Ecology and Geographical Expansion of Japanese Encephalitis Virus

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Abstract
Japanese encephalitis virus (JEV) (*Flavivirus: Flaviviridae*) is a leading cause of encephalitis in eastern and southern Asia. The virus is maintained in a zoonotic cycle between ardeid wading birds and/or pigs and *Culex* mosquitoes. The primary mosquito vector of JEV is *Culex tritaeniorhynchus*, although species such as *Cx. gelidus*, *Cx. fuscoccephala*, and *Cx. annulirostris* are important secondary or regional vectors. Control of JEV is achieved through human and/or swine vaccination, changes in animal husbandry, mosquito control, or a combination of these strategies. This review outlines the ecology of JEV and examines the recent expansion of its geographical range, before assessing its ability to emerge in new regions, using the hypothetical establishment in the United States as a case study.
INTRODUCTION

Japanese encephalitis virus (JEV) is a member of the JEV serological complex, the members of which cause significant morbidity and mortality. The complex consists of eight species and two strains/subtypes: Japanese encephalitis (JEV), Murray Valley encephalitis (MVEV), St. Louis encephalitis (SLEV), West Nile (WNV), Kunjin (KUNV), Alfuy, Cacipacore, Yaounde, Koutango, and Ustusu viruses (131). JEV is the most important member of the JEV group, with an estimated worldwide annual incidence of 45,000 human cases and 10,000 deaths. However, because of insufficient medical facilities and inadequate data collection, JE cases are underreported. Thus, the true annual incidence of encephalitis cases is estimated to be closer to 175,000 (133). Recent outbreaks of JEV in northern India and Nepal between 2005 and 2007 have resulted in at least 11,000 cases and over 2000 deaths, highlighting the continued burden of disease in developing countries (93, 148). This review examines the ecology of JEV, details its emergence in new areas, and then assesses the potential for JEV to establish in the Americas, Europe, or Africa.

DISCOVERY OF AGENT AND ELUCIDATION OF TRANSMISSION CYCLE

JEV was originally isolated from the brain of a fatal human encephalitis case in Tokyo in 1934 (74) and from Culex tritaeniorhynchus mosquitoes in 1938 (73). Seminal experiments conducted near Tokyo in the 1950s elucidated the transmission cycle of JEV, with pigs and wild birds identified as amplifying hosts and Cx. tritaeniorhynchus incriminated as the primary vector species (summarized in Reference 6). Pigs are necessary for pre-epizootic amplification of the virus, although some epidemics do occur in the absence of high pig populations (119). Humans and horses can develop fatal encephalitis, but they are only incidentally infected and are dead-end hosts of the virus.

HUMAN INFECTION WITH JAPANESE ENCEPHALITIS VIRUS

Infection of humans with JEV produces a broad spectrum of clinical manifestations, ranging from asymptomatic infection, through mild febrile illness, to acute and lethal meningomyeloencephalitis (118). Most infections are asymptomatic or cause a nonspecific influenza-like illness. Only 1 in 50 to 1 in 1000 infections result in encephalitic illness, although the reason why clinical disease is so rare is unknown (143). The ratio of symptomatic to asymptomatic cases has occasionally been higher [e.g., up to 1:25 in nonindigenous U.S. servicemen (34)]. The case fatality rate of JE can be as high as 67%, although between 20% and 40% is more typical, with children and the elderly at the greatest risk of fatal infection (33, 59, 89). Neurological sequelae occur in 45% to
70% of survivors and can last for many years, with the remaining patients making a full recovery (21). Sequelae are more frequent in patients whose acute disease is severe, prolonged, and associated with coma and focal neurological deficits (60).

**GEOGRAPHICAL RANGE AND EPIDEMIOLOGICAL PATTERNS**

JEV is distributed in temperate and tropical areas of eastern and southern Asia. Its geographic range extends from eastern Asia (China, Japan, Korea, maritime Siberia, Taiwan, the Philippines, and Vietnam), to Southeast Asia and northern Australasia (Cambodia, Indonesia, Laos, Malaysia, Papua New Guinea, Thailand, and the Torres Strait islands of northern Australia), and to southern Asia (Bangladesh, Bhutan, India, Myanmar, Nepal, and Sri Lanka) (10, 26, 70, 143) (Figure 2). A single report has also suggested that JEV may occur in Pakistan (47).

JE is largely a disease of rural areas, especially associated with irrigated rice agriculture. In general, two epidemiological patterns of JEV have been recognized: endemic activity in tropical regions, such as southern Thailand (11), and epidemic activity in temperate and subtropical regions, as first described in Japan (59, 106). In endemic areas, no seasonal pattern exists and sporadic cases of encephalitis occur throughout the year, most often in infants and young children, although cases may peak after the onset of the rainy season. Serological tests have shown that by the time children reach adulthood all have been exposed to JEV and possess neutralizing antibodies. Epidemic activity in temperate and subtropical areas occurs most commonly in summer or early autumn after the rainy season, although this may extend from spring to late autumn, or even throughout summer.
the year in more southern regions. Encephalitis cases in temperate areas are observed most often in children and young adults, although when epidemics occur in new areas or after long periods with no virus activity, all age groups may be affected (136). However, where childhood immunization has been included in the expanded program of immunization in temperate countries, such as Japan, South Korea, and Taiwan, JEV infections are becoming most common in the elderly. The two patterns of endemic and epidemic transmission tend to blur in subtropical regions such as northern Thailand and Vietnam, where epidemic activity may be superimposed on low-level endemic or year-round transmission.

**Epidemics and the Evolution of JEV**

Historically, epidemics of encephalitis attributable to JEV infection have been reported in Japan since 1871, but the first well-described large outbreak occurred in Japan in 1924, with over 6000 cases and a fatality rate of 60%. Subsequent frequent summer epidemics suggested a seasonal occurrence and the possibility that transmission was through mosquito vectors. Summer epidemics were reported regularly.
every 2–3 years in Japan, Korea, and Taiwan until the mid-1960s, but no epidemics occurred thereafter owing to the introduction of childhood immunization programs and widespread use of pesticides on rice paddies. The records of the first epidemics or isolations of JEV in other Asian countries have appeared to follow in a southeasterly direction (70, 116). Major epidemics were reported in northern Vietnam in 1965; in the Chiang Mai Valley of Thailand in 1969 and 1970 (33, 151); in West Bengal in 1973 (14); in the lowland (Terai) region of Nepal in 1978 (136); and in Sri Lanka in 1985–86 (144), with a larger epidemic in 1987 (90). Large outbreaks continue to occur in India, but the recent introduction of major immunization programs by state governments in collaboration with the Program for Appropriate Technology in Health (PATH) offers hope that epidemic activity may soon be controlled (17).

Despite this apparent spread southward from Japan, molecular studies conversely suggest that JEV may have evolved in Southeast Asia and spread north (117). Studies of the geographic occurrence of JEV genotypes using nucleotide sequencing indicate that at least four, and possibly five, genotypes of JEV can be distinguished. The only geographic area where all genotypes are found is the Indonesia-Malaysia region. Moreover, the oldest genotypes are also confined to this region.

**ECOLOGY OF JAPANESE ENCEPHALITIS VIRUS**

**Vertebrate Hosts of JEV**

Viremia and/or seroconversion to JEV has been observed in over 90 wild and domestic bird species belonging to a number of different avian families. However, ardeid wading birds are considered the primary enzootic hosts of JEV, and they can play a role in epizootic viral amplification in some areas (7, 101, 119). Field studies by Buescher and colleagues (7, 107) established the role of ardeids in the ecology of JEV. During a five-year period, 54 strains of JEV were recovered from the black-crowned night heron (Nycticorax nycticorax), plumed egret (Egretta intermedia), and little egret (Egretta garzetta), and all three species consistently displayed neutralizing and hemagglutination-inhibiting antibodies to JEV. Subsequent laboratory experiments demonstrated that postinoculation viremia was sufficient to infect Cx. tritaeniorynchus (9, 32). Because of their close association with humans and varying levels of seroprevalence, pigeons, sparrows, ducks, and chickens have been implicated in natural transmission cycles of JEV, although their actual role in these cycles has not been clearly defined.

Despite high seroprevalence rates in many mammal species (e.g., cattle, dogs, goats, and rodents), pigs are the only mammals that are important in the JEV transmission cycle. Pigs serve as amplifying hosts because they fulfill the following criteria: (a) high natural infection rate (98%–100%); (b) high viremia; (c) viremia that remains high enough to infect mosquitoes for up to 4 days; (d) propensity for vector mosquitoes to feed on swine; and (e) high birth rate, providing a source of susceptible pigs every year (32, 108, 109). Although pigs are the major amplifying hosts of JEV, they can also act as maintenance hosts in endemic areas (53, 114). Although clinical disease is relatively rare, the primary illness associated with JEV infection in pigs is fetal abortion and stillbirth in infected sows and aspermia in boars (12, 129).

**Vectors of JEV**

Although JEV has been isolated from over 30 species, paddy-breeding mosquitoes of the *Culex vishnui* subgroup, particularly *Cx. tritaeniorynchus*, are the major vectors of the virus. This is not surprising, as this species shares a similar ecological niche to *Cx. tarsalis* and *Cx. annulirostris*, the major vectors of JEV serological group viruses in the western United States and Australia, respectively (68, 95). A number of other species, such as *Cx. gelidus*, *Cx. fuscocephala* and *Cx. annulirostris*, have yielded numerous isolates, implicating them as important secondary or regional vectors (90, 99, 145).
Mosquito infection rates display considerable geographical and temporal variation, reflecting the complex interaction of climatic patterns, agricultural practices, mosquito population dynamics, and the presence of susceptible amplifying hosts. Infection rates can be as high as 1:233 or as low as 1:369,146, as have been observed in mosquitoes processed from Japan (8) and India (18), respectively. Distinctive seasonal patterns of virus activity in mosquitoes occur, with increases in infection rates linked to the warmer summer months in temperate areas (8) and the onset of the monsoon season in tropical areas (28).

Laboratory experiments have confirmed the vector competence of *Cx. tritaeniorhynchus* for JEV. This species is susceptible to infection, with titers of only 1–2 log10 infectious units per mosquito required to infect some strains (126, 146). Subsequent transmission rates of 100% are commonly obtained and are a function of the virus titer of the initial blood meal and the temperature at which the mosquitoes are held. Other *Culex* spp. that are efficient laboratory vectors include *Cx. pseudovishnui*, *Cx. gelidus*, *Cx. fuscocephala*, *Cx. annulirostris*, and *Cx. sitiens* (25, 30, 79, 140).

*Cx. tritaeniorhynchus* displays intraspecific variation in susceptibility to JEV infection, with Japanese strains generally more susceptible to infection than strains from Taiwan and Pakistan (127). The genetic basis for this difference in *Cx. tritaeniorhynchus* susceptibility has not been established. However, the discovery of multiple cytochrome oxidase I lineages of *Cx. annulirostris* may potentially explain differences in vector competence of these mosquitoes for JEV genotypes I and II in Australasia (40).

**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* (22, 24). 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 postinfection at 28°C to 20 days at 20°C (126). At low temperatures the transmission rate was reduced.

**Host Feeding Patterns**

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 (42, 81), reportedly as a result of physiological conditioning rather than inherent genetic factors (80). 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 on pigs when available (4, 92). Indeed, pig feeding rates of 30%–40% have been recorded from South Korea (111) and northern India (5). However, throughout much of its geographical range, *Cx. tritaeniorhynchus* obtains most blood meals from cattle (16, 31, 75, 97), and because bovines do not produce sufficient viremia to infect mosquitoes, they may impede transmission of JEV and provide passive zooprophylaxis (48, 80). Humans account for only a small proportion (less than 5%) of blood meals for most *Culex* vectors of JEV in Asia.

**VIRUS SURVIVAL AND REINTRODUCTION**

A variety of mechanisms may explain the ability of JEV to survive during interepidemic or
interepizootic periods, adverse conditions associated with winter or dry season, or the period before the virus is reintroduced. Possible mechanisms include persistence in enzootic foci within vertebrate hosts and/or mosquitoes and reintroduction of the virus by migratory birds and/or mosquitoes.

**Overwintering Mechanisms**

The duration of viremia of JEV in birds and pigs is too short for these animals to effectively maintain the virus during adverse conditions. However, experimentally inoculated bats can sustain low levels of virus in the blood and brown adipose tissue during simulated hibernation at low temperatures; when the bats were returned to 24°C, virus multiplication was activated, raising viremia that invaded other tissues (123). Transplacental transmission in bats has also been demonstrated, which could enhance viral persistence (124).

Experimentally infected lizards, snakes, and frogs also develop a viremia under simulated hibernation (23, 63, 87). However, these results are difficult to interpret because field isolations from poikilothermic vertebrates have only been obtained during summer or autumn rather than spring, which would provide evidence for these animals maintaining the virus through the winter (102).

Experimentally infected *Cx. tritaeniorhynchus* and *Cx. quinquefasciatus* transmit the virus to susceptible hosts following overwintering (44, 71). Despite this, JEV has only been isolated once from field-collected overwintering *Cx. tritaeniorhynchus*, although only low numbers have ever been processed during the winter months (39). Importantly, the female *Cx. tritaeniorhynchus* rarely takes a blood meal prior to hibernation, thus reducing its exposure to viremic animals (86). In Korea, JEV has been isolated twice during winter from *Cx. pipiens* (63).

Vertical transmission can facilitate overwintering of JEV when an infected female mosquito passes the virus to its progeny, which may then harbor the virus during adverse conditions during the egg, larval, pupal, or adult stage. It appears that JEV, like other flaviviruses, enters the fully formed egg through the micropyle at the time of fertilization just prior to oviposition, unlike true transovarial transmission (103). Laboratory transmission studies have demonstrated that vertical transmission occurs through the F1 generation of larvae and adults of numerous species, including *Cx. tritaeniorhynchus*, *Cx. p. pallens*, *Cx. p. molestus*, *Cx. quinquefasciatus*, *Cx. vishnui*, *Aedes albopictus*, *Ae. alcatrani*, *Ae. japonicus*, *Ae. togoi*, *Ae. vexans*, and *Armigeres flavus* (104, 105, 128). However, these results are difficult to interpret in terms of natural transmission cycles, as parenteral inoculation was used as the mode of infection in many instances and JEV is rarely isolated from field-collected immatures or adult male mosquitoes. Indeed, over a 3.5-year period in Taiwan, only one isolate of JEV was obtained from almost 400,000 *Cx. tritaeniorhynchus* larvae, compared with 164 isolates obtained from about 142,000 adult females (103). Additional field isolates have been obtained from larvae or adult males of *Cx. tritaeniorhynchus*, *Cx. pseudovishnui*, *Cx. p. pallens* and *Ae. albopictus* (20, 43, 85).

**Introduction by Migrating Birds, Bats, or Mosquitoes**

Migratory birds or bats and/or wind-borne mosquitoes could reintroduce JEV into temperate regions (84, 117). Alternatively, wind-borne mosquitoes may periodically reintroduce the virus from endemic southern areas of Asia. Indeed, JEV transmission does not occur until after the onset of the southwest winds in temperate areas of Asia (112), and *Cx. tritaeniorhynchus* has been collected up to 500 km offshore in the Pacific Ocean (1) and at an altitude of over 380 m (72). Subsequent long-distance southern migration of *Cx. tritaeniorhynchus* before winter is also observed in northern latitudes during autumn, with a potential dispersal of 200 km per night (72). Finally, backtrack simulations indicated that northerly winds associated with tropical lows west of Cape York Peninsula could have transported mosquitoes from the
island of New Guinea to northern Australia in 1998 (100).

PREVENTION AND CONTROL OF JEV

The prevention and control of JEV transmission can be achieved by three possible strategies, each targeting a specific part of the transmission cycle: (a) vaccination of the human population, (b) control of amplifying hosts by either swine vaccination or changes in animal husbandry, or (c) vector control. Human vaccination is considered the most reliable method of preventing JE in humans.

Vaccination

Until recently, the only vaccine available internationally and recommended for travelers was the mouse brain-derived, formalin-inactivated vaccine developed using the prototype Nakayama strain, which was manufactured and exported by the Biken Institute in Japan (113). Similar vaccines were manufactured in China, Japan, Korea, Taiwan, India, Thailand, and Vietnam (27). Use of this vaccine has almost eliminated the incidence of disease in Japan, Korea, and Taiwan (46, 115, 150), although other activities such as vector control and alternative agricultural practices have all contributed to the reduction in disease (46). Inactivated cell culture vaccines prepared in primary hamster kidney (PHK) or African green monkey kidney (Vero) cells, and a live attenuated SA14-14-2 vaccine have been used in China (64). The SA14-14-2 vaccine has also been used successfully in Nepal (130), and most recently in India (3). The latter recorded some adverse events, although the WHO Global Advisory Committee on Vaccine Safety (WHOGACVS) concluded that no serious adverse events related to the vaccine had occurred; however, they recommended that improved monitoring should be undertaken (149).

There has been a long-held perception of significant risk in using the inactivated mouse brain-derived Biken vaccine, particularly with regard to neurological and allergic reactions (125). Indeed, the Japanese government stopped recommending its use following a single case of acute disseminated encephalomyelitis after vaccination. However, as the link between the vaccine and this severe reaction could not be definitively proven, the WHOGACVS concluded that there was no reason to change current immunization recommendations (147). Despite this, manufacture of the Biken vaccine was discontinued in 2005, necessitating the development of suitable replacement vaccines. Currently, a number of vaccines are under development or in clinical trial for international use (3). These include the inactivated Vero-cell-derived SA14-14-2 vaccines (66, 121) and a live attenuated chimeric vaccine based on the ChimeriVax infectious clone of 17D yellow fever vaccine containing the prM and E genes of SA14-14-2 virus (78). These vaccines, together with the potential licensing of the SA14-14-2 attenuated vaccine in the future, suggest that replacements for the discontinued Biken vaccine will soon be available.

Control Measures Targeting Vertebrate Hosts

Some protection against swine abortion is afforded by vaccination of sows (56). However, swine vaccination is not effective or practical for preventing transmission to humans. Because most pigs are slaughtered at 6–8 months of age, annual vaccination of newborn piglets is required and maternal antibodies render the live-attenuated vaccine ineffective against pigs less than 6 months of age (46). The reduction of JE incidence in Japan, Taiwan, and Korea has been partially linked to the relocation of domestic pigs to specialized farms sited away from human habitation (120, 136, 150). Finally, in Japan, vaccination of horses has been carried out during the JE transmission season each year since 1948 (82), resulting in a decrease in incidence of equine encephalitis (29). Indeed, vaccination of race horses is mandatory in several countries, including Singapore, Malaysia, and Hong Kong.
Mosquito Control

Although ultralow-volume insecticide application has had some success in Southeast Asia (110), it is generally accepted that such vector control is impractical and costly during large, widespread outbreaks (10, 45, 133, 143). The extensive rice paddies that provide larval habitats for *Cx. vishnui* subgroup mosquitoes, coupled with the isolation of rural villages, make it virtually impossible to employ large-scale chemical treatment to control JEV transmission (62). However, the widespread application of pesticides to control agricultural insects has had the added benefit of also reducing mosquito populations (61, 76, 150). Indoor residual spraying using DDT and other chemicals to control malaria is largely ineffective in reducing JEV transmission, owing to the largely exophilic resting behavior of the vector mosquitoes, although JE incidence was reduced in China, where pyrethroid-impregnated bed nets were deployed (65). In most JEV endemic rural settings, where vaccination rates are often low, an integrated vector management approach incorporating alternating wet and dry irrigation and larvivorous fish can reduce vector populations and potentially JEV transmission (54, 62).

THE SPREAD OF JAPANESE ENCEPHALITIS VIRUS

In common with other members of the JEV serological complex, JEV has shown a propensity to spread and establish in new areas, most recently in the eastern Indonesian archipelago, the island of New Guinea, the Torres Strait of northern Australia (68), and southwest India (96). The incidence and spread of JEV has recently been extensively reviewed by Mackenzie et al. (70).

Mechanisms of JEV Movement

Several mechanisms could explain the spread of JEV. Natural cycling involving mosquitoes, pigs, and ardeid birds is thought to have spread the virus regionally (70). Viremic migratory birds (84) and even bats, especially fruit bats (Megachiroptera) (2, 122), may be involved in distant transport. Wind-blown infected mosquitoes have been suggested for the dispersal of JEV in China (72) and into Australia (100). The incidental transport of infected mosquitoes on aircraft has been implicated (38), and it has been suggested that Japanese troop movements in World War II may have introduced JEV into areas of Southeast Asia, possibly via transport of infected mosquitoes in aircraft or equipment, or via infected pigs (70). Rice irrigation and fertilizers that have resulted in increased vector populations, and increased pig production, have all been associated with the spread and establishment of JEV in new areas (54, 70, 132).

The Australian Experience

While JEV has recently appeared in Australia, it is noteworthy that after more than 10 years, the virus has apparently not become established on the mainland, let alone spread beyond the region. This is despite predictions that when JEV first appeared in a widespread outbreak on the Torres Strait islands in 1995 (37), it was feared that it would spread to the mainland’s Cape York Peninsula, where populations of feral pigs, wading birds, and mosquitoes were prolific (67). In light of the explosive spread of WNV in the Americas, the apparent inability of JEV to establish warrants a detailed discussion.

When compared to the Torres Strait islands, where intense activity has resulted in repeated mosquito isolates and widespread rapid seroconversion of pigs, recorded JEV transmission has been of a low level on Cape York Peninsula. First, fewer domestic pigs are housed in Cape York Peninsula communities and seroconversion of these pigs to JEV during two incursions of JEV into Cape York Peninsula in 1998 and 2004 has been asynchronous, thus limiting the pool of available epizootic hosts (36). Second, collections of 48,495 *Culex sitiens* subgroup mosquitoes on Cape York Peninsula in 1998 and 2004 yielded only one JEV isolate.
(in 2004) (138, 139), whereas comparable collections during JEV outbreaks on Badu Island in the Torres Strait yielded 66 JEV isolates (51, 99, 141). Finally, with the exception of a single human clinical case, there is no serological evidence of human JEV infections on Cape York Peninsula from sera surveys of 1092 individuals in seven communities following the 1998 outbreak (36). Furthermore, no JEV activity has been detected in mainland sentinel pigs in years following the Cape York Peninsula incursions (139). Although it is possible that some JEV transmission is cryptically maintained in foci on Cape York Peninsula, JEV certainly has not become a significant public health issue on the Australian mainland.

Several mechanisms have been proposed to account for the failure for JEV to become established on the Australian mainland. Cape York Peninsula has several endemic flaviviruses, such as KUNV, MVEV, and Kokobera virus (50, 138), that might cross-protect pigs from infection with JEV (36). However, JEV cocirculates with related flaviviruses in Papua New Guinea (52) and India (101). Different lineages of Cx. annulirostris occur on Cape York Peninsula and southern Papua New Guinea and may differ in their vector competence for JEV. Indeed, there is preliminary evidence to suggest that the most widely distributed mainland Australian lineage of Cx. annulirostris is a relatively inefficient laboratory vector of genotype I JEV that has been circulating in northern Australia since 2000 (40).

The presence of alternative hosts may serve to minimize blood feeding by Cx. sitiens subgroup mosquitoes on feral pigs. Analysis of host feeding patterns revealed that only 5% and 1% of bloodfed Cx. sitiens subgroup mosquitoes collected from rural locations on western Cape York Peninsula had fed on birds and pigs, respectively (137). Most (75%) of these blood meals were of marsupial origin and were retrospectively identified to be from the agile wallaby (Macropus agilis) (142), which is abundant on Cape York Peninsula but absent from most Torres Strait islands. When experimentally inoculated with JEV, both Tammar wallabies (Macropus eugenii) and agile wallabies failed to develop a viremia (69). Indeed, if agile wallabies are an unsuitable JEV host, then preferential blood feeding by Culex on this species could effectively dampen transmission and prevent virus maintenance (137). The only areas on Cape York Peninsula where JEV has been detected in pigs or mosquitoes had local concentrations of domestic or feral pigs accompanied by a high incidence of blood feeding by Cx. sitiens subgroup mosquitoes on pigs (137, 139).

**POTENTIAL EMERGENCE AND PUBLIC HEALTH RISK TO REGIONS OUTSIDE ITS NATURAL RANGE**

Considering its propensity to spread, JEV could become established in new ecosystems outside of its current range. However, unlike the recent expansion into India and Australasia, where overlapping natural cycles are thought to have introduced the virus, JEV would have to traverse great distances over the Pacific Ocean, the Indian Ocean, and the deserts and mountains to the northwest of Pakistan to infest the Americas, Europe, or Africa. Introduction by migratory birds is plausible, although the migration routes of ardeid birds are generally north-south rather than east-west, and birds would no longer be viremic after the long journey from Asia. Instead, human-transported mosquitoes or viremic vertebrates would be more likely mechanisms, as has been proposed for the potential introduction of WNV into Hawaii from the United States (55). However, owing to animal quarantine procedures and the short duration of viremia in birds and pigs, the most likely mechanism of introduction would be via an infected mosquito transported by aircraft from a JEV endemic area. Indeed, high populations of adult Cx. tritaeniorynchus occur near Narita International Airport, Japan, and this species has been collected in aircraft originating from other locations in Asia (38).

Should JEV be introduced into a new region, a range of factors will influence its establishment, as illustrated using a hypothetical
North American scenario. The areas at potentially greatest risk are those with intensive pig rearing, such as the Midwestern United States, where swine populations exceed 30 million head (83). However, many of these are reared within purpose-built buildings and may not be exposed to significant mosquito feeding, and the Australian experience suggests that JEV may be unable to establish itself in areas if vectors cannot or do not access significant swine populations.

For it to establish, JEV may have to exploit native or introduced vertebrate species in much the same way as WNV, for which endemic birds (57), reptiles (49), and mammals (88) have been implicated in transmission cycles. Numerous species of ardeid wading birds occur in North America, including widespread resident populations of _N. nycticorax_ and the cattle egret (_Bubulcus ibis_), two species involved in JEV transmission in Asia (98). Other bird species common to both Asia and North America, including English sparrows, house finches, pigeons, ducks, and chickens, produce viremia after experimental infection (15, 19, 35). However, these bird species do not contribute to epidemic JEV transmission to the same extent as ardeid birds and pigs, possibly because they only produce a low-level viremia, and _Cx. tritaeniorhynchus_ rarely feeds on them. Nonetheless, there are guilds of ornithophilic _Culex_ species (77) that may facilitate enzootic transmission through these birds in North America. Should common urban fauna act as vertebrate hosts, JEV could become established in both urban and rural locations.

Prior infection of vertebrate hosts with endemic flaviviruses, such as SLEV and WNV, may provide some level of immunity against JEV, thus reducing the pool of available hosts to facilitate enzootic transmission. However, the cocirculation of JEV and MVEV in Papua New Guinea, and of JEV and WNV in Pakistan, suggest that sufficient populations of susceptible hosts exist to allow concurrent circulation of these closely related flaviviruses in the same ecosystem (52, 101).

A number of secondary or moderately susceptible JEV vectors already occur in North America, including _Cx. pipiens_, _Cx. quinquefasciatus_, _Ae. albopictus_, _Ae. japonicus_, and _Ae. vexans_. However, endemic _Culex_ spp. are more likely to be involved in local transmission, especially vectors of endemic JEV serological group flaviviruses, such as _Cx. tarsalis_, _Cx. pipiens_, _Cx. salinarius_, and _Cx. nigripalpis_. Vector competence experiments with North American mosquitoes conducted in the 1940s not only confirmed that _Cx. tarsalis_ and _Cx. pipiens_ could serve as laboratory vectors of JEV but also incriminated non-_Culex_ species, including _Ae. dorsalis_ and _Culiseta inornata_ (94).

The introduction and establishment of JEV in a virgin ecosystem, such as the United States, could have dramatic consequences for human and animal health. JEV has a higher rate of severe neurological disease compared with other endemic encephalitic flaviviruses, such as SLEV and WNV (134, 135). Additionally, SLEV and WNV more often cause severe disease in the elderly, whereas both children under 5 years of age and the elderly typically develop severe clinical manifestations following JEV infection. Furthermore, when JEV emerges in an immunologically naïve population, clinical disease occurs in all age groups (13, 41). Finally, in terms of animal disease, significant equine morbidity and mortality could occur, and severe disease may develop in previously unexposed vertebrate species, as has occurred with WNV infection in birds (57) and alligators (49).

A comprehensive contingency plan is necessary to limit the potential impact of JEV should it emerge in a region outside of its current geographical distribution. This plan should include an active surveillance system, comprising human and animal clinical case diagnosis, coupled with JEV-specific diagnostic assays for rapid detection of an incursion. Should the virus become established, control programs will have to be implemented, potentially at significant economic cost to all levels of government. Widespread immunization of residents at risk
may be needed, with introduction of the vaccine into the early childhood vaccination schedule. Tourism could be affected, especially if vaccination is recommended for travelers visiting endemic areas, as is the case with visitors to Southeast Asia (113). The economic impact on agriculture would also be considerable, as there would be concerns regarding the export of livestock and associated products, resulting in a requirement for widespread testing to verify freedom from infection. Importantly, the potential role that endemic mosquitoes and vertebrate species could play in transmission cycles needs to be assessed using laboratory-based infection and transmission experiments before an outbreak occurs.

**SUMMARY POINTS**

1. JEV is distributed throughout Southeast Asia and the Indian subcontinent, through the Indonesian archipelago, and into the Australasian zoogeographical region.

2. Recent outbreaks in northern India and Nepal have resulted in almost 11,000 cases and 2000 deaths.

3. JEV is maintained in an enzootic cycle between ardeid wading birds and *Culex* mosquitoes; pigs are important for epizootic transmission.

4. Although humans and horses develop fatal encephalitis, they are dead-end hosts.

5. Proposed overwintering mechanisms include persistence in vertebrates and/or mosquitoes and vertical transmission in the mosquito. Alternatively, migrating birds and/or bats and wind-assisted dispersal of mosquitoes may reintroduce the virus.

6. Control measures include human vaccination and, to a limited extent, alternative pig husbandry and vector control.

7. While JEV has expanded its range into northern Australia, it does not appear to have become established in natural transmission cycles. Possible reasons for this include (a) the presence of related flaviviruses that may provide cross-protection against JEV infection in susceptible hosts; (b) different lineages of *Cx. annulirostris*, which may vary in their vector competence to the different genotypes of JEV; and (c) a propensity for *Cx. annulirostris* to feed on marsupials and not pigs or wading birds (experimentally infected marsupials, especially wallabies, do not produce high levels of viremia).

8. There is the potential for JEV to spread to the Americas, Europe, or Africa, but the long distance from endemic areas makes this difficult, and modern pig husbandry may impede virus amplification.

**FUTURE ISSUES**

1. Modeling should be used to investigate why JEV has failed to establish in mainland Australia, as well as its potential for spread and establishment in North America, Europe, and Africa.

2. Assessment is needed whether JEV could establish in a new region by conducting vector competence experiments with native mosquito species, especially those belonging to the genus *Culex*. In addition, conducting laboratory-based infection studies of common
vertebrate fauna with JEV may determine their potential role in JEV transmission cycles. This will provide information to facilitate a targeted response should the virus be introduced.

3. The genetic basis for the variation in vector competence between populations of *Cx. tritaeniorhynchus* and other vector species should be investigated.

4. *Culex* ecology, especially blood-feeding behavior, near modern intensive pig-rearing facilities or ardeid roosts should be investigated.

5. It should be assessed whether infection of vertebrates with endemic flaviviruses, including MVEV, WNV and SLEV, provides cross-protection or immune enhancement following infection with JEV.

6. More specific serological assays are required to differentiate JEV from related flaviviruses.

7. Development and adoption of safe, low-cost, and effective vaccine candidates is needed.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED


6. Summarizes the epidemiological and ecological experiments that elucidated the transmission cycle of JEV in Japan.


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37. Details the original outbreak of JEV in northern Australia and therefore the emergence of the virus in the Australasian region.

59. Kono R, Kim KH. 1969. Comparative epidemiological features of Japanese encephalitis in the republic of Korea, China (Taiwan) and Japan. *Bull. WHO* 40:263–77


117. Suggests that the different JEV genotypes evolved from an ancestral virus in Indonesia-Malaysia and spread across Asia.

126. Details the intrinsic and extrinsic factors that influence the vector competence of Cx. tritaeniorhynchus for JEV.


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