Japanese encephalitis (JE) virus is a mosquito-borne, zoonotic flavivirus that infects vertebrate hosts, primarily birds and swine, in an enzootic cycle. Multiple host contacts in a gonotrophic cycle increase the chance of acquiring and transmitting the pathogen. The increase in the number of host contacts as a result of multiple feeding may increase disproportionately the rate of encephalitis virus transmission by Culex tarsalis. Thus, host vector contact is an important parameter in JE epidemiology. However, many epidemiologic models on vector-borne diseases assume that mosquitoes contact one host per gonotrophic cycle. Multiple feeding was reported in field populations of vectors of malaria, eastern equine encephalitis, St. Louis encephalitis, and western equine encephalitis. However, no information is available regarding frequency of multiple feeding among the major JE vector, Cx. tritaeniorhynchus. The study reports the observation of multiple-feeding behavior of Cx. tritaeniorhynchus in a JE-endemic area in Kerala in southern India. We examined field population of Cx. tritaeniorhynchus associated with JE virus transmission in the study area for multiple feeding to determine the frequency of contact with more than one host in a gonotrophic cycle.

The study was conducted in the Kuttanadu region of Kerala, India. The area is devoted primarily to rice culture, but the agro-ecosystem is interspersed with rivers and streams. Six villages where at least one confirmed case of JE had occurred were selected as index villages for longitudinal studies. Cattle, goats, dogs, fowl, and ducks are the common domestic animals in Kuttanadu. Pigs are reared in backyards of some houses.

Each study village was sampled at monthly intervals. Mosquitoes resting on vegetation and bushes around cattle sheds and pig sties were collected for one hour after dusk by oral aspirator and transported to the laboratory for identification and enumeration. Mosquito (only females) abundance was calculated as number collected per human-hour.

Outdoor resting mosquitoes were collected from vegetation and bushes by using drop nets to get an unbiased sample of engorged female mosquitoes to study the host feeding patterns. Engorged female mosquitoes were placed on ice, transported to the laboratory, and identified. Stomach contents were smeared on Whatman (Brentford, United Kingdom) no. 1 filter paper strips, dried, and stored at 4°C. The agarose gel diffusion method with minor modifications was used to identify blood meals from wild-caught mosquitoes. Antisera to cattle, pig, duck, goat, fowl, and humans were obtained from Serologist (Government of India, Kolkata, India).

A total of 150,454 female mosquitoes representing 6 anophelines and 12 culicine species were collected. Culex tritaeniorhynchus was the most abundant species, comprising 66.9% of the total collected. This was followed in decreasing order by Cx. gelidus (11.1%), Mansonia uniformis (9.6%), Ma. indiana (8.2%), and Ma. annulifera (3.0%); the remaining mosquito species comprised less than 2% of the mosquitoes collected.

The abundance of Cx. tritaeniorhynchus was lowest in June–August, increased in September, and reached a maximum in December–March. The increase corresponded with the period of rice cultivation. Entomologic assessment indicated that Cx. tritaeniorhynchus was the primary vector for JE virus based on relative abundance, widespread distribution, and frequent virus infection.

A total of 3,067 blood-engorged Cx. tritaeniorhynchus females collected from outdoor resting places during the period 1998–2001 were tested and 2,553 (83.2%) of the samples could be identified. Culex tritaeniorhynchus had predominantly fed on cattle (56.6%) and to a lesser extent on ducks, fowl, goats, and humans (< 2%). Culex tritaeniorhynchus had also fed on pigs, which accounted 6.3% of the total samples. Nine hundred eighty samples proved to be of serologic mixed origin. Of 980 mixed blood-fed mosquitoes, 975 (99.5%) had imbibed blood from two distinct hosts and 5 (0.5%) imbibed blood from three distinct hosts. Mixed blood meals were mostly (96.7%) from cattle and goats. The epidemiologic implications of multiple feeding of Cx. tritaeniorhynchus on dampening (dead-end) hosts such as cattle and goats in the transmission of JE virus is discussed.
reason for the high percentage of mixed feeding in *Cx. tritaeniorhynchus*.

The frequency of multiple feeding on cattle and goats is very high in *Cx. tritaeniorhynchus*. It may be due to the availability of the animals under the same roof. It is clearly evident that JE vectors prefer to feed on cattle. When disturbed, they readily feed on goats, although goats are not the preferred hosts because single feedings on goats accounted for less than 1% of the feeds.

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 endophagic, bringing them into the proximity of humans. However, the high proportion of multiple feeding of exophagic 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. *Culex 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 virus during exploratory movements. *Culiceta melanura* infected with equine encephalomyelitis virus could transmit the virus to susceptible chicks in the laboratory by merely probing. In malaria transmission, the loss of certain number of sporozoites in non-human hosts during mixed feeding could be of importance in malaria control. 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. 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. 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. 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. 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. 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. However, in countries such as India, where the cattle population outnumbered that of pigs, more than 80% of the blood meals of JE vectors were in cattle. Cattle are considered as dead end hosts because they do not develop a high enough viremia to infect mosquitoes. 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. 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 seroconversion 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. In Yangon, Myanmar, the zoophilic nature of JE vectors was attributed to the lack of JE virus transmission to humans.

Pigs are considered essential for pre-epizootic amplification of JE virus in all southeast Asian countries experiencing JE epidemics. According to the 2000 figures of the Food and Agriculture Organization of the United Nations, more than 500 million pigs are present in developing Asian nations. In Australia, a country at risk for JE epidemics, there are many domestic and feral pigs. Japanese encephalitis will remain a public health problem in southeast Asian countries because of the high pig population. By collating the available information from the literature and based on the present studies, it may be deduced that JE transmission can be damped by adopting zooprophylaxis (by using cattle) because the principle vector, *Cx. tritaeniorhynchus*, has a high affinity for bovine blood.

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