How Zoophilic Japanese Encephalitis Vector Mosquitoes Feed on Humans

著者	Tuno Nobuko, Tsuda Yoshio, Takagi Masahiro
著者別表示	都野 展子
journal or publication title	Journal of medical entomology
volume	54
number	1
page range	8-13
year	2017-01-01
URL	http://doi.org/10.24517/00049519

doi: 10.1093/jme/tjw165

Tuno, Tsuda, and Takagi: How Zoophilic JE
Vectors Feed on Humans

N. Tuno
Graduate School of Natural Science and
Technology, Kanazawa University, Ishikawa 920-
1192, Japan.
Phone and Fax : +81-76-264-6214
E-mail: tuno@staff.kanazawa-u.ac.jp

1

2 How Zoophilic Japanese Encephalitis Vectors Mosquitoes Feed on Humans

3 N. Tuno¹, Y. Tsuda², K. M. Takagi³

4 ¹ Graduate School of Natural Science and Technology, Kanazawa University, Ishikawa 920-1192, Japan.

5 E-mail: tuno@staff.kanazawa-u.ac.jp

6 ² Department of Medical Entomology, National Institute of Infectious Diseases, Tokyo 162-8640. E-mail:

7 tsudayso@nih.go.jp

8 ³ Department of Vector Ecology and Environment, Institute of Tropical Medicine, Nagasaki University,

9 Nagasaki 852-8523. E-mail: mstakagi@nagasaki-u.ac.jp

10

11

12

ABSTRACT (250 words)

Japanese encephalitis virus (JEV) is the most frequent cause of mosquito-borne encephalitis in Asian countries. Several culicine species are potential vectors. The primary JEV vectors feed mainly on cows (a dead-end host for JEV), pigs (an amplifying host), and occasionally humans (a dead-end host). It is essential to determine blood feeding patterns to understand the transmission cycle of the disease. Here we review blood feeding characteristics of the primary JEV vector *Culex tritaeniorhynchus*, *Cx. vishnui*, and *Cx. gelidus* based on experimental works and field surveys conducted in Asian countries. Several studies showed that these JEV vectors have an innate preference for cows, however, the former two species often showed higher rates of blood feeding on pigs than on cows, probably because pigs are more abundant than cows. On the other hand, the latter species *Cx. gelidus* fed mostly on cows. Thus, the first two species showed higher plasticity to compromise host availability than the last. By reviewing the available articles and based on our relevant studies, it may be deduced that JEV transmission cannot be reduced by zooprophylaxis. We emphasize the need of keeping cows away from the human residences to dampen the human risk of JEV. These primary JEV vector species exhibit pre-biting resting. The adaptive significance of this behavior remains to be unexplored, but it may have a function to avoid defensive attack of host animals. Application of recent quantitative analysis of gene expression in this phase may enable us to come up with novel vector control strategies.

Keywords: Host preference, *Culex tritaeniorhynchus*, *Culex vishnui*, *Culex pseudovishnui*, *Culex gelidus*, Japanese Encephalitis.

Japanese encephalitis virus (JEV) is an arthropod-borne virus (an arbovirus) that circulates among wild animals and is the most frequent cause of mosquito-borne encephalitis (Vaughn and Hoke 1992, Endy and Nisalak 2002). JEV was first isolated in Japan in the 1935 (Kamimura 1998) and is the main cause of viral encephalitis, with an estimated 68,000 cases annually in South-East Asia and the Western Pacific regions, exposing more than 3 billion people to the risk of infection (WHO 2015). JEV prevalence is associated with rice fields (which are the breeding sites for the vector mosquitoes) and the densities of large non-human mammals (which are the sources of blood meals) (WHO 2011, Solomon 2006). The primary JEV vectors are not anthropophilic; the mosquitoes feed more commonly on pigs and cows than on chickens or humans (Gajanana et al. 1995, Gingrich et al. 1992, Gould et al. 1974, Leake et al. 1986, Peiris et al. 1993, Vythilingam et al. 1997, Bhattacharyya et al. 1994, Arunachalam et al. 2005, Samuel et al. 2008, Reuben et al. 1992, Wang 1975). If JEV vector mosquito species feed only on non-human hosts or humans, they are no longer vectors, because human JEV infection requires virus preservation or amplification in a non-human host prior to transmission to a human as a dead-end host where JEV is unable to amplify enough for further infection. Wild birds (especially herons) are reservoirs of JEV and they carry JEV over long distance by their seasonal migration (Kamimura 1998). Domestic pigs act as an amplifying host and has an important role in the epidemiology. Human, cattle and horse are dead-end hosts as the disease manifests as fatal encephalitis. There is no human-to-human transmission. Therefore, an effective JEV vector should have catholic host preferences. The individual mosquitoes must bite multiple host species, including humans.

In Asian countries, *Culex tritaeniorhynchus* Giles, *Culex vishnui* sensu lato (sl.), *Culex fuscocephala* Theobald, *Culex gelidus* Theobald, *Culex whitmorei* (Giles), and *Mansonia uniformis* (Theobald) have been implicated as JEV vectors (Gajanana et al. 1995, Gingrich et al. 1992, Gould et al. 1974, Leake et al. 1986, Peiris et al. 1993, Vythilingam et al. 1997). Although these mosquitoes feed more commonly on pigs and cows than on chickens or humans (Pennington and Phelps 1968, Reisen and Boreham 1979, Reuben et al. 1992, Bhattacharyya et al. 1994, Arunachalam et al. 2005, Samuel et al. 2008, LY 1975), the feeding pattern varies by host availability. The feeding patterns of mosquitoes are largely influenced by two parameters: an innate tendency to respond to particular cues, and the relative availability of hosts in combination with the capacity of the vector to be mobile. The term “host preference” can be used to

describe an integration of these parameters (Clements 1999). Therefore, studies on the feeding patterns of JEV vectors in Asia have produced varying results, depending on the relative abundances of host populations and the sampling procedures used. The relative abundance of pigs compared to cows can be low in countries dominated by Muslims. In India, where the cow population is greater than the pig population, 86–98% of all blood meals ingested by vectors are from cows (Christopher and Reuben 1971). In Okinawa, Singapore, and Taiwan, where the pig populations are greater than the cow populations, up to 60% of vector blood meals are from pigs (Pennington and Phelps 1968, Colless 1958, Mitchell et al. 1973). Some researchers investigated the JEV vector host feeding patterns in Asia (Japan, Thailand, and Vietnam) and explored the innate host preferences and the actual field feeding habits of the primary vector species (Mwandawiro et al. 1999, 2000, Hasegawa et al. 2008).

Innate Host Preference and How this Can be Distorted

Primary JEV vectors have been reported to feed on pigs and cows rather than chickens or humans (Clements 1999, Christopher and Reuben 1971, Pennington and Phelps 1968, Reisen and Boreham 1979, Reuben et al. 1992, Bhattacharyya et al. 1994, Arunachalam et al. 2005, Samuel et al. 2008, LY 1975). The pig is an amplifying host but the cow is a dead-end host for JEV; thus, the nature of the blood meals taken by vector mosquitoes is critical in terms of disease transmission. Host preference tests were performed by our group using these two host animals (Mwandawiro et al. 1999, 2000) and field-collected mosquitoes (Mwandawiro et al. 1999, Hasegawa et al. 2008) to determine the innate preferences of, and blood meals taken by, wild mosquitoes. Release-and-recapture tests and light trapping were conducted on the Mae Joh University campus in Chiang Mai, where various animals, including cattle and pigs, are kept and JEV vectors are abundant (Mwandawiro et al. 1999). Wild-collected mosquitoes or offsprings of them were released and recaptured in experimental mosquito nets in which host animal (a cow, a pig or both) were confined to evaluate host preference in terms of the blood taken (Mwandawiro et al. 1999, 2000). Under non-choice conditions (either a cow or a pig was confined), all three species tested, *Cx. tritaeniorhynchus*, *Cx. vishnui*, and *Cx. gelidus*, fed on cows in significantly higher proportions (65.2–66.1%) than on pigs (42.4–56.6%). Under choice conditions (both animals were confined), they fed on cows almost 10-fold more often (39.0–45.3%) than on pigs (2.4–5.3%) (Mwandawiro et al. 2000). Thus, the JEV vectors exhibited a higher preference for cows than pigs but the difference was not large when no choice was

available (Mwandawiro et al. 2000). When mosquitoes that had fed on, or had been attracted to, cow or pig were released, they tended to bite the same host animals to which they had originally been attracted. However, laboratory-reared offspring of pig-fed or cow-fed mothers did not exhibit such differences, rather showing a uniform preference for cows (Mwandawiro et al. 2000). Therefore, the three JEV vector species underwent physiological or behavioral conditioning in terms of host preference. Mosquitoes (n= 34,708) were collected in light traps baited with dry ice and placed in animal sheds (housing cows, pigs, chickens, sheep, and goats) to evaluate feeding preferences in the field (Mwandawiro et al.1999). Unlike what they found with the bait experiments, *Cx. tritaeniorhynchus* and *Cx. vishnui* took more meals from pigs than from cows, probably because pigs are more abundant than cows. On the other hand, *Cx. gelidus* fed significantly more often on cows than on pigs. Interestingly, individuals of *Cx. tritaeniorhynchus* and *Cx. vishnui* caught in pigsties drastically increased in late night (02.00-06.00), whereas those caught in cowsheds (feeding on cows) remained constant throughout the night. On the other hand, *Cx. gelidus* fed on cows in significantly higher proportions than on pigs throughout the night. Thus, *Cx. gelidus* had a fixed feeding preference (cows), while *Cx. tritaeniorhynchus* and *Cx. vishnui* preferred cows but exhibited more flexibility in feeding. The two species exhibited higher feed ratios on pigs in the late night; they may have changed their preferences according to the availability of host. Our data gained from the field do not explain the cause of their host shift; however, we suggest host defensive behavior as a possible cause. Host defensive behavior triggered by high density of mosquitoes that may cause attacking mosquitoes' fatality. The relationships between mosquitoes density and their biting success will be discussed later.

Host Animal Distributions Change the Risk to Humans

We have described how host availability (thus influencing host choice) may differ among vector species. This raises the following questions: Does the host animal distribution affect the risk of a human being bitten by a JEV vector? If animals are kept in the vicinity of humans, does this increase or reduce the risk to humans? Hasegawa et al. (2008) conducted a study to seek answers to these questions. They performed a field investigation in a rice production area of northern Vietnam to elucidate the relationship between host species and mosquito distributions. We determined mosquito and host abundances in 50 compounds (where both humans and animals lived), and host abundances in an additional 29 compounds, to examine

the relation between mosquito and vertebrate host densities.

Cattle increase the human risk:

In Vietnam, Hasegawa et al. (2008) found *Cx. quinquefasciatus* (not a JEV vector) as the most dominant species that occurs indoors, followed by the *Cx. vishnui* subgroup, and *Cx. gelidus* (the latter was the most dominant species outdoors). They applied PCR analyses on parts of the samples and found that 79% of the captured specimens were *Cx. tritaeniorhynchus* and 21% were *Cx. vishnui*. They treated these two species as the “*Cx. vishnui* subgroup” because a few specimens classified as *Cx. tritaeniorhynchus* by morphology were assigned as *Cx. vishnui* by PCR. The numbers of *Cx. vishnui* subgroup and *Cx. gelidus* mosquitoes were larger in outdoor collections. Individuals of the *Cx. vishnui* subgroup and *Cx. gelidus* had fed mainly on cows and pigs, even though they were sampled indoors (Table 1, Hasegawa et al. 2008). These species had also fed on humans. The number of individuals of the *Cx. vishnui* subgroup that fed human blood correlated positively with the number of cows kept in the compound. Thus, they found that the presence of cows increased human mosquito bites (Fig. 1, Hasegawa et al. 2008). The number of individuals with mixed blood meals was examined in these species and also in *Culex quinquefasciatus*, a non-JEV-vector that mainly feeds on humans and chickens; it was 15 (9%) of the 164 *Cx. vishnui* subgroup mosquitoes, 3 (4%) of the 70 *Cx. gelidus* mosquitoes, and 16 (5%) of the 299 *Cx. quinquefasciatus* mosquitoes. The mixed blood meal combinations were as follows: 2 of the *Cx. vishnui* subgroup mosquitoes had ingested human and cattle blood, and 13 of these mosquitoes along with 3 of the *Cx. gelidus* mosquitoes had ingested cattle and pig blood. In *Cx. quinquefasciatus* mosquitoes, three had ingested human and pig blood; two had ingested human and cattle blood; five had ingested human and chicken blood; two had ingested pig and cattle blood; one had ingested pig and chicken blood; two had ingested cattle and chicken blood; and one had ingested human, pig, and chicken blood. This shows how different types of arbovirus are mixed.

Mosquito abundance and environmental factors:

Culex gelidus, *Cx. tritaeniorhynchus*, and *Cx. vishnui* are exophilic (i.e., they mainly remain outdoors), but they sometimes occur indoors. These indoor individuals predominantly feed on cattle and pigs (Table 1, Hasegawa et al. 2008). This suggests that these vectors enter the houses even after feeding. The number of

cows significantly affected the indoor collection numbers of the *Cx. vishnui* subgroup, but less significantly with the distance from mosquito breeding sites (the nearest rice field: the location of breeding sites was assessed by the abundance of male mosquitoes, because they usually remain in the vicinity of breeding sites; see Hasegawa et al. 2008 for details). This indicates that the distributions of the *Cx. vishnui* subgroup in the villages were not constrained by their breeding sites. On the other hand, the numbers of *Cx. gelidus* mosquitoes were mainly influenced by the proximity to their breeding sites and were only slightly affected by the number of cow hosts; this result was consistent with Mwandawiro et al.'s (1999, 2000) findings that *Cx. gelidus* prefers cows to pigs or chickens. However, these results imply that for *Cx. gelidus* the distance between available hosts and breeding sites is more critical than is host preference. It has been reported that this species breeds in a variety of habitats in Malaysia (Gould et al. 1962). In the study area, people washed their animal sheds and thus created polluted ground pools that served as larval habitats for *Cx. gelidus*. When the hosts and the breeding sites are closely located, mosquitoes do not need to disperse over long distances; this may be important to a species with limited flight ability. The number of female *Cx. quinquefasciatus* mosquitoes also correlated positively with the proximity to the breeding sites. *Cx. quinquefasciatus* is reported to breed in any type of habitat that contains water (ranging from fresh clear water to polluted water with decayed organic matter, [Reid 1968]). In the study area, the larval habitats of *Cx. quinquefasciatus* were assumed to locate within the villages, similar to those of *Cx. gelidus*. Human blood comprised 76% of the diet of this species. The number of female *Cx. quinquefasciatus* mosquitoes did not correlate with the abundance of any animal host.

How Do Mosquitoes Achieve Plasticity in Blood Feeding?

The primary JEV vectors prefer cows to pigs although the actual blood meals taken do not necessarily coincide with host preference; species-specific plasticity is in play (Mwandawiro et al. 1999, 2000, Hasegawa et al. 2008,). The *Cx. vishnui* subgroup exhibited more flexibility than did *Cx. gelidus*. The distribution of the latter species is thought to be limited to the vicinity of breeding sites, whereas the distribution of the former species is less limited by the breeding sites (rice fields). This raises a question whether there is relation between the plasticity in terms of host preference and the ability to move away from breeding sites. In this context, Tuno et al (2003) studied an interesting behavior, termed pre-biting

resting. They speculated that vector breeding sites and vector density might affect blood-feeding plasticity. Mosquito blood-feeding behavior is composed of several phases, i.e., searching for a host, attraction to the host, attack, feeding, and resting. Among these phases, the marked interval between the appearance of mosquitoes near a host and the actual alighting on the host is termed the “pre-biting rest” (Reid 1968) or “pre-attack rest” (Clements 1999). Pre-biting resting has been reported in several taxa (Service 1993) including the *Anopheles leucosphyrus* subgroup (Colless 1956ab), *An. dirus* (Scanlon and Sandhinand 1965), *An. gambiae* s. l. (Smith 1958), *Cx. quinquefasciatus* (De Meillon and Sebastian 1967), *Cx. tritaeniorhynchus* (Wada 1969), and *Mansonia* species (Service 1969, Wharton 1962). The biological significance of pre-biting resting has not been elucidated, but it may have evolutionary significance. Tuno et al. (2003) studied micro-spatial distribution of mosquitoes around a cow host in the countryside of Northern Thailand. Forty sticks were arranged in 4 rays in vicinity of a cow tethered. All mosquitoes resting on the sticks were collected, sexed, identified their species and blood feeding status. A total of 1,566 mosquitoes of 25 species of five genera were captured (Tuno et al. 2003). *Anopheles aconitus* was the most abundant, followed by *An. peditaeniatus*, *Cx. vishnui*, and *Cx. pseudovishnui*. There was no directional difference in mosquito abundance. Mosquitoes were randomly distributed before they approached the cow. More unfed mosquitoes were collected at sites closer to the host (i.e., they were engaged in pre-biting resting), and the feeding ratio correlated negatively with mosquito density (Tuno et al. 2003). Thus, the numbers of fed mosquitoes were almost constant despite fluctuations in the daily numbers of mosquitoes captured. They also found that mosquito species can be separated into two groups in terms of distributions of fed and unfed mosquitoes around the host. One group, represented by five species, showed higher proportions of fed individuals irrespective of mosquito density, while the other, represented by seven species, aggregated around the host to close distances of 1–4 m but contained lower proportions of fed mosquitoes. A characteristic of mosquito blood-feeding is that the amount of blood available is enormous compared to what is required. Thus, mosquitoes do not need to hurry to bite because of a shortage of blood. Possible factors limiting feeding might include the host body surface area (Clements 1999), or (more likely) host defenses triggered by excessive attacks. A negative correlation between mosquito density and feeding success, possibly caused by density-dependent defensive host behavior, has been reported (Tuno et al. 2003). If this is a general rule, pre-biting resting may be an adaptation used by mosquitoes to avoid aggressive host defenses. Dawkins and Krebs (1979) called life-

dinner principle about asymmetric relationships between prey and predator. In case of mosquitoes' blood feeding, mosquitoes bet their life for attacking but host are not killed by their attacks (Kweka et al. 2010). Therefore evolutionary selection would work more severely on mosquitoes than host animal. However, if so, why is pre-biting resting behavior observed in only some mosquito species? Indeed, they found that density-dependent feeding ratios were not evident in all species. Then they returned to the two different feeding groups. They compared the specific breeding habitats and adult host preferences to seek any common characteristics within a group that differed between the groups and found that members of the second group, that aggregated around the host exhibited lower proportions of fed mosquitoes than did the first group, used larger breeding sites (such as rice fields, ponds, swamps, and streams). However, no clear difference in host preference was evident between the two groups. If host animal defensive behavior is triggered by only high mosquito density, then mosquito species that form large populations will have more experience of such host defensive behavior. From this point of view, members of the second group, using larger breeding sites, must have been subjected to the density-dependent evolutionary selection. In other words, the pre-biting rest allows the mosquito to decide whether the host is to be attacked. Gillies (1980) showed that carbon dioxide generally attracts host-seeking mosquitoes. Most mosquitoes are attracted by general host cues such as carbon dioxide, odors, and heat. But we raise a question; why can mosquitoes that engage in pre-biting resting stop their attack so close to the host where the host cues are strongest? We suggest that a form of density effect may be in play. The closer the host, the more mosquitoes are present. Mosquitoes may evaluate their densities by sensing wing vibrations or certain volatiles that remains unknown.

Future Directions.

Finally, we integrated our thoughts to suggest how to control the transmission rates of JEV vectors. Some researchers have shown experimentally that the JEV vectors *Cx. tritaeniorhynchus*, *Cx. vishunui*, and *Cx. gelidus* have an innate preference for cows over pigs; these likes and dislikes are clearer when they can choose between the two animals. Contrary to these preferences, the pig blood feed ratios were often higher than the cow blood feed ratios in the former two species, while the latter species, *Cx. gelidus*, fed mainly on cows in Thailand (Mwandawiro et al. 1999, 2000) and in Vietnam (Hasegawa et al. 2008). In a village in northern Vietnam where people lived in close proximity to many types of animals, mosquito abundance

was most affected by cow abundance (Hasegawa et al. 2008). The abundance of the *Cx. vishnui* subgroup was positively associated with cow abundance (Fig. 2). The number of human blood meals taken by this species increased with the number of cows in the compound (Fig. 1). However, the abundances of *Cx. gelidus* and *Cx. quinquefasciatus* were primarily affected by closeness to their breeding sites. In another words, their distributions were limited by the distance from their breeding grounds (Hasegawa et al. 2008). A study of the micro-distributions of pre-biting mosquitoes (including the *Cx. vishnui* subgroup) around a cow found that many unfed mosquitoes remained in vicinity of the host (Tuno et al. 2003). There was previous study that discussed the adaptive aspect of pre-biting resting. Wada (1969) used various methods to observe the nocturnal biting activities of *An. sinensis* and *Cx. tritaeniorhynchus*, and counted mosquitoes alighting on pigs and on plates or tapes set near dry-ice baits in Japan. In the cited study, *Cx. tritaeniorhynchus* showed a sharp peak in nocturnal activity when dry-ice baits were used; however, mosquito counts on pigs did not exhibit a peak, being instead almost constant. This difference was attributed to pre-attack resting. The dry-ice baited counts indicated only the flight activity rhythm; this differs from attack behavior; the lack of a peak was explained by suggesting that the “missing” mosquitoes (that should have formed a peak) were engaging in pre-attack resting.

We gave a schematic illustration of blood feeding by JEV vectors based on the data of Hasegawa et al (2008) (Figure 2). The primary vectors aggregate around cow, most beloved host, resulting in a high vector density due to their comparatively high mobility. If the high density of mosquitoes attack altogether they will more likely fail to get blood meals because of host defensive behavior. To avoid it, many of them engage in pre-biting resting instead of direct attacks. A proportion of them will successfully take a blood meal from cow in course of time and a certain proportion change their mind to turn to pigs, humans, and chickens (in successively smaller ratios) to feed; more than half of such “alternative feeders” will remain unfed (Table 2). Figure 1 presents us that mosquitoes attracted to cow changed their target into human. Therefore, it is important to keep cows away from human residences and keep pigs (the amplifying host) away from cows (dead end host) to reduce the human risk suffering from JEV. This was realized in Japan in the 1960s (Kamimura 1998). Thousands of JE cases were reported in Japan prior to 1960, but the last outbreak occurred in 1966. Japan became JE-free for several reasons; e.g., a nationwide human immunization program, and isolation of pigsties and cowsheds from human dwellings (Kamimura 1998).

We receive reports every year that naïve piglets kept in pigsties are becoming JEV preservers, but have few opportunities to contact JEV vectors. It is important to isolate human dwellings from cows and pigs, as achieved in Japan. But it is not easy to achieve social changes over a short period. Low-cost interim solutions are to keep cows and humans apart, and to keep cows and pigs apart. The spatial isolation can be less than 100 m. Mosquitoes judge the abundance of preferable hosts on the small spatial scale (Hasegawa et al. 2008). *Culex gelidus*, the other important JEV vector species, is less mobile. Therefore, its attack can be effectively reduced by locating its breeding sites apart from human habitats. Thoughtful village and city planning can achieve costless control of the transmission rate of JEV. In this study, we pointed out evolutionally aspects of pre-biting resting. Now we are able to study the effect of high density of mosquitoes or defensive behavior of host on the occurrence of pre-biting resting by analysing the expression of thousand genes with next generation sequencing. If we apply the emerging quantitative gene expression analysis to unsolved mosquitoes pre-biting behavior, we will be able to clarify what kind of physiological processes are operating and these processes are activated by what kind of environmental stimuli. Understanding of pre-biting resting will lead to a novel finding in mosquitoes biting behavior and will enable us to design new program to suppress vector contacts and disease transmission not only JEV.

Acknowledgement

We thank editor Dr. Theodore Andreadis and two anonymous reviewers for their constructive discussion, and Dr. Kabirul Bashar, and Dr. Masahito T. Kimura for their proof reading and modification of the manuscript.

280 REFERENCES

- 281 **Arunachalam, N, P. P. Samuel, J. Hiriyar, R. Rajendran, A. P. Dash.(2005).** Short report: observations
 282 on the multiple feeding behavior of *Culex tritaeniorhynchus* (Diptera: Culicidae), the vector of
 283 Japanese encephalitis in Kerala in southern India. Am. J. Trop. Med. Hyg. 72:198-200.
- 284 **Bhattacharyya, D. R., Handique, R., Dutta, L. P. Dutta, P. , Doloi P., Goswami, B. K., Sharma, C. K.**
 285 **and J. Mahanta. (1994).** Host feeding patterns of *Culex vishnui* sub group of mosquitoes in
 286 Dibrugarh district of Assam. J. Commun. Dis. 26:133-8.
- 287 **Endy, T.P., and A. Nisalak. (2002).** Japanese encephalitis virus: ecology and epidemiology. Cur Top
 288 Microbiol Immunol 267: 11–48.
- 289 **Christopher, S., and R. Reuben. (1971).** Studies on the mosquitoes of north Arcot district, Madras state,
 290 India. Part4: Host preferences as shown by precipitin test. J. Med. Entomol. 8: 314-318.
- 291 **Clements, A.N. (1999).** TheBiology of Mosquitoes. CABI Publishing Ltd.
- 292 **Cliff, A.D. and J.K. Ord. (1981).** Spatial Processes: Models and Applications. Pion Ltd. London.
- 293 **Colless, D.H. (1956a).** Observations on anopheline mosquitoes of the Akah River, 4th Division, Sarawak.
 294 Bull. Entomol. Res. 47: 115-23.
- 295 **Colless, D.H. (1956b).** The *Anopheles leucosphyrus* group. Trans. R. Entomol. Soc. Lond. 108: 37-116.
- 296 **Colless, D.H. (1958).** Notes on the culicine mosquitoes of Singapore VII.-Host preference in relation to the
 297 transmission of disease. Ann. Trop. Med. Parasitol. 53: 259-267.
- 298 **Dawkins, R. and J.R. Krebs. (1979).** Arms races between and within species. Proc. R. Soc. Lond. B.
 299 205:489-511.
- 300 **De Meillon, B. and A, Sebastian. (1967).** Cane-sugar feeding in *Culex pipens fatigans*. Bull. World
 301 Health Organ. 36: 174-176.
- 302 **Edman, J.D., Webber, L.A, and H.W. Kale. (1972).** Effect of mosquito density on the interrelationship

303 of host behavior and mosquito feeding success. *Am. J. Trop. Med. Hyg.* 21: 487-91.

304 **Kweka, E. J., Mwang'onde, B. J., Lyaruu, L., Tenu, F, and A. M. Mahande. (2010).** Effect of different
 305 hosts on feeding patterns and mortality of mosquitoes (Diptera: Culicidae) and their implications on
 306 parasite transmission. *J. Glob. Infect. Dis.* 2: 121–123.

307 **Gajanana, A., Thenmozhi, V., Samuel P.P, and R. Reuben. (1995).** A community-based study of
 308 subclinical flavivirus infections in children in an area of Tamil Nadu, India, where Japanese
 309 encephalitis is endemic. *Bull. World Health Organ.* 73: 237-244.

310 **Gillies, M.T. (1980).** The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a
 311 review. *Bull. Entomol. Res.* 70: 525-532.

312 **Gingrich, J. B., Nisalak, A., Latendresse, J.R., Sattabongkot, J., Hoke, C. H., Pomsdhit, J.,**
 313 **Chantalakana, C., Satayaphanta, C., Uechiewcharnkit, K, and B. L. Innis. (1992).** Japanese
 314 encephalitis virus in Bangkok: factors influencing vector infections in three suburban communities. *J.*
 315 *Med. Entomol.* 29: 436-444.

316 **Gould, D.J., Barnett, H.C, and W. Suyemoto.(1962).** Transmission of Japanese encephalitis virus by
 317 *Culex gelidus* Thobald. *Trans. R. Soc. Trop. Med. Hyg.* 56: 429-435.

318 **Gould, D.J., Edelman, R., Grossman, R.A., Nisalak, A, and M. F. Sullivan. (1974).** Study of Japanese
 319 encephalitis virus in ChiangMai valley, Thailand IV. *Vector Studies. Am. J. Epidemiol.* 100: 49-56.

320 **Hasegawa, M., Tuno, N., Yen, N.T., Nam, V.S, and M. Takagi. (2008).** Influence of the distribution of
 321 host species on adult abundance of Japanese encephalitis vectors *Culex vishnui* subgroup and *Culex*
 322 *gelidus* in a rice-cultivating village in northern Vietnam. *Am. J. Trop. Med. Hyg.* 78:159-68.

323 **Kamimura, K. (1998).** Studies on the population dynamics of the principal vector mosquito of Japanese
 324 encephalitis. *Med. Entomol. Zool.* 49: 181-185.

325 **Leake, C.J., Ussery, M.A., Nisalak, A., Hoke, C.H., Andre, R.G, and D. S. Burke. (1986).** Virus
 326 isolations from mosquitoes collected during the 1982 Japanese encephalitis epidemic in northern

327 Thailand. Trans. R. Soc. Trop. Med. Hyg. 80: 831-837.

328 **Mitchell, C.J., Cheng, P.S, and P.F.L. Boreham. (1973).** Host-preference patterns of four *Culex* species
329 in an endemic area of Japanese encephalitis. Bull. World Health Organ. 49: 293-299.

330 **Mwandawiro, C., Tuno, N., Suwonkerd, W., Tsuda, Y., Yanagi, T, and M. Takagi. (1999).** Host
331 preference of Japanese encephalitis vectors in Chiangmai, Northern Thailand. Med. Entomol. Zool.
332 50: 323-333.

333 **Mwandawiro, C., Boots, M., Tuno, N., Suwonkerd, W., Tsuda, Y, and M. Takagi. (2000).**
334 Heterogeneity in the host preference of Japanese encephalitis vectors in Chiang Mai, northern
335 Thailand. Trans. R. Soc. Trop. Med. Hyg. 94: 238-242.

336 **Peiris, J.S.M., Arunagiri, F.P., Perera, L.P., Karunaratne, S.H.P.P., Ratnayake, C.B., Kulatilaka,**
337 **T.A, and M.R.N. Abeysinghe. (1993).** Japanese encephalitis in Sri Lanka: comparison of vector and
338 virus ecology in different agro-climatic areas. Trans. R. Soc. Trop. Med. Hyg. 87: 541-548.

339 **Pennington, N.E, and C.A.Phelps. (1968).** Identification of the host range of *Culex tritaeniorhynchus*
340 mosquitoes on Okinawa, Ryukyu islands. J. Med. Entomol. 5: 483-487.

341 **Reid, J.A. (1968).** Anopheline mosquitoes of Malaya and Borneo. Stud. Inst. Med. Res. Malaya. 31: 1-520.

342 **Reisen, W.K, and P.F.L. Boreham. (1979).** Host selection patterns of some Pakistan mosquitoes. Am. J.
343 Trop. Med. Hyg. 28: 408-421.

344 **Reuben, R., Thenmozhi, V., Samuel, P., Gajanana, A, and T. Mani. (1992).** Mosquito blood feeding
345 patterns as a factor in the epidemiology of Japanese encephalitis in southern India. Am. J. Trop. Med.
346 Hyg. 46: 654-663.

347 **Ryan, P.A., Lyons, S.A., Alsemgeest, D., Thomas, P, and B.H. Kay. (2004).** Spatial Statistical analysis
348 of adult mosquito (Diptera: Culicidae) counts: an example using light trap data, in redland shire,
349 southeastern queensland, Australia. J. Med. Entomol. 41: 1143-1156.

350 **Samuel, P. P., N. Arunachalam, J. Hiriyan, B. K. Tyagi. (2008).** Host Feeding Pattern of Japanese

351   Encephalitis Virus Vector Mosquitoes (Diptera: Culicidae) from Kuttanadu, Kerala, India. J. Med.
352   Entomol. 45: 927-932.

353   **Solomon, T. (2006).** Control of Japanese encephalitis-within our grasp?. New Eng. J. Med. 355: 869-871.
354   10.1056/NEJMp058263.

355   **Scanlon, J.E, and U. Sandhinand. (1965).** The distribution and biology of *Anopheles balbacensis* in
356   Thailand (Diptera: Culicidae). J. Med. Entomol. 2: 61-69.

357   **Service, M.W. (1969).** Observations on the ecology of some British mosquitoes. Bull. Entomol. Res. 59:
358   161-94.

359   **Service, M.W. (1993).** Mosquito Ecology. Elsevier Applied Science. London.

360   **Smith, A.(1958).** Outdoor cattle feeding and resting of *A. gambiae* Giles and *A. pharoensis* Theo. in the
361   Pare-Taveta area of East Africa. East African Inst. Med. Res. 35: 559-567.

362   **Tuno, N., Tsuda, Y., Takagi, M, and W. Swonkerd. (2003).** Pre-and post-prandial mosquito resting
363   behavior around cattle hosts. JAMCA. 19: 211-219.

364   **Vaughn, D.W., C.H. Hoke. (1992).** The epidemiology of Japanese encephalitis: prospects for prevention.
365   Epidemiol. Rev. 14: 197–221.

366   **Vythilingam, I., Oda, K., MAhadevan, S., Abdullah, G., Thim, C.S., Hong, C.C., Vijayamalar, B.,**
367   **Sinniah, M, and A. Igarashi. (1997).** Abundance, parity, and Japanese encephalitis virus infection of
368   mosquitoes (Diptera: Culicidae) in Sepang district, Malaysia. J. Med. Entomol. 34: 257-262.

369   **Wada, Y. (1969).** Ecological studies of *Culex tritaeniorhynchus summosus* (Diptera: Culicidae) 1. biting
370   rhythm of the mosquito. Jpn. J. Sanit. Zool.20: 21-26.

371   **Wang, L.Y. (1975).** Host preference of mosquito vectors of Japanese encephalitis. Chinese J. Microbiol. 8:
372   274-9.

373

374 **Wharton, R.H. (1962).** The biology of *Mansonia* mosquitoes in relation to the transmission of filariasis in
375 Malaya. Bull. Inst. Med. Res. Malaya.11. 114pp.

376 **WHO. (2011).** Estimated global incidence of Japanese encephalitis: a systematic review, Bull. World
377 Health Org. 89:766-774. E. doi: 10.2471/BLT.10.085233.

378 **WHO. (2015).** Fact sheet No 386. retrieved April 16th, 2016, from
379 <http://www.who.int/mediacentre/factsheets/fs386/en/>

380

381 Figure captions

382 Fig 1. Relationships between numbers of human blood meals in the *Culex vishnui* subgroup sampled in
383 respective house compounds and the numbers of cows kept in the compound in a village in Vietnam.
384 Effect of cow was significant ($P < 0.0001$).

385 Fig 2. Relative numbers of vertebrates species in the village were, cow: human: pig: chicken=1: 8: 16: 80,
386 and the relative blood meals taken from them were, cow: human: pig: chicken=60: 3: 35: 1 in the
387 *Culex vishnui* subgroup sampled in Vietnam.

388

389

Table 1. Species composition (%) of blood meal identified of *Culex* mosquitoes sampled in a village in Vietnam.

Host	Numbers of host	the <i>Cx. vishnui</i>	<i>Cx. gelidus</i>	<i>Cx. quinquefasciatus</i>
		subgroup n=175	n=71	n=314
Human	(370)	2.9	2.8	75.8
Swine	(787)	35.2	28.2	4.5
Cow	(48)	60.3	67.6	4.5
Chicken	(3852)	1.2	1.4	15.3

390

391

Table 2. Unfed ratio in dominant mosquito species sampled by light traps in early night and late night hours in a village in Vietnam

Species	Early night (18-23PM)		Late night (23PM-+09AM)		Total unfed ratio	Total numbers of individuals
	Unfed ratio	Numbers of individuals	Unfed ratio	Numbers of individuals		
<i>Cx. gelidus</i>	0.55	549	0.58	1573	0.57	2122
the <i>Cx. vishnui</i> subgroup	0.53	276	0.62	398	0.58	674
<i>Mansonia annulifera</i>	0.54	28	0.44	25	0.49	53
<i>Cx. quinquefasciatus</i>	0.19	16	0.33	15	0.26	31

Fig 1. Relationships between numbers of human blood meals in the *Culex vishnui* subgroup sampled in respective house compounds and the numbers of cows kept in the compound in a village in Vietnam. Effect of cow was significant ($P < 0.0001$).

Fig 2. Relative numbers of vertebrates species in the village were, cow: human: pig: chicken=1: 8: 16: 80, and the relative blood meals taken from them were, cow: human: pig: chicken=60: 3: 35: 1 in the *Culex vishnui* subgroup sampled in Vietnam.