (0.57) was higher in Kurnool compared with the MIR of *Cx. gelidus* (0.52) in Cuddalore, India [18]. MIR and maximum likelihood estimates of JEV infections of *Cx. gelidus* were lower compared with the values obtained for *Cx. tritaeniorhynchus*, which indicate the primary role played by *Cx. tritaeniorhynchus. Cx. gelidus* is highly zoophagic and poorly anthrophagic; therefore, it may have an important role in amplifying JEV transmission [31, 32, 33].The seasonality of JEV transmission depends on various factors, among which the relative abundance of the vector species is important [34].

## 5. Isolation of virus from vectors

In India, JE virus has been isolated from 16 mosquito species *viz*., *Cx. bitaeniorhynchus, Cx. epidesmus, Cx. fuscocephala, Cx. gelidus, Cx. infula, Cx. psedo vishnui, Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cx. vishnui, Cx. whitmorei, An. barbirostris, An. peditaeniatus, An.subpictus, Ma. annulifera, Ma. indiana*, and *Ma. uniformis.* Among these species, *Cx. tritaeniorhynchus* has yielded maximum number of JE isolates [30, 11].

Nine other species *viz*., *An. barbirostris, Cx. bitaeniorhynchus, Cx. fuscocephala, Cx. gelidus, Cx. pseudovishnui, Cx. vishnui, Cx. whitmorei, Ma. uniformis* and *Ma. annulifera,* are regarded as JE vectors in India and they together contributed only 0.13 per cent in combined population density. Hence, their role in JE epidemiology seems to be very feeble [30].

*Cx. tritaeniorhynchus* was the most abundant species and showed two density peaks, first in February and second in October. This species is known to breed in rice fields and its population dynamics are closely associated with paddy cultivation. In Bellary, two rice crops are grown from January to April and July to December thereby showing incidences in both the seasons [35].

## 6. Rice cultivation and JE

Several studies in India analyzed the presence of rice irrigation and vector abundance in relation to the incidence of JE occurring in humans. In two districts viz. Gorakhpur district in Uttar Pradesh, and the Mandya district of Karnataka, areas extensively developed for irrigated rice agriculture, the occurrence of JE was closely associated with high vector densities, breeding in the fields or the canal system. The highest numbers of JE cases were observed shortly after the mosquito densities peaked [36, 9]. In addition, in the Mandya district, a high incidence of JE was found in extensively irrigated areas, while few cases occurred in villages with less irrigation or no irrigation systems [32]. In Assam, 78.6% of the JE cases occurred in families practicing rice cultivation [5].

In 1985–1986 in the Mahaweli System H, Sri Lanka, an epidemic of JE occurred, resulting in more than 400 cases and 76 deaths. In 1987–1988 a second outbreak took place with >760 cases and 138 deaths. The promotion of smallholder pig husbandry was suspected to be responsible for these outbreaks. The highest number of cases occurred in areas with irrigation and pig husbandry, while no cases were reported from non-irrigated areas with a few pigs [37]. Linear relationship between the proportion of rice fields and mosquito abundance was observed in this report [11].

JE cases occurred in two spells in a year, one during April-June (summer epidemic) and another during October-December (winter epidemic) in Mandya district of Karnataka. There was very high incidence of JE cases in extensively irrigated areas and a low incidence in some of the taluks with less or no irrigation systems. Among culicines, *Cx. tritaeniorhynchus* was the most predominant species (20.54%), followed by *Cx. fuscocephala* (16.94), *Cx. vishnui* (16.48%), *Cx. gelidus* (10.70%) and other species. The overall mosquito population showed two peaks in a year, one during the March April and another during September, usually preceding the human epidemics. Relative abundance of certain species varied in different years [32] and no cases were reported in non-rice cultivated provinces [38].

## 7. Habitat and environmental factors

Most predominant mosquito species inhabited in rice fields and marshlands during the study period in Srilanka were *Cx. gelidus*, *Cx. tritaeniorhynchus*, *Cx. fuscocephala* and *Cx. pseudovishnui* which are major Japanese encephalitis vectors [1, 39]. Other than that *Cx. bitaeniorhynchus* was also recorded from marshlands which were considered as a vector of Japanese encephalitis [2]. Density And Diversity Of Mosquito Larvae Associated With Rice Field And Marshland Habitats In Two Climatically Different Areas In Sri Lanka [40].

Relative humidity is a critical factor affecting the lifecycle pattern of mosquitoes [41]. It has been observed that temperature at 28°C with 50–55% RH is the most appropriate condition for the elevation in mosquito density than the condition of lower temperature with higher humidity (22°C/80–85% RH). During the investigation carried out, the temperature was between 22°C and 34°C with low to medium humidity (42.7 to 69.6%), which might have facilitated the higher mosquito density in both rural and urban areas. Similar kinds of results have also been reported at the maximum survival rate of mosquitoes for the same temperature and humidity [42].The pattern of rainfall also affects larval habitats and vector population size. In some cases, increased rainfall may increase larval habitat and vector population by creating a new habitat, while excessive rain would eliminate habitats through flooding, thus, decreasing the vector population [43, 44, 25]. During the dry season limited rainfall can also create new habitats, when water in the rivers is drawn into pools, providing the perfect breeding sites for a number of mosquito species and thus favouring diseases transmission [43]. It was reported that among many mosquito species collected, the two main vectors of JE, *Cx. tritaeniorhynchus* and *Cx. gelidus* were considered in support of their abundance. It was noticed that the prevalence of *Cx. tritaeniorhynchus* varied among different years during the study period. This difference in their population was may be due to the change occurring in the agricultural practices during each year [33].

**8. Multiplication of JEV in Mosquitoes**

A series of studies using fluorescent antibody techniques was undertaken in the 1960s to determine the mode of development of JEV in infected *Cx. tritaeniorhynchus*, *Cx. pipiens pallens* and *Cx. quinquefasciatus* [45, 46]. Following ingestion of a viremic blood meal, JEV rapidly infected the epithelial cells of the posterior portion of the midgut, followed by high titer replication in the anterior section of the midgut. The second stage of multiplication occurred when the virus infected the fat body cells adjacent to the midgut, followed by infection of fat body cells in the hemocoel and, especially, between the thoracic muscles. The final stage of multiplication occurred in the salivary glands and other susceptible organs, including the compound eyes, thoracic ganglia, and Malpighian tubules. The EIP was temperature dependent and ranged from 6 days post infection at 28◦C to 20 days at 20◦C (126). At low temperatures the transmission rate was reduced [45, 46].

**9. Host Feeding Patterns**

Multiple feeding within the same gonotrophic cycle increases the potential for human-vector contact, especially in zoophagic vectors such as *Anopheles culicifacies* and *An. subpictus*, which are also quite endophilic, bringing them into the proximity of humans [47]. However, the high proportion of multiple feeding of exophilic vectors such as *Cx. tritaeniorhynchus* on dampening (dead-end) hosts such as cattle and goats may impede the transmission of JE virus to humans by diverting host-seeking mosquitoes away from potential hosts such as pigs and birds. *Cx. tritaeniorhynchus* contacting hosts of lesser importance such as cattle and goats may not be a favorable factor in the transmission of JE virus. Mosquitoes attempting to feed on the host by repeated penetration of mouth parts may be of epidemiologic significance even when blood is not ingested because the infected mosquitoes secrete fluids along with the virus during exploratory movements [48, 49, 50]. Thus, multiple feeding on various hosts may favor the transmission if the vector feeds on potential hosts or reduce transmission if the vector feeds on unimportant hosts. The presence of domestic animals has been associated with a decrease in malaria transmission rates due to zoophilic deviation [51]. In some parts of Africa, zooprophylaxis is used and cattle are intentionally kept near or inside houses to divert mosquitoes from humans to cattle. It was suggested that cattle could play a role in reducing transmission of malaria by *An. arabiensis* by distracting the vector from humans [52]. An outbreak of JE on Badu Island in the Torres Strait near Australia was attributed to the presence of domestic pigs and high mosquito populations in close proximity to humans on many of the islands [53, 54]. In northern Australia, marsupials divert host-seeking *Cx. annulirostris* away from pigs and since marsupials are poor JE virus hosts, the prevalence of marsupials impedes the establishment of JE virus [55]. In Chiang Mai, Thailand, field studies on host preference of JE vectors indicate that effective control of JE could be achieved by increasing the availability of cows to deflect the vector mosquitoes from pigs [56]. In countries such as Singapore, Japan (Okinawa and the Ryuku Islands), and Taiwan where the pig population was high relative to that of cattle feedings on pigs (up to 60%), have been reported for JE vectors [57, 58, 27]. However, in countries such as India, where the cattle population outnumbers that of pigs, more than 80% of the blood meals of JE vectors were in cattle [31]. Cattle are considered as dead end hosts because they do not develop a high enough viremia to infect mosquitoes [59, 60] The relative availability of cattle and pigs to mosquitoes was the major factor responsible for the epidemiologic differences in JE between the areas studied in India [31, 61, 62]. In a non-endemic area, the low prevalence of JE was attributed to a higher number of cattle relative to pigs. Children in villages with a high ratio of cattle to pigs showed lower sero-conversion rates to JE virus than those in villages where there was a relatively lower proportion of cattle to pigs, which suggests that the higher cattle density may be an important factor that may reduce the risk of infection in humans [64, 65, 53]. In Yangon, Myanmar, the zoophilic nature of JE vectors was attributed to the lack of JE virus transmission to humans [11].

An essential component of the JEV transmission cycle is the degree of contact between vectors and amplifying hosts. In classic host preference studies, cattle generally attracted more *Cx. tritaeniorhynchus* than pigs did [64, 66], reportedly a result of physiological conditioning rather than inherent genetic factors [66]. Throughout their geographical range, most JEV vectors are opportunistic blood feeders, with host availability being the key factor influencing host feeding patterns. High porcine feeding rates are generally reflective of high pig populations and *Cx. tritaeniorhynchus* readily feeds for pigs when available [67, 58]. Indeed, pig feeding rates of 30%–40% have been recorded from South Korea [10] and northern India [68]. However, throughout much of its geographical range, *Cx. tritaeniorhynchus* obtains most blood meals from cattle [57, 69, 27, 31] and because bovines do not produce sufficient viremia to infect mosquitoes, they may impede transmission of JEV and provide passive zooprophylaxis [56]. Humans account for only a small proportion(less than 5%) of blood meals for most *Culex* vectors of JEV in Asia.

In a study out of the total mosquitoes caught in light trap 25% of this species were blood fed. Also in landing catches the *Cx. tritaeniorhynchus* was caught in small numbers compared to other species. All night catches need to be carried out using a pig baited trap to determine the peak biting time and the man: pig biting ratio [71].

**10. JEV vectors identified in Assam**

In an entomological study of various Primary Health Centres of Sivasagar district of Assam nine known JE vectors were collected. Out of this *Cx. bitaeniorhynchus*, *Cx. quinquefasciatus* and *Cx. whitmorei* were collected in very low number. Population density of *Cx. vishnui* group was substantially high among all the sampling villages during peak JE period, this species is most likely to be a potential JE vector in the area [71]. *Cx. vishnui* and *Cx. gelidus* have been reported to be a more prevalent JE vector in many parts of Assam [72]. In many parts of India and Southeast Asian countries, *Cx. tritaeniorhynchus* has been recorded in abundance and incriminated as a major vector JE vector [35].

The population dynamics of JE vectors largely depends upon rice cultivation, water bodies, temperature and humidity in the rural areas [5].The practices of paddy cultivation, domiciliary surroundings with adjacent water bodies and high temperature and humidity were found to be the environmental factors influencing the abundance of the potential mosquito vectors responsible for transmission of the virus in the rural community [73]. The association of pigs as viremic amplifying hosts complemented the JE virus activity in such populations [8].Ten species of mosquitoes in 9 localities represented by JE cases during the period June-August of 2001-2002 in Dibrugarh district of Assam, were identified, where *Cx. vishnui*, followed by *An. hyrcanus* and *Cx. gelidus*, were found to be most prevalent. Peak incidence was observed when the maximum temperature ranged from 31-32.5ºC with rainfall 426-587 mm and humidity from 94-97% [5]. Most of the cases were reported during the monsoon and the period after monsoon [74]. Higher prevalence of JE in adult is not only due to shifting of disease to adult population, as mentioned by some of the authors, it may be due to higher exposure to mosquito bite in paddy fields. The study revealed a significant number of JE cases in the monsoon season which is similar to others findings [75, 76, 5].

## 11. Conclusion

Japanese encephalitis is a public health problem, not only in Asia but for the entire world. However, JE is rising throughout Asia, because epidemics are typically noticed only after outbreaks and because the disease may go largely unobserved in endemic regions. Environmental and ecological factors are responsible for the spread of JEV. There is no specific treatment for JE; only prevention can control the disease. Vectors play a pivotal role in the transmission of the virus to humans. The presence of known JEV vectors in Assam has thereby opened a discussion regarding the means for early detection and control the spread of JE in the state. Vector indexing along with serological studies in amplifier animals such as pigs and reservoir animals such as egret is an excellent approach which can be used as an early warning system. Which shall invariably help the authority concerned with it to take preventive measures against JE and also take suitable steps to control the vector population.

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