Japanese encephalitis (JE) virus is a mosquito-borne, zoonotic flavivirus that infects vertebrate hosts, primarily birds and swine, in an enzootic cycle.\(^1,^2\) Multiple host contacts in a gonotrophic cycle increase the chance of acquiring and transmitting the pathogen.\(^3\) 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*.\(^4\) 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.\(^5–^7\) Multiple feeding was reported in field populations of vectors of malaria, eastern equine encephalitis, St. Louis encephalitis, and western equine encephalitis.\(^8–^12\) 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 epidemiologic 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.\(^13,^14\) 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 *C. 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.

**SHORT REPORT: OBSERVATIONS ON THE MULTIPLE FEEDING BEHAVIOR OF CULEX TRITAENIORHYNCHUS (DIPTERA: CULICIDAE), THE VECTOR OF JAPANESE ENCEPHALITIS IN KERALA IN SOUTHERN INDIA**

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Abstract. A study was undertaken in villages endemic for Japanese encephalitis (JE) in Kerala in southern India during the period 1998–2001 to determine the host-feeding pattern of *Culex tritaeniorhynchus*, the major vector of JE in southeast Asia. A total of 3,067 blood-engorged *C. tritaeniorhynchus* were tested and 2,553 (82.2%) of the samples could be identified. *Culex tritaeniorhynchus* had fed mainly (56.6%) on cattle. Pig feeding accounted 6.3% of the total samples. Some samples (n = 980, 38.3%) were 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.
Fluids along with viruses during exploratory movements. 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 find 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 endophilic, bringing them into the proximity of humans. 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. *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.

In countries such as Australia, a country at risk for JE epidemics, there are more than 500 million pigs 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 dampened by adopting zooprophylaxis (by using cattle) because the principle vector, *Cx. tritaeniorhynchus*, has a high affinity for bovine blood.

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