Quantification of vector and host competence for Japanese Encephalitis Virus: a systematic review and meta-analyses of the literature.

by

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Abstract

Japanese encephalitis virus (JEV) is a virus of the *Flavivirus* genus that may result in encephalitis in vertebrate hosts. This vector-borne zoonosis occurs in Eastern and Southeastern Asia and an intentional or inadvertent introduction into the United States (US) would lead to important public health and economic consequences. The objective of this study was to gather, appraise, and synthesize primary research literature to identify and quantify vector and host competence for JEV, using a systematic review-meta-analysis (SR-MA) approach.

After defining the research question, we performed a search in selected electronic databases. The title and abstract of the identified articles were screened for relevance using a defined set of exclusion and inclusion criteria, and relevant articles were subjected to a risk of bias assessment followed by data extraction. Random-effects subgroup meta-analysis models were fitted by species (mosquito or vertebrate host species) to estimate pooled summary measures as well as to compute the variance between studies. Meta-regression models were fitted to assess the association between different predictors and the outcomes of interest and to identify sources of heterogeneity among studies.

Data were extracted from 171 peer-reviewed articles. Most studies were observational (59.06%) and reported vector competence (60.2%). The outcome measures reported pertained to transmission efficiency, host preference, and vector susceptibility to infection within vector competence; and susceptibility to infection within host competence.

All outcome measures (JEV proportion of infection in vectors and hosts from observational studies; and JEV infection, dissemination, and transmission rates in vectors from experimental studies) had high heterogeneity. Mosquito species, diagnostic method, country, and capture method represented important sources of heterogeneity associated with the proportion of JEV infection in vectors; host species and region were considered sources of heterogeneity associated with the proportion of JEV infection in hosts; and diagnostic and mosquito capture methods were deemed important contributors of heterogeneity for the minimum infection rate (MIR) outcome. Mosquito species and administration route represented the main sources of heterogeneity associated with JEV infection rate in vectors.

Quantitative estimates resulting from this SR-MA will be inputted into risk assessment models to evaluate risks associated with the introduction of JEV in the US.

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Chapter 1 - Literature review

The Japanese encephalitis virus (JEV) is the causative agent of Japanese encephalitis (JE), a vector-borne zoonosis that affects most countries in South and Southeast Asia, and the Western Pacific Rim. It is considered the main cause of viral encephalitis in that region, affecting mainly children up to 14 years old. The virus is transmitted by mosquitoes, particularly of the *Culex* genus, and the transmission cycle is complex, involving pigs and ardeid birds as reservoir hosts. Environmental, ecological, and social determinants play a paramount role on the epidemiology of JE and JEV, as well as on its geographical expansion over new territories. Japanese encephalitis has no cure; thus, efforts are put forth towards prevention and control by reducing exposure to potentially infected mosquitoes or vaccination. The sections below aim at covering the most important aspects of JE and JEV, providing background information for a better understanding of our current knowledge and laying the ground work for the following chapters, which comprise the research work of this thesis.

Historical background

The history of JE dates to the 19th century, when it was first recognized in humans and horses in Japan in 1871. Later, in 1924, an epidemic occurred and from then on, approximately every ten years a severe epidemic took place in Japan. The agent could be extracted from a human brain and passed to rabbits, but it was not until 1934 that it was inoculated intracerebrally in monkeys and better characterized. The following year, the Nakayama strain was established after being isolated from a human brain in Tokyo (Misra and Kalita, 2010). Japanese encephalitis was initially called Japanese B encephalitis to distinguish it from von Economo encephalitis, also known as encephalitis lethargica (Solomon, 2006).

The transmission cycle and the involvement of pigs and birds as reservoir hosts were elucidated in 1959 though the acknowledgement of JE as a mosquito-borne disease had already taken place in the early 1930s when the agent was isolated from a *Culex tritaeniorhynchus* mosquito (Misra and Kalita, 2010).

The first studies on JEV transmission ecology were performed in Tokyo, circa 1952, by US Army personnel (Scherer and Buescher, 1959). Viral sequencing was completed in 1990 with genetic studies contributing to further our understanding of the virus and its evolution and adaptation to newer habitats. It is suggested that JEV originated in the Indonesia-Malaysia region, as it has been found that all five JEV genotypes overlap on that geographical area, having then spread over Asia (Erlanger *et al.*, 2009; Misra and Kalita, 2010). The last region JEV has reached was Australia (Torres Strait), probably from Papua New Guinea through wind-blown mosquitoes (Solomon, 2006).

In 2011, about 67,900 JE cases were reported annually worldwide, with an estimated overall incidence of 1.8 per 100,000 people, of which only approximately 10% were reported to the World Health Organization. Accurate incidence estimates are cumbersome to calculate due to differences in intensity and quality of JE surveillance in affected countries and the availability of laboratory diagnostic tests, which differ throughout the world (Campbell *et al.*, 2001). It is also estimated that half the cases occur in China (excluding Taiwan) and 75% occur in children aged between 0 and 14 years old, as prior to gaining immunity by natural exposure to JEV, subclinical infection, or vaccination, they comprise the most susceptible part of the population (Misra and Kalita, 2010; Campbell *et al.*, 2001).

Virology

Japanese encephalitis virus is a single-stranded positive-sense RNA virus with an envelope. It has an open reading frame (ORF) that encodes three structural proteins (the capsid protein C, the precursor to the membrane protein PrM, and the envelope protein E) and seven nonstructural proteins. The protein E gene sequences are considered to be responsible for JEV virulence and are important for the entry of the virus into the host cell, and also for being a major target of humoral immune response. The virus enters the host cell by endocytosis, then fusing the lipid membrane with the endosome membrane by conformational change, which leads to the penetration of the viral RNA into the cytoplasm of the now infected host cell (Misra and Kalita, 2010).

By being a member of the genus *flavivirus* and *Flaviviridae* family, JEV is genetically close to the West Nile virus (Africa and the Middle East) and the Saint Louis encephalitis virus (North America), sharing both clinical and ecologic features. Other neurotropic viruses related to JEV and found around the world include: the *alphavirus* equine encephalitis virus (North America) and other members of the *Flaviviridae* family – Murray Valley encephalitis virus (Australia), Roccio virus (South America), and tick-borne encephalitis virus (Russia). These viruses have probably evolved from a common ancestor around 10,000 to 20,000 years ago, further developing apart to adapt to the specific ecological niches they encountered (Solomon, 2006; Misra and Kalita, 2010).

There are five JEV genotypes that differ among them at the nucleotide level by 10%, though differences in neurovirulence are debated. Genotype III, located in the temperate regions of Asia, has spread more widely and the oldest lineages are genotypes IV (Indonesia) and V (Singapore) (Solomon, 2006). Genotype I is located in Northern Thailand, Cambodia, South Korea, China, Japan, Vietnam, and Taiwan; and genotype II is located in Southern Thailand, Malaysia, Indonesia, Papua New Guinea, and Northern Australia. According to Le Flohic *et al.* (2013), 98% of the strains isolated from 1935 to 2009 belong to genotypes I, II, and III and no straightforward relationship between genotype and clinical features of JE has been established (Le Flohic *et al.*, 2013).

Changes in genotype may be attributed to JEV fitness to new competent vectors or, potentially, to new host availability, but no differences in terms of transmission mechanisms, pathogenicity, vector or host preference have been established among genotypes (Le Flohic *et al.*, 2013).

Enzootic cycle

The epidemiology of JEV is dependent on the biology of vectors and hosts, as well as environmental factors (Le Flohic *et al.*, 2013). Because JEV is an arthropod-borne virus (ARBO virus), it requires a blood sucking arthropod to complete its life cycle (Misra and Kalita, 2010).

There are three epidemiological patterns of JEV that occur in Southeast Asia: an endemic pattern in the southern regions (South of India, South of Vietnam, South of Thailand, the Philippines, Malaysia, and Indonesia); an intermediary pattern in the subtropical region (Northern India, Burma, Northern Thailand, Northern Vietnam, Southern China, and Bangladesh), characterized by low intensity but year-round transmission with peaks in the rainy season; and an epidemic pattern in the temperate regions (North of China, Korea, Japan, Taiwan, and South of Russia), where JEV transmission varies according to temperature, with epidemics occurring in the Summer and Fall (Misra and Kalita, 2010). Besides humans, domestic animals may also become infected and develop clinical signs (Misra and Kalita, 2010).

The two main transmission cycles are expanded on the sections below. Both cycles, domestic (pig-associated) and wild (bird-associated), may coexist, as vectors may feed on both types of hosts concurrently (Le Flohic *et al.*, 2013).

Pig-associated transmission cycle

Pigs act as amplifying hosts, bringing the virus closer to human dwellings, particularly in Asia, where backyard farming is common (Solomon, 2006).

Pigs, both domestic and wild, are considered the most important JEV reservoir because there is an increased incidence of natural swine infection. Furthermore, they develop high titers of viremia when infected, which lasts for two to four days, *Culex* mosquitoes tend to feed on pigs in nature, and the number of susceptible pigs is large due to a high turnover rate in the pig industry (Misra and Kalita, 2010).

Though usually asymptomatic, JEV can cause reproductive disease in pigs, with losses, such as abortions in sows, stillbirths or mummified fetuses, and reduced fertility in boars, reaching up to 70%. Newborn piglets may have clinical neurological signs, such as tremors and convulsions, with mortality in non-immune, infected piglets reaching up to 100%. Non-pregnant females may have mild clinical signs (fever) or subclinical JE. Naturally infected pigs have long-lasting immunity (and therefore are no longer able to amplify JEV) and mortality rates in adult pigs is nearly zero (Misra and

Kalita, 2010; OIE Technical Disease Cards, 2013). More recently, Ricklin *et al.* (2016) published evidence that vector-free transmission between pigs without the intervention of arthropod vectors is possible, further increasing the importance of the role pigs play in JEV transmission.

Bird-associated transmission cycle

There are over 90 bird species, both wild and domestic, that can become infected and transmit JEV. Once infected, birds become immune to JEV and are no longer able to amplify the virus (van den Hurk *et al.*, 2009; Misra and Kalita, 2010). However, migration or hormonal stress could lead to a reactivation of latent JEV in birds.

Wading birds of the *Ardeidae* family, such as egrets (*Egretta garzetta*) and herons (*Nycticorax nycticorax*), are highly susceptible to JEV infection, reaching high viremia titers that last as long as four days and representing an important source of infection for mosquitoes (Gresser *et al.*, 1958; Le Flohic *et al.*, 2013). Furthermore, Cleton *et al.* (2014) compared JEV mean peak viremia between two days old and 42 days old ducklings and chicks that were experimentally infected and demonstrated that viremia decreases as the age of infection increases, demonstrating that magnitude of viremia is related to young age in birds.

Interpretation of JEV serosurveillance in birds could be complicated by other existing circulating flaviviruses, as cross-protection between flaviviruses, such as West Nile virus and JEV, exist (Nemeth *et al.*, 2012).

Vertical transmission

Vertical transmission of JEV between mosquitoes and their offspring had been suggested as a potential strategy for JEV to survive the cold season and restart the transmission cycle every year in the northern regions where an epidemic pattern occurs. Takashima and Rosen (1989) and Rosen *et al.* (1989) evaluated vector competence of several mosquito species for JEV, as well as their ability to transmit the virus vertically to their offspring. Vertical transmission in A*edes japonicus* was demonstrated by Takashima and Rosen (1989). Furthermore, Rosen *et al.* (1989) demonstrated vertical transmission in the F1 adult stage in *Culex tritaeniorhynchus*, *Culex annulus*, *Culex quinquefasciatus*, and *Armigeres subalbatus*, and in the F1 larval stage in *Culex pipiens*, *Aedes vexans*, *Aedes alcasidi*, and *Armigeres flavus*.

Overwintering

Besides vertical transmission in mosquitoes, literature suggests that the virus survives the cold season by overwintering in hibernating mosquitoes, mosquito eggs, or reptiles. Another theory is that JEV is reintroduced every year by migrating birds. An argument against it, however, is based on the fact that adult birds become immune after infection, thus reinfection is unlikely, though migration stress or

hormonal stress could play a role in the reactivation of latent JEV in birds. Nonetheless, the fact that different JEV strains have distinct geographical locations between the northern (genotypes I and III) and southern regions (genotypes II, IV, and V) dismisses this possibility. If migrating birds were responsible for JEV introduction in the northern, epidemic regions, all genotypes would be scattered across Asia, with no evident genotype distribution pattern (Misra and Kalita, 2010). Regardless, it is possible that migratory birds may still play a role on JEV overwintering.

Ecological factors

The mechanisms by which JEV transmission takes place are related to a change in land usage and agricultural practices. As the economy improves and the rice industry flourishes at the expense of deforestation, increased opportunities for mosquito breeding develop, as rice paddy fields are considered ideal breeding grounds for mosquito development. Furthermore, paddy fields also attract migrating birds, adding to the complex interplay of factors characterizing JEV transmission and spread (Solomon, 2006; Misra and Kalita, 2010).

Higher humidity rates and temperatures lead to mosquito development and consequent higher number of vectors available to infect (Misra and Kalita, 2010). Besides climate, altitude may also play an important role on JEV transmission (Keiser *et al.*, 2005).

In the most western and southeastern parts of Asia where JEV has spread and outbreaks occurred (Pakistan and the Torres Strait in northern Australia, respectively), other factors may be implicated in JEV transmission. In Australia, Solomon (2006) suggested that infected wind-blown mosquitoes might have crossed the strait between Papua New Guinea and Australia, carrying the virus along. Climate change may also be implicated in the expansion of JEV, as higher temperatures increase the span of the mosquito season in temperate regions, such as Pakistan (Erlanger *et al.*, 2009).

Bird movement, implicating both resident and migratory birds, are associated with JEV transmission over short distances, although the mechanism involved is not well understood (Brown and O'Brien, 2011).

Japanese encephalitis in vectors and other hosts

Culex tritaeniorhynchus and *Culex vishnui* are important JEV vectors because they breed in rice paddies and other dirty water, but there is evidence pointing to more than 30 mosquito species that may carry JEV (Solomon, 2006; Le Flohic *et al.*, 2013).

Among mosquito species that are competent to JEV, the following species may be found in the United States (US): *Culex tritaeniorhynchus, Culex tarsalis, Culex annulirostris, Culex gelidus, Culex fuscocephala, Culex vishnui, Culex bitaeniorhynchus, Culex pseudovishnui, Culex whitmorei, Culex sitiens, Culex pipiens (Molestus form), Culex pipiens pipiens, Culex quinquefasciatus, Culex salinarius, Culex nigripalpus, Culiseta inornata, Ochlerotatus dorsalis, Ochlerotatus nigromaculis,*

Ochlerotatus japonicus, Aedes albopictus Skuse, Aedes japonicus, Aedes vexans, Anopheles spp., and *Mansonia uniformis* (Darsie and Ward, 2005).

Besides pigs and birds, other domestic animals that may have subclinical disease, but likely do not contribute to JEV transmission, are horses and other equids (donkeys), cattle, sheep, goats, dogs, cats, chickens, ducks, sylvatic mammals, reptiles, and amphibians (OIE Technical Disease Cards, 2013).

Vector competence for JEV refers to the ability of arthropods to acquire, maintain, and transmit JEV, while host competence for JEV is related to the ability of the JEV infected vertebrate hosts to make the virus available to vectors during feeding, thus maintaining the transmission cycle. Vector competence is determined by mosquitoes' transmission efficiency, as well as host preference, and susceptibility to infection. Conversely, host competence is determined by hosts' susceptibility to infection. These parameters are essential to further our knowledge and clarify the epidemiology of JEV and its transmission patterns, thus leading to a better understanding of potential paths of introduction of JEV and aiding in the implementation of methods of prevention and control (Huang *et al.*, 2014).

Japanese encephalitis in humans

Humans are dead-end hosts, as they have a low-level, transient viremia and though they do not transmit the virus, they can become infected (Solomon, 2006).

In endemic areas, children and travelers are usually those who become infected. The highest attack rates occur in children between 3 and 6 years old and become increasingly lower after 14 years old, time at which the levels of neutralizing antibodies rise, either by natural exposure to JEV, by subclinical infection or vaccination (Misra and Kalita, 2010).

The reason why symptomatic JE occurs only in a small proportion of infected humans is unclear. Some hypotheses include viral factors related to the route of entry, viral titers, and neurovirulence; host factors, such as age, genetic make-up of the individual, general health status and immunity; and endemicity of JEV (Misra and Kalita, 2010).

Pathogenesis

It is unclear how JEV crosses the blood-brain barrier and reaches specific regions of the central nervous system (Solomon, 2006).

After an infected mosquito bite, incubation period lasts between 5 and 15 days. The virus amplifies peripherally in the dermal tissue and then the lymph nodes, producing a transient viremia. By unknown mechanisms, JEV then crosses the blood-brain barrier and reaches the central nervous system. Japanese encephalitis virus infection triggers both humoral and cellular responses. IgM increases before central nervous system invasion and neutralizes extracellular virus by facilitating the lysis of infected cells. T-cell response has been observed in convalescent JE patients, as well as

vaccinated people, thus possibly playing an important role in the control of JEV. Moreover, CD4+ cells and JEV-specific CD8+ T lymphocytes recognize JEV's E protein, activating an inflammatory response through the action of chemotactic cytokines, such as IFN-alpha, beta and gamma, and TNF-alpha (Misra and Kalita, 2010).

Clinical features

JEV affects the thalamus, corpus striatum, brainstem, and spinal cord. There is a reported co-infection of JE with neurocysticercosis, which may suggest that one disease predisposes to the other or simply that they coexist, as both have the pig as a common reservoir. Furthermore, meningitis or head injuries may increase the risk of neuroinvasion (Misra and Kalita, 2010).

Most JE patients experience nonspecific illness with acute short-lived symptoms, such as headache, fever, rigor, and gastrointestinal symptoms (nausea, anorexia, vomiting, and abdominal pain), that last from two to four days. However, more severe JE include altered senses, seizures, neurological deficit (hemiplegia, quadriplegia, or cerebellar signs), acute flaccid paralysis and movement disorders, such as dystonia (of the limbs, orofacial, or axial). Prolonged convalescence is a hallmark of clinical JE (Misra and Kalita, 2010).

In endemic areas, JE may predispose to Guillain–Barré syndrome, an auto-immune disease that attacks the peripheral nerves and damages their myelin insulation (Misra and Kalita, 2010).

About 20% to 40% of JE patients die during the acute stage of the disease, with 50% of the survivors having chronic severe neurological sequelae, which include cognitive dysfunction, abnormal behavior, seizures, and movement disorders (Solomon, 2006; Misra and Kalita, 2010).

Diagnosis

Diagnostic confirmation of JE should be based on clinical, biological, neurophysiologic, and cerebral imaging findings criteria (Diagana *et al.*, 2007).

Besides the clinical symptoms described above, brain imaging findings aid in the diagnosis of JE. Such findings include bilateral hemorrhagic lesions of the thalamus that can be seen on cerebral computerized tomography (CT scan) and are characteristic of JE (Diagana *et al.*, 2007).

Laboratory findings include classical signs of inflammation (polymorphonuclear leukocytosis and increased sedimentation rate), hyponatremia (due to decreased secretion of antidiuretic hormone), normal or slightly increased pressure in the cerebrospinal fluid (CSF), atypical lymphocytes type I (resulting from abnormal CD5 cells), and atypical lymphocytes type II with typical basophilic cytoplasm and chromatin nucleus (resulting from abnormal CD8 cells) (Diagana *et al.*, 2007).

Final diagnosis is achieved by identification of IgM by ELISA in the blood or CSF, which has high sensitivity and specificity, detecting antibodies in 75% of cases. The ELISA method demonstrating

IgM to IgG seroconversion in two successive blood samples is considered the best diagnostic tool (Diagana *et al.*, 2007; Misra and Kalita, 2010).
Western Blot, hemagglutination inhibition test, and rapid complement fixation may lead to confusing results as there may be cross-reactivity with other flaviviruses (dengue and West Nile virus), which limits their application. RT-PCR used for viral genome amplification also allows rapid detection of RNA in the CSF, but is rarely used (Diagana *et al.*, 2007).
Differential diagnosis includes other encephalitis, such as herpes encephalitis, dengue, and West Nile virus encephalitis, and other infections involving the central nervous system, such as tuberculosis, bacterial meningitis, and cerebral malaria (Diagana *et al.*, 2007; Erlanger *et al.*, 2009).

Treatment and prognosis

There is no specific treatment for JE. Treatment is aimed at controlling immediate complications, such as seizures and intracranial pressure, as well as long-term consequences of the disease that include limb contractures, malnutrition, and bed sores (Solomon, 2006; Solomon *et al.*, 2000).
Though corticosteroids had been administered to JE patients for some years, double blind randomized placebo controlled trials failed to show their benefit. Interferon-alpha was reported as a promising potential treatment (Solomon *et al.*, 2000).
Misra *et al.* (1999), investigated the role of clinical, neurophysiological, laboratory, and radiological parameters in the prognosis of JE and concluded that the best set of prognosis predictors were age, Glasgow coma scale (method for assessment of the level of consciousness), and reflex changes.

Public health and economic implications

The assessment of the economic impact of JE in Southeast Asia depends on JE case reporting, which is not always accurate due to differences in quality of health information systems, as well as the ability to perform clinical and serological JE diagnoses (Erlanger *et al.*, 2009). Furthermore, difficulties estimating disease burden in Asia are related to different case-definitions (defining acute encephalitis), challenges in confirming the diagnosis in rural areas, and problems with quantitatively assessing disease outcomes (survivors pose a higher burden than fatal cases) (Solomon, 2006).
The impact of JE should take into account aspects related to the collective burden, as opposed to individual burden of disease, the cost of preventive measures *versus* treatment costs, and morbidity and mortality in contrast to financial burden. Moreover, aspects such as acute *versus* chronic JE and disease in humans *versus* animals should also be considered (Tarantola *et al.*, 2014). However, Tarantola *et al.* (2014) concluded that regardless of the nature and scope of disease burden being assessed, it is usually the rural populations located in economically and socially fragile areas and having little access to health and prevention care, on which most of the impact is reflected.

The estimated global impact for JE in 2002 was 709,000 disability-adjusted life years (DALYs), though it fluctuated between 1,046,000 DALYs in 1999 and 426,000 in the year 2000 (Erlanger *et al.*, 2009).

According to Griffiths *et al.* (2013), the total costs related to acute encephalitis syndrome in Nepal, in 2011, were 10 times the median monthly income of the participants' families for children with severe or moderate functional impairment, and 4.6 times for children with mild or no functional impairment following JEV infection. Furthermore, a higher proportion of caretakers of children with severe or moderate disabilities due to JE, took time off work after discharge (and longer time), compared to children with mild or no impairment. The child's participation within their home, community, or school was also affected by the JE-related impairment (Griffiths *et al.*, 2013).

Prevention and control

Prevention and control methods for mosquitoes have included the use of larvicides and insecticides, however they have proved ineffective to control breeding in rice paddies. More natural methods, including neem cake, a by-product of cold-pressed neem tree fruits and kernels that may be used to manage insects and pests, has also been tried as a control method. Intermittent draining of paddy fields and use of larvivorous fish in rice paddies are also control methods that have been applied in affected regions (Solomon, 2006).

Another prevention strategy that has been debated is vaccination of pigs. Though theoretically it could represent a strategy to target the main amplifying host, it is not cost-effective in most settings due to the high pig turnover in industrial farming. Moreover, there is no evidence this intervention decreases human or mosquito infection and the effectiveness of live attenuated vaccines is decreased in young pigs due to the presence of maternal antibodies (Erlanger *et al.*, 2009). Moving pigs at least 5 km away from human habitats could improve prevention but its benefit has yet to be proven (Solomon, 2006).

Prevention of human infection is usually targeted by advocating reducing the time spent outdoors, especially in the evening, time at which mosquitoes usually take blood meals. Wearing protective clothing and insect repellants are other preventive measures recommended for humans (Solomon, 2006).

Human vaccination is the main prevention method currently in use in most Southeast Asia, with vaccination programs well-established in many of those countries. The first vaccines were mouse brain-derived and proved effective in controlled trials performed in Taiwan in the 1960s and in Thailand in the 1980s. However, due to the reporting of side effects, potentially related to the presence of gelatin and murine neural proteins in the vaccines, newer vaccines were developed (Solomon, 2006; Yun *et al.*, 2015). These include inactivated and live attenuated vaccines, with the

brain mouse-derived type being replaced by cell culture-derived vaccines. These are now considered safe, cheap, and effective (Solomon, 2006; Misra and Kalita, 2010).

All vaccines are genotype III vaccines, but because neurovirulence is not related to JEV genotype (Le Flohic *et al.*, 2013), genotype III vaccines are equally effective against all JEV genotypes. A hindrance of vaccination is the fact that it does not provide herd immunity because humans are not the primary JEV host (Solomon, 2006).

Final remarks

Knowledge of flavivirus-mosquito interactions is crucial for a better understanding of the changing epidemiology and transmission patterns of JEV, as well as its application in disease control and prevention strategies, particularly in JEV-free regions of the globe, such as the US (Huang *et al.*, 2014).

The observed geographical expansion of JEV to contiguous regions, globalization, an increase in travel and trade across continents, and past experiences with exotic diseases emerging in susceptible regions call for action.

In the US, specifically, the rapid spread of the West Nile virus after its introduction in 1999, and the inability of the public health services to control the outbreaks and prevent the establishment of the disease raised an important issue regarding the emergence of exotic vector-borne diseases in the country.

Nett *et al.* (2009) proposed that California, due to its geographical position, has a high potential risk for the introduction of JEV. California is the most important state when reaching continental US after crossing the Pacific Ocean, representing a strategic geographical point for tourism and trade between the Asian and the North American continents. Moreover, California has a large Asian community frequently crossing the Pacific, which leads to increased opportunities for importing mosquitoes via air or maritime transportation. Furthermore, the warm climate and the presence of potential JEV susceptible vectors and vertebrate hosts creates a suitable environment for viral replication and transmission (Reeves and Hammon, 1946; Nett *et al.*, 2009; Nemeth *et al.*, 2012; Huang *et al.*, 2015).

The lack of active JEV surveillance programs that could spot the virus at an initial stage and the encountering of a naïve population of both adults and children would add up to the high impact of the disease once introduced and established in the country (Nett *et al.*, 2009).

The introduction, establishment, spread, and persistence of JE in the US largely depend on the availability, abundance, competence, and distribution of amplifying hosts and vectors; the contact rates between both; the virulence and genotype of the viral strain; and the environmental and climatic conditions found (Nemeth *et al.*, 2012; Huang *et al.*, 2014).

To date and despite many reviews describing the susceptibility of hosts, vectors, and environmental parameters that could sustain the introduction of the virus and its further spread in the US, the role of

different vectors and hosts, along with their competence, has not been quantitatively evaluated (Reeves and Hammon, 1946; Nett *et al.*, 2009; Nemeth *et al.*, 2012; Huang *et al.*, 2015). This is an important data gap that this thesis proposes to address, providing data that can support future surveillance programs for preventing the introduction of JEV in susceptible regions, and particularly in the US.

Objectives of the thesis

The following chapters of this thesis focus on assessing and synthesizing information regarding vector and host competence for JEV, using a systematic review of the literature and meta-analysis methodology.

A systematic review (SR) of the literature is a replicable, transparent, and reliable methodological approach for identifying, assessing, and summarizing current evidence on a research question, with reduced bias (Sargeant *et al.*, 2006; Sargeant and O'Connor, 2014).

A meta-analysis (MA) is a quantitative method that combines the results of the data gathered from the literature (e.g., SR), providing a more accurate estimate of the outcome (a summary effect measure). A MA also allows exploring the sources of heterogeneity between results from different studies, and considers possible sources of confounding and bias, while increasing the power of a SR and providing valuable information to answer the research question or to identify potential knowledge gaps (Egger *et al.* 2001; Sutton *et al.*, 2001; Sargeant *et al.*, 2006; O'Connor *et al.*, 2014; Sargeant and O'Connor, 2014).

In chapter 2 of this thesis, we used a systematic review of the literature approach to identify research gaps and information regarding vector and host competence for JEV worldwide. In chapter 3, a meta-analysis was performed to identify and quantify vector and host competence for JEV from observational studies, specifically regarding the proportion of viral infection in vectors and vertebrate hosts. Finally, in chapter 4, a quantitative assessment of infection, dissemination, and transmission rates of mosquitoes was performed to study vector competence in experimental studies using a meta-analysis approach.

Through a quantitative assessment of vector competence, we aimed to achieve a better understanding of the data currently available. The estimates provided by the meta-analyses can be used in risk assessment models to estimate the risk of introduction, transmission and persistence, support future surveillance programs and aid in decision-making in public health interventions. These ultimately aim at preventing the introduction of JEV or minimize the effects of an inadvertent or intentional introduction, should it occur, into currently JEV-free geographical regions, including the US. Prevention and control of other animal or human mosquito-borne diseases may also use the information gathered in this systematic review and meta-analyses, as the data can similarly be used to populate other models within the risk assessment framework.

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Chapter 2 - Quantification of vector and host competence for Japanese Encephalitis Virus: a systematic review of the literature

Summary

Japanese encephalitis virus (JEV) is a virus of the *Flavivirus* genus that may result in encephalitis in human hosts. This vector-borne zoonosis occurs in Eastern and Southeastern Asia and an intentional or inadvertent introduction into the United States (US) will have major public health and economic consequences. The objective of this study was to gather, appraise, and synthesize primary research literature to identify and quantify vector and host competence for JEV, using a systematic review (SR) of the literature.

After defining the research question, we performed a search in selected electronic databases. The title and abstract of the identified articles were screened for relevance using a defined set of exclusion and inclusion criteria, and relevant articles were subjected to a risk of bias assessment followed by data extraction.

Data were extracted from 171 peer-reviewed articles. Most studies were observational studies (59.1%) and reported vector competence (60.2%). The outcome measures reported pertained to transmission efficiency, host preference, and vector susceptibility to infection within vector competence; and susceptibility to infection within host competence. Regarding vector competence, the proportion of JEV infection reported across all 149 mosquito species in all observational studies ranged from 0 to 100%. In experimental studies, infection, dissemination, and transmission rates varied between 0 and 100%. Minimum infection rates (MIR) varied between 0 and 333.3 per 1,000 mosquitoes. Maximum likelihood estimation (MLE) values ranged from 0 to 53.78 per 1,000 mosquitoes. The host species in which mosquitoes mostly fed consisted of pigs and cattle (84 blood meals), cats and dogs (73 blood meals), and horses and donkeys (57 blood meals). As for host competence, the proportion of JEV infection varied between 0 (in rabbits, reptiles, and amphibians) and 88.9% (cattle).

This SR presents comprehensive data on JEV vector and host competence, which can be inputted to quantify risks associated with the introduction of JEV into the US.

Keywords: Japanese encephalitis virus, Japanese encephalitis, systematic review, vector, host, competence.

Introduction

Japanese encephalitis (JE) is a mosquito-transmitted disease that may result from infection by the Japanese encephalitis virus (JEV), an arbovirus (arthropod-borne virus) of the *Flavivirus* genus. Virus transmission extends from Southeastern Asia to the Western Pacific islands. Japanese encephalitis virus is the most important cause of encephalitis worldwide, with approximately 68,000 JE human cases occurring every year, particularly in children (Weaver and Barrett, 2004; Campbell *et al.*, 2011). The mechanism of transmission is based on interactions between vectors (over 30 species of mosquitoes) and hosts (pigs and ardeid birds) that maintain an enzootic cycle not yet fully understood (Le Flohic *et al.*, 2013). According to Le Flohic *et al.* (2013), JEV can easily shift between the domestic and the wild cycles, with no viral adaptation needed, if competent hosts and vectors are present, which is consistent with the observed geographical expansion of the virus to contiguous regions. This expansion puts the more than three billion people who live in currently JE-endemic countries at risk of infection (Le Flohic *et al.*, 2013). The spread of JE is also related to the exponential human population growth in the affected regions, the increase in the number of pig production systems, and the changes in land usage and agricultural practices. Changes in agricultural practices are related to an increase in rice production, leading to more opportunities for mosquito breeding, as rice paddy fields are a suitable and prolific habitat for the development of mosquito vector populations (Mackenzie *et al.*, 2004; Erlanger, 2009). The most competent JEV vectors are *Culex* mosquitoes, such as *Culex tritaeniorhynchus*, *Culex annulirostris*, *Culex annulus*, *Culex fuscocephala*, *Culex gelidus*, *Culex sitiens*, and the *Culex vishnui* species complex, which are widely distributed over the JEV-endemic areas, thus contributing to further maintaining the transmission cycle. Regarding vertebrate host species, the most competent amplifying and reservoir hosts are wild ardeid birds, especially egrets (*Egretta garzetta*) and herons (*Nycticorax nycticorax*), and pigs, both feral and domestic. The rise in intensive pig farming observed in East and Southeast Asia over the past decades also contributes to JEV transmission, as it increases the number of susceptible vertebrate hosts available (Le Flohic *et al.*, 2013).

To date and despite many reviews describing the susceptibility of hosts, vectors, and environmental parameters that can sustain the introduction of the virus and its further spread in JEV-free countries, such as the United States (US), the role of different vectors and hosts, and their competence, has not been quantitatively evaluated (Reeves and Hammon, 1946; Nett *et al.*, 2009; Nemeth *et al.*, 2012; Huang *et al.*, 2015).

Furthermore, Lord *et al.* (2015) refer to the fact that the basis of our current knowledge about the JEV transmission cycle was established during the initial research in the 1950s in Japan, and that it reflected the context of the region and time period when it was carried out. Japanese encephalitis virus transmission should therefore be reconsidered for other regions where the transmission context differs from the one first described in Japan. Limited evidence supporting the need of viral adaptation between different cycles, as well as recent evidence that vector-free transmission between pigs without the intervention of arthropod vectors is possible (Ricklin *et al.*, 2016), further reiterate knowledge gaps. A better understanding of the relative importance of vectors and hosts will determine optimal mitigation strategies, such as JEV vaccination or insect vector control, but will depend on the accurate assessment of parameters that include vector and host abundance, mosquito host preference, and vector competence for JEV (Le Flohic *et al.*, 2013; Lord *et al.*, 2015).

Globalization and increasing international travel create additional opportunities for the introduction of exotic pathogens into new regions of the globe. In the US, specifically, the rapid spread of the West Nile virus (WNV) after its introduction in 1999, and the inability of the public health services to control the outbreaks and prevent the establishment of the disease raised an important issue regarding the emergence of exotic vector-borne diseases in the country. Agencies responsible for the prevention and control of the introduction of foreign pathogens and the timely response to potential outbreaks, should they occur, require comprehensive information regarding the previously mentioned parameters, of which vector and host competence play a major role (Reeves and Hammon, 1946; Nett *et al.*, 2009; Nemeth *et al.*, 2012; Huang *et al.*, 2015).

A systematic review (SR) of the literature provides a replicable, transparent, and reliable method of identifying, assessing, and summarizing available evidence on a research question, with reduced bias (Sargeant *et al.*, 2006; Sargeant and O'Connor, 2014a). The objective of this study was therefore to identify research gaps and information regarding vector and host competence for JEV worldwide, using a systematic review of the literature.

Materials and Methods

Steps of the SR consisted of posing a research question, searching the literature, conducting a relevance screening, extracting data, assessing the risk of bias, as well as analysis and presentation of the extracted data.

Research question

The original research question was as follows: Which vectors and hosts are competent for Japanese encephalitis virus transmission in the United States?

Due to the low number of publications originating from the US, the research question and search were refined to include peer-reviewed literature worldwide.

Because the research question was related to descriptive parameters (competence of vectors and hosts), rather than interventions, we used a PO (population, outcome) question format to define the research question (O'Connor *et al.*, 2014b). Population (P) referred to vectors and hosts, while outcomes (O) concerned competence, in terms of transmission efficiency, host preference, and susceptibility to infection.

The working team was comprised of four reviewers (AO, LH, ES, NC), each participating in different steps of the review.

We followed the protocols and guidelines for performing systematic reviews in veterinary medicine described by Sargeant and O'Connor (2014a; 2014b) and O'Connor *et al.* (2014a; 2014b) and adapted from the Cochrane group's guidelines (Higgins and Green, 2011).

Searching the literature

The search was restricted to the English language, without limitations to year of publication, and was performed using eight electronic databases and journals (Table 1). The journals included in the search (Armed Forces Pest Management Board, The American Journal of Tropical Medicine and Hygiene, Journal of the American Mosquito Control Association, and Vector-Borne and Zoonotic Diseases) were selected based on the relevance of the topics covered, which are aligned with our research question. Databases were accessed until April 25th, 2016 and the search terms were related to the PO components. A complete list of the search terms, and their combinations, used for each database and journal is available in Appendix A.

A hand-search was also used to identify additional articles cited in the reference list of nine articles considered as key publications by the reviewers based on a priori identification of relevant articles (Solomon *et al.*, 2000; Mackenzie *et al.*, 2004; Weaver and Barrett, 2004; Erlanger *et al.*, 2009; Nett *et al.*, 2009; van den Hurk *et al.*, 2009; Misra and Kalita, 2010; LeFlohic *et al.*, 2013; Huang *et al.*, 2014). A summary of the search results including number of original articles, duplicates, and total abstracts searched and selected, by database source, is presented in Table 1. Only peer-reviewed articles were considered for further evaluation.

All articles were given a unique number that was kept throughout the SR for identification purposes.

Relevance screening

To determine their relevance, the title and abstract of all identified articles were subjected to a set of inclusion and exclusion criteria comprised of language, time period, population, study type, outcome measures, and location fields. A detailed description of the inclusion and exclusion criteria is included in Table 2.

Based on this set of criteria, we created a relevance screening tool composed of six questions, using an Excel® (Microsoft Corp., Redmond WA, 2013) spreadsheet. The first five questions were deemed crucial to establish relevance, and based on the answers to those questions, the abstracts were considered relevant or not.

The first version of the tool was pre-tested using 10 abstracts by three reviewers (AO, LH, NC). After reviewing the sources of disagreement, we improved the tool and performed a second testing using the same 10 abstracts and three new ones.

Two reviewers (AO, LH) working independently performed the final relevance screening, and compared the answers for conflict resolution. When both reviewers determined that the abstract was not relevant, it was not considered further in the SR. The two reviewers resolved all conflicts by consensus, and a third reviewer (NC) intervened whenever the first two reviewers were not able to reach consensus. The relevance screening process and conflict resolution were completed by June 27th, 2016.

Following this process, we downloaded all full articles whose abstracts were considered relevant. A second relevance screening was performed by two reviewers (AO and ES), after appraisal of the full articles. This second relevance screening aimed at resolving uncertainties raised in the first screening for some articles, and sorting relevant articles into two categories: competence of hosts and/or vectors for JEV and competence of hosts and/or vectors for other *Flavivirus*. Articles pertaining to the second category (competence of hosts and/or vectors for other *Flavivirus* other than JEV) were not considered further. Again, reviewers resolved all conflicts by consensus, consulting a third reviewer (NC) whenever consensus could not be reached.

Figure 1 presents a flowchart of the relevance screening tool, including all questions and possible answers.

Data extraction

Similar to the relevance screening step, a data extraction tool was created to guide data extraction from the relevant papers.

A pre-testing of five relevant articles was performed by two reviewers (AO and ES) in a first version of the tool, which was improved according to the flaws observed during this process. Conflicts were resolved by consensus or by the intervention of a third reviewer (NC) when consensus could not be reached.

The data were extracted and incorporated into an Excel® (Microsoft Corp., Redmond WA, 2013) spreadsheet. Information extracted consisted of general information about the article, which included the identification number, authors, year of publication, title, journal, population type (vector or host), and study type (observational or experimental). Data related to host and vector competence in experimental and observational studies were extracted and pertained to the outcome measures of interest: transmission efficiency, host preference, and susceptibility to infection. A detailed description of the outcome measures extracted from the articles is summarized in Table 3.

Studies could contribute to more than one population type (vector and host) and/or study type component (observational and experimental). For this reason, data from articles containing information on more than one type or component were split into multiple entries, according to the type of information they included, but maintaining the same unique identification number that linked those entries to the article from which they originated. Therefore, an article could have multiple entries under the same identification number.

Assessment of the risk of bias

Following the Cochrane Review Handbook guidelines (Higgins and Green, 2011), we created a tool for each type of study design (observational and experimental) to assess the risk of bias in articles from which data were extracted (Tables 4 and 5), using eight criteria for each. These criteria aimed to address internal and external validity of the relevant studies. Criteria were designed to objectively determine if the study question, study population, inclusion and exclusion criteria, study period, study area, exposures, outcomes, and bias, were reported and/or defined for observational studies. Similarly, criteria pertaining to study question, study population, intervention, experimental conditions, experimental setting, randomization, blinding, and outcomes, were assessed for experimental studies.

We determined three levels of risk of bias for the articles being evaluated: low risk of bias, which is defined as the article having plausible bias that is unlikely to seriously alter the results; high risk of bias, defined as plausible bias that seriously weakens confidence in the results; and unclear risk of bias, defined as plausible bias that raises some doubt regarding the results of the study (Higgins and Green, 2011).

To assign articles a low, high, or unclear risk of bias, we established key domains that determined to which risk category each article belonged (Tables 4 and 5). Key domains pertained to questions considered critical by the authors for the overall risk of bias, given the relative importance of the different domains. A low risk of bias was assigned to the articles with a low risk of bias in all key domains; a high risk of bias was attributed when at least one key domain had a high risk of bias; and finally, an unclear risk of bias was assigned to articles with at least one unclear key domain.

The risk of bias assessment tool was pre-tested by two reviewers (AO and NC) working independently in a set of 10 articles, with all remaining articles being assessed by one reviewer (AO).

Different entries from the same article were assessed individually for their risk of bias. Thus, for instance, data from an article containing both observational and experimental components for vector competence were assessed twice for their risk of bias (one for each of the study type components).

Data analysis

A descriptive summary of results, presented in tabular form, was performed using Stata-SE 12.0 (StataCorp., College Station TX, USA) and the pivot tables function in Excel® (Microsoft Corp., Redmond WA, 2013).

The outcomes that were summarized are described below and pertained to vector transmission efficiency in terms of infection, dissemination, and transmission rates; host preference of vectors; and susceptibility to infection, measured as minimum infection rates (MIR) and maximum likelihood estimation (MLE) for vectors and as proportion of JEV infection for both vectors and host species.

Infection rates refer to the sum of individual mosquitoes (or pool of mosquitoes) divided by the total number of mosquitoes (or pools of mosquitoes) tested in experimental studies; dissemination rates refer to the proportion of mosquitoes containing virus in their legs, regardless of their infection status (Golnar *et al.*, 2015); and transmission rates refer to the

proportion of mosquitoes with a disseminated infection that transmits the virus after refeeding (Golnar *et al.*, 2015). Because these outcome measures were extracted from experimental studies, the administration route used for infecting mosquitoes in the different articles was recorded and included oral feeding (pledgets/membranes or hosts), intrathoracic inoculation, or vertical transmission (parents infected either intrathoracically or by oral feeding).

Host preference is defined as the host species from which mosquito blood meals originate.

Minimum infection rate (MIR) is defined as the ratio of the number of positive mosquito pools to the total number of mosquitoes in the sample, assuming that only one infected individual is present in a positive pool, while maximum likelihood estimation (MLE) values represent the proportion of infected mosquitoes that maximizes the likelihood of the number of pools of a specific size to be virus positive, where the proportion is the parameter of a binomial distribution (Bustamante and Lord, 2010).

Despite being referred to as rates, however, we note that infection, dissemination, and transmission rates are actually proportions, as a rate is a ratio in which the denominator is the number of subject-time units at risk, mosquito-time units at risk in these examples, which is not the case, as no time component is involved (Dohoo *et al.*, 2009). However, because this is the common terminology used to refer to these measures, particularly among entomologists, we will keep using it across this manuscript.

Proportion of JEV infection is the sum of positive mosquito pools/vertebrate hosts divided by the total number of pools/host samples tested in observational studies.

In observational studies, we also extracted data pertaining to the methods used to capture vectors, which included manual passive (aspirations) or active (sweep or drop nets) methods; and mechanical visual (use of visual attractants like UV or white light) or olfactory (use of olfactory attractants like CO2 and other lures, such as octanol) methods.

The diagnostic methods recorded for measuring vector-related outcomes varied and included Real-time RT-PCR, reverse transcription PCR (RT-PCR), antigen-capture enzyme assays (e.g., ELISA), and virus isolation (using cell culture techniques, insect bioassays, immunofluorescence assays, hemagglutination inhibition tests, or neutralization tests). These methods were used exclusively or in different combinations.

As for hosts, diagnostic methods reported aimed at detecting antibodies using ELISA or immunochromatography, hemagglutination inhibition assays, neutralization tests (e.g., plaque reduction neutralization test), and virus isolation, exclusively or combined.

Data presentation for proportion of JEV infection in vectors were organized alphabetically by species and by author and year of publication (Table 7). Minimum infection rates and MLE values were organized by author and year of publication and then alphabetically by mosquito species (Tables 8 and 9, respectively). Data regarding transmission efficiency were presented by days post infection (DPI), by author and year of publication, and alphabetically by mosquito species (Table 10). As for proportion of JEV infection in host species and mosquito host preferences, data from all articles were combined and presented by vertebrate host species (Table 11).

Results

Searching the literature and relevance screening

We identified a total of 1,855 abstracts, 450 of which were duplicates or non-primary research (non-peer reviewed articles, conference proceedings, thesis dissertations, and other non-peer reviewed publications), leading to a final selection of 1,405 abstracts, which were downloaded for assessment of relevance (Table 1).

During the relevance screening process 14 more abstracts were identified as duplicates, leading to a total of 1,391 abstracts that were screened for relevance and subjected to conflict resolution. A total of 568 abstracts were considered relevant.

During the process of downloading the 568 full-text articles, 67 were excluded for not meeting the inclusion criteria, mainly due to not fulfilling the language (full-text not in English) and study type (literature reviews) criteria.

The second relevance screening further narrowed down relevant studies to 288, however, during data extraction, 116 more articles were excluded for not having extractable data, resulting in a final number of 171 articles, which we then assessed for the risk of bias and from which we extracted data.

A complete flowchart of the articles identified, screened, and included for data extraction is presented in Figure 2.

Data extraction

Characteristics of the studies

The study characteristics of the 171 articles included in the final SR, including their source (author and year of publication) are summarized in Table 6.

Most studies were observational (59.1%) and reported vector competence (60.2%). Seven articles (4.1%) had both an experimental and an observational component and 18 (10.6%) reported more than one population type (Table 6).

The year of publication ranged from 1946 to 2016, with half of the articles (n = 85) published after 1992.

The countries represented in all studies were: Vietnam, Taiwan, China, Nepal, Saipan (Mariana islands), South Korea, Indonesia, India, Malaysia, Thailand, Australia, Papua New Guinea, Japan, Bangladesh, Sri Lanka, Myanmar, USA, Singapore, and Guam (US). Only two articles reported information from continental US.

Outcome measures: vector competence

Data on proportion of JEV infection in vectors extracted from articles reporting positive mosquito pools are depicted in Table 7. A complete list of results from all mosquito species reported across all studies is available in Appendix B.

The proportion of JEV infection, reported in a total of 149 mosquito species, across all observational studies ranged from 0 to 100%. For *Culex tritaeniorhynchus*, one of the most important vector species, the proportion of positive pools also ranged from 0 to 100% across all 44 articles that reported results for this species. The countries where mosquitoes were captured and tested for JEV infection belonged to Southeast Asia, including Australia, Bangladesh, China, India, Indonesia, Japan, Malaysia, Saipan (Mariana islands), South Korea, Sri Lanka, Taiwan, Thailand, and Vietnam.

Minimum infection rates (MIR) from observational studies were reported in 16 studies in 28 species and 10 countries. Minimum infection rates varied between 0 per 1,000 mosquitoes in several species and 333.3 per 1,000 mosquitoes in *Culex gelidus*. Information related to MIR is presented in Table 8.

Maximum likelihood estimation (MLE) values from observational studies were reported in 6 studies, in 30 mosquito species and 5 countries, ranging from 0 per 1,000 mosquitoes in different species to 53.8 per 1,000 mosquitoes in *Anopheles minimus*. Results are presented in Table 9.

In experimental studies, infection, dissemination, and transmission rates from 50 different mosquito species and 30 studies varied between 0 and 100%. *Culex quinquefasciatus* and *Ochlerotatus detritus* were the mosquito species reported having up to 100% dissemination rates, while the species reported as having 100% transmission rates were *Culex tritaeniorhynchus*, *Culex gelidus*, *Mansonia uniformis*, and *Ochlerotatus purpureus*.

JEV infection, dissemination, and transmission rates for 14 days post-infection (DPI), or the closest to 14 DPI, are reported in Table 10 and a complete list of all results is available in Appendix C.

Concerning mosquito host preference, mosquitoes preferred to feed on pigs and cattle (84 blood meals from 14 and 13 observational studies, respectively), followed by cats and dogs (73 blood meals from 6 observational studies), and horses and donkeys (57 blood meals from 6 observational studies).

Mosquito host preferences in all observational studies are depicted in Table 11.

There was only one experimental study reporting host feeding preferences (Mwandawiro *et al.*, 2000). This study reported data from three mosquito species: *Culex gelidus*, *Culex tritaeniorhynchus*, and *Culex vishnui*. Mosquitoes were released in nets where pigs, cows, or both host species were present, marked with a fluorescent dye, and then tested to reveal the origin of the blood meals. Results showed that mosquitoes preferred to feed on cows, rather than pigs (Mwandawiro *et al.*, 2000).

Outcome measures: host competence

Regarding JEV infection in vertebrate host species, reported in 33 observational studies, proportions varied between 0 and 88.9%. Information pertained to 13 countries: Nepal, India, South Korea, USA, China, Japan, Sri Lanka, Myanmar, Thailand, Australia, Guam (US), Saipan (US), and Vietnam. The total number of host species categories represented was 15 and included: pigs, birds, sylvatic mammals, cattle, sheep and goats, cats and dogs, chickens, ducks, rabbits, herons, horses and donkeys, wild pigs, bats, rats, and reptiles and amphibians. Host species tested but JEV-negative included rabbits, reptiles, and amphibians. Host species reporting the highest JEV infection proportions were cattle (88.9%) and cats and dogs (85.4%).

Detailed information on proportion of JEV infection in host species across all observational studies is presented in Table 11.

Assessment of the risk of bias

For observational studies, the risk of bias assessment revealed that all 101 articles had a low risk of bias. The number of entries corresponding to studies that did not report or control for bias was 188, while 6 entries included information on bias reporting but not control. Seventeen entries did report and control for bias.

Experimental studies were all considered having a high risk of bias and articles with both observational and experimental components all had a low risk of bias.

The key domain that contributed to all articles reporting experimental studies being considered as having a high risk of bias was the randomization criterion, as none of the entries defined randomization or provided evidence of having performed randomization. One entry also did not define the outcome measures, though it reported them. Furthermore, and despite not being considered as a key domain, 73 entries did not define or perform blinding.

Discussion

This study is the first SR evaluating vector and host competence for JEV that compiles the body of evidence on vector transmission efficiency and host preference along with data on vector and host susceptibility to infection.

Data on vector and host competence outcomes for JEV are very broad varying, depending on the specific outcome, from 0 to 100% across different genera and species, making it difficult to interpret and contrast. This is typical for most arboviruses where only a small number of species are important vectors. Nevertheless, by gathering information on JEV infection from various mosquito species and a large number of articles, this SR provides a comprehensive resource of mosquito species and their reported JEV infection. This review also demonstrates that similar to other JE group viruses, JEV is invasive to a number of mosquito species of medical and veterinary importance and known disease vectors of other arboviruses.

Moreover, this SR provides a better understanding of the geographical distribution of the information available regarding vector competence, revealing that data are more readily available on countries where JE is prevalent, as expected, and pointing to the need of conducting similar studies in other countries where hosts and vectors are present and thus may be potentially at risk.

It is important to highlight that variation across studies may result from between-study and within-study variation. Within-study variation is associated with random sampling error, while between-study variation is considered to be related to the study characteristics, although other factors may be involved. Unexplained variation is usually incorporated into random-effects models in meta-analyses, the statistical analysis of individual studies for integrating the findings of a SR (Dohoo *et al.*, 2009).

Data on JEV infection were reported most frequently in *Culex tritaeniorhynchus* (44 articles), which is in line with our current understanding of this species being the most significant JEV vector, playing a paramount role in the transmission dynamics of the disease (Solomon, 2000; Mackenzie *et al.*, 2004; Weaver and Barrett, 2004; van den Hurk *et al.*, 2009; Le Flohic *et al.*, 2013). However, Lord *et al.* (2016) proposes that sampling design of JEV studies, which tend to be based on capturing mosquitoes from around cattle sheds at dusk, may influence the observed dominance of *Culex tritaeniorhynchus* as the primary JEV vector reported in the literature. In fact, our results show a great variability for the proportion of JEV infection in *Culex tritaeniorhynchus* among all observational studies, with infection proportions as low as 0 and as high as 100% (Table 7). This variability is likely due to differences in data

collection, study design and reporting, sample size, methods used for testing mosquito infection, as well as the sensitivity and specificity of those methods, geographical regions and environmental factors specific to those regions.

Furthermore, the highest values for minimum infection rates (MIR) in observational studies belong to mosquito species other than *Culex tritaeniorhynchus*: *Culex gelidus* (333.3 per 1,000 mosquitoes), *Culex vishnui* (0.4 per 1,000 mosquitoes), and *Culex rubithoracis* (30.8 per 1,000 mosquitoes) (Table 8). Accordingly, maximum likelihood estimation (MLE) values were the highest in *Anopheles minimus* (53.8 per 1,000 mosquitoes), *Aedes vexans* (29.7 per 1,000 mosquitoes), and *Culex annulus* (26.3 per 1,000 mosquitoes) (Table 9). Additionally, Bustamante and Lord (2010) point to evidence that supports that infection in vectors is not always a straightforward indicator of risk and that other indicators, such as mosquito population size (vector abundance), age, and climatic conditions, should be taken into account for assessing the risk of arbovirus transmission.

In experimental studies, results on JEV infection, dissemination, and transmission rates are also very broad, ranging from 0 to 100% in different mosquito species and days post-infection, across all articles (Table 10). For *Culex tritaeniorhynchus*, for instance, JEV infection rates varied between 0 and 100%, although the lowest infection rates pertained to 1 DPI, time at which mosquitoes have probably not developed infection yet. Transmission rates for *Culex tritaeniorhynchus* varied, again, between 0 and 100%, as well as for other mosquito species reported. Data about transmission efficiency are therefore variable across experimental studies.

Transmission experiments are important, as they lead to a better understanding of which factors determine the mosquito's ability to acquire, maintain, and transmit the virus (virus competence), thus clarifying the mechanisms under which mosquitoes become infected, disseminate infection, and transmit the virus to hosts.

It is important to note that the relevance of JEV infection lies on the fact that it is a persistent and amplifying infection in the mosquito and is generally a requirement prior to midgut penetration, resulting in diffused infection of the insect vector.

Furthermore, it is important to stress that in observational studies mosquito-related outcomes are measured in terms of pools of mosquitoes (with variable number of mosquitoes per pool, up to 800), while in experimental studies mosquitoes are tested individually, except for three studies reporting vertical transmission that used pools of mosquitoes with sizes up to 120.

This distinction is important as it contributes to the variability reported across outcomes, especially in observational studies, where the number of mosquitoes per pool varies greatly. Regarding host preference of vectors, information extracted revealed that mosquitoes preferred to feed on pigs and cattle, according to the number of blood meals originated from those species (Table 11). Nonetheless, and according to Lord *et al.* (2015), poor mixing of hosts and mosquitoes across the spatial areas where mosquitoes are captured may be a source of bias leading to a potential overestimation of the proportion of blood meals taken from certain species. Therefore, reported feeding patterns may depend on the availability of hosts, rather than the actual feeding preference of mosquitoes, as some species of mosquitoes just feed on certain animals while others are more opportunistic. Furthermore, trap placement to collect blood fed mosquitoes for the observational studies may be biasing these studies, as few traps are placed in trees or on the water where wading birds are most frequently present. The importance of host feeding preferences, based on the only experimental study included in this SR reporting this outcome (Mwandawiro *et al.*, 2000) is questionable, because the hosts' surface area or biomass are not considered when analyzing the results. Results showing that mosquitoes preferred to feed on cows, rather than pigs, may reflect that mosquitoes simply chose to feed on the larger animal which has higher surface area and volume available (Tuno *et al.*, 2017).

Regarding host competence, pigs were not the host species with the highest proportion of JEV, as it would be expected because pigs are considered the main amplifying host for JEV (Solomon, 2000; Mackenzie *et al.*, 2004; van den Hurk *et al.*, 2009; Le Flohic *et al.*, 2013). In fact, across all observational studies, the proportion of JEV positive pigs was 20.4%, which is low compared to cattle (88.9%) or cats and dogs (85.4%). Proportion of JEV infection in ardeid birds was also lower than in other species (29.7%) (Table 11). Because host competence is not a constant parameter though, assuming constant proportions of JEV infection in hosts may lead to failure in recognizing regional differences due to environmental or ecological factors that are not being taken into consideration when accounting for transmission potential of competent species (Lord *et al.*, 2015).

An evaluation of the risk of bias allows us to determine whether the study has asked the appropriate question (external validity: generalizability of study findings) and it answered the question correctly (internal validity: the study is free from bias). As such, we can better appraise if the results from included studies are valid. The fact that all experimental studies (n=63) had a high risk of bias is related to the fact that a randomization criterion was

considered to be a key domain for determining that a study had a low risk of bias. Randomization is considered an important criterion in experimental studies, as an inadequate randomization may lead to non-comparable experimental groups, which, in turn, may lead to selection bias (Higgins and Green, 2011). However, due to the nature of the experimental studies included in this SR, mostly challenge trials performed in a small sample of subjects, randomization was not performed or reported in these articles. For this reason, none of the experimental studies passed the randomization criterion, which would otherwise lead to a low risk of bias. Although all experimental studies were deemed to have high risk of bias, this assessment did not warrant exclusion of these studies. However, it is important to consider the potential of bias (due to lack of randomization and/or blinding) arising from these studies when interpreting their results.

In observational studies, on the other hand, all studies had a low risk of bias, because all of them successfully met the key domain criteria established for observational studies. The bias criterion was not met by most of the articles, as authors either did not report or did not control for bias (e.g., selection bias) in the design or analysis stages, but because this was not considered to be a key domain in the assessment of the risk of bias, it did not influence the final assessment. Reporting and controlling for bias was not considered a key domain for determining the risk of bias in observational studies, as it is usually difficult to assess and it constitutes an important source of heterogeneity between studies, according to the Cochrane Review Handbook guidelines (Higgins and Green, 2011).

Outcome measures for vector and host competence of JEV had a large variability in terms of values reported across all studies included in this SR, which constitutes a major limitation of this study.

Differences in study methodology, data collection, detection methods, data reporting, and results presentation were in part responsible for this variability and constituted a challenge when combining and summarizing the data. Furthermore, geographical diversity and environmental factors related to differences in the locations where studies were performed also determined the variability found across articles. Nevertheless, the large amount of information retrieved substantially contributed to further our understanding on the role that different vectors and hosts have on the epidemiology of JEV. Moreover, the span of the research question posed in the beginning of this study played a major role on the large amount of data collected and their variability, making it challenging to compare and contrast studies, as data were collected under different field or experimental conditions, outcomes

were measured using different methods and also differed in terms of sample size. In addition, the specifics of each study in terms of design were often lacking or not presented in sufficient detail, also failing to report measures of variability around point estimates (e.g., standard errors, variance, confidence intervals), preventing the possibility of extrapolation and of being able to extract and summarize their data. As an example of extrapolation challenges, data regarding JEV infection on vectors from observational studies are preferred to data from experimental studies, because in general, challenge trials, in which conditions are artificially controlled by researchers, although may have higher internal validity, they have limited external validity. Moreover, in observational studies, methods employed for measuring and testing mosquito infection, including the number of mosquitoes per pool tested and diagnostic tests, along with their sensitivity and specificity, vary. Due to the likely heterogeneity (within and between-study variability) of the data extracted, we did not attempt to summarize the data quantitatively (to calculate summary effect sizes), though a quantification of specific outcomes could be pursued using meta-analyses. Variability of the data was also evident when summarizing the actual proportions of JEV infection in vectors (both in experimental and observational studies) that ranged from 0 to 100%. The same occurred when examining infection, dissemination, and transmission rates in mosquitoes in experimental studies (values varying between 0 and 100%) and was likely related to the small sample size of some of these studies. Some of the 100% dissemination and transmission rates were calculated for sample sizes of one mosquito, erroneously leading to high rates for some mosquito species. The large quantity of articles from which data were extracted (n=171) is a strength of this SR, as well as the assessment of the risk of bias of the primary articles, which allowed for a critical appraisal of their internal and external validity, thus providing information regarding the relevance and validity of the extracted information.

Future efforts for combining the results from this SR include performing meta-analyses for some of the outcome measures of interest. This type of analyses will be crucial for obtaining quantitative estimates to be inputted into risk assessment models.

This SR presents comprehensive data on competent vectors and hosts in JEV endemic and epidemic countries, which may lead to a better understanding of the paths of introduction of JEV and other arboviruses in the US and other relevant JEV-free regions, such as South America. Vector abundance, along with climatic conditions and availability of hosts, are important factors in arbovirus transmission (Bustamante and Lord, 2010). In the US, specifically, mosquito size populations should be taken into account when assessing JEV

transmission, as availability and abundance of vectors have a strong implication on the transmission potential of JEV. Data on vector and host competence can be used to generate parameters to quantitatively evaluate the potential role arthropods and vertebrate hosts may play in the transmission of transboundary foreign vector-borne diseases.

These efforts will ultimately support future surveillance actions and public health interventions for predicting the risk of introduction and maintaining the JEV-free status of the US. Similarly, data obtained from this study can help populate risk models to predict risk and determine the effectiveness of mitigation strategies for other foreign mosquito-borne disease threats in the US.

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Tables and Figures

Table 1. Summary of search results including number of original (n=1,137), duplicate (n=680), non-primary research (n=38), and total abstracts searched (n=1,855) and selected (n=1,405), by database source, for further relevance screening

Source	Originals[1]	Duplicates[2]	Non-primary research[3]	Total
Web of Science	77	35	0	112
PubMed	93	36	0	129
Armed Forces Pest Management Board	35	11	22	68
The American Journal of Tropical Medicine and Hygiene	14	19	0	33
Journal of Medical Entomology	93	33	0	126
Journal of the American Mosquito Control Association	71	68	0	139
Vector-Borne and Zoonotic Diseases	5	10	0	15
Google Scholar	115	65	0	180
Hand Search	634	403	16	1,053
Total	1,137	680	38	1,855
Eliminated				450
Selected articles				1,405

[1]Originals refer to abstracts identified as unique during the literature search.
[2]Duplicates refer to repeated abstracts found during the literature search (could be repeated more than once).
[3]Non-primary research refers to abstracts from non-peer reviewed articles, conference proceedings, thesis dissertations, and other non-peer reviewed publications.

Table 2. Inclusion and exclusion criteria for relevance screening.

	Inclusion	Exclusion
Language	English	Other than English
Time period	No restriction regarding time	-
Population	Vectors (mosquitoes, other insects) and/or Hosts (vertebrate[1] hosts)	Vectors other than insects Non-vertebrate hosts
Study type	Challenge trial (laboratory and field) Field studies (e.g., trapping, capture) Observational or experimental studies	Non-primary research (thesis) [Literature reviews]
Outcomes and outcome measures	**Vector AND/OR Host <u>Competence</u> to JEV** Transmission efficiency Feeding patterns Host preference Infectiousness Susceptibility to infection Incubation time Duration of viremia	Vector and/or host competence for other flaviviruses transmitted by ticks[2]
Type of evidence	Peer-reviewed articles	Non-peer reviewed articles, conference proceedings, thesis dissertations, and other non-peer reviewed publications
Location	World-wide	-

[1] Vertebrate (with a backbone or spinal column; includes mammals, birds, reptiles, amphibians, and fishes).
[2] Include other flaviviruses transmitted via mosquitoes (West Nile Virus, St. Louis Encephalitis, Yellow Fever, Dengue fever, Zika virus), but not via ticks (Tick-borne encephalitis, Kyasanur Forest Disease, Alkhurma disease, Omsk hemorrhagic fever).

Table 3. Outcome measures documented and extracted during data extraction.

	Vector competence	Host competence
Transmission efficiency	Infection[1], Dissemination[2] and transmission[3] rates	-
Host preference	Host species preference[4]	-
Susceptibility to infection	Proportion of JEV infection[5]	Proportion of JEV infection[6]
	Minimum infection rate[7]	-
	Maximum likelihood estimation[8]	-

[1] Infection rate refers to the sum of individual mosquitoes (or pool of mosquitoes) that are positive to JEV divided by the total number of mosquitoes (or pools of mosquitoes) tested in experimental studies.
[2] Dissemination rate refers to the proportion of mosquitoes containing virus in their legs, regardless of their infection status (Golnar *et al.*, 2015).
[3] Transmission rate refers to the proportion of mosquitoes with a disseminated infection that transmit the virus during blood refeeding (Golnar *et al.*, 2015).
[4] Host preference pertains to the host species from which mosquito blood meals originate.
[5]Proportion of JEV infection is the sum of positive mosquito pools divided by the total number of pools tested in observational studies.
[6] Proportion of positive vertebrate hosts equals the sum of positive samples divided by the sum of samples tested.
[7] Minimum infection rate (MIR) is defined as the ratio of the number of positive mosquito pools to the total number of mosquitoes in the sample, assuming that only one infected individual is present in a positive pool (Bustamante and Lord, 2010).
[8] Maximum likelihood estimation (MLE) represents the proportion of infected mosquitoes that maximizes the likelihood of the number of pools of a specific size to be virus positive, where the proportion is the parameter of a binomial distribution (Bustamante and Lord, 2010).

Table 4. Description of criteria, outcomes and identification of key domains for risk of bias assessment in observational studies.

OBSERVATIONAL STUDIES			
Criteria	**Description**	**Outcome**	**Notes**
1. **Study question***	Is the study question clearly defined?	Yes No Not reported	*Not reported*: study question is unclear or not well defined.
2. **Study population***	Is the study population properly described?	Yes No Partially	**KEY DOMAIN** *Study population*: vertebrate hosts (age, breed, gender, location) and mosquito populations (age, species, gender) clearly reported. *Partially*: some information is provided.
3. **Inclusion/exclusion criteria***	Are inclusion/exclusion criteria properly described?	Yes No Not reported	*Not reported*: criteria are unclear or not well defined.
4. **Study period****	Was time/duration (month/year/season) of the study reported?	Yes No Partially	**KEY DOMAIN** *Partially:* some information is provided.
5. **Study area****	Was the area (country/region) of the study reported?	Yes No Partially	**KEY DOMAIN** *Partially*: some information is provided.
6. **Exposures***	Are exposures clearly defined and reported?	Yes No Not defined	*Not defined:* exposures are reported but not clearly defined.
7. **Outcomes***	Are outcome measures clearly defined and reported?	Yes No Not defined	**KEY DOMAIN** *Not defined:* outcome measures are reported but not clearly defined.
8. **Bias***	Was bias reported and controlled for in the statistical analyses?	Yes No Not controlled for	*Not controlled for*: bias is reported but controlling for bias is unclear or not reported.

*Questions that assess internal validity.
**Questions that assess external validity.

Table 5. Description of criteria, outcomes and identification of key domains for risk of bias assessment in experimental studies.

EXPERIMENTAL STUDIES			
Criteria	**Description**	**Outcome**	**Notes**
1. **Study question***	Is the study question clearly defined?	Yes No Not reported	*Not reported:* study question is unclear or not well defined.
2. **Study population***	Is the study population properly described?	Yes No Partially	**KEY DOMAIN** *Study population:* vertebrate hosts (age, breed, gender, location) and mosquito populations (age, species, gender) clearly reported. *Partially:* some information is provided.
3. **Intervention***	Is intervention clearly defined (dose, route, viral strain, incubation period, with details sufficient for assessment and reproducibility)?	Yes No Not reported	**KEY DOMAIN** *Not reported:* information concerning intervention is unclear or not well defined.
4. **Experimental conditions (challenge trials)****	Are results generalizable (e.g., infection by oral feeding *vs* intrathoracic in vector studies/ infection by mosquito bite *vs* needle in host studies)?	Yes No Not applicable	-
5. **Experimental setting (controlled trials)****	Are results generalizable (e.g., cage *vs* farm/slaughterhouse)?	Yes No Not applicable	-
6. **Randomization***	Is randomization performed and defined?	Yes No Not defined	**KEY DOMAIN** *Not defined:* evidence that randomization is performed but not clearly defined.
7. **Blinding***	Is blinding performed and defined?	Yes No Not defined	*Not defined:* evidence of blinding but not clearly defined.
8. **Outcomes***	Are outcome measures clearly defined and reported?	Yes No Not defined	**KEY DOMAIN** *Not defined:* outcome measures are reported but not clearly defined.

*Questions that assess internal validity.
**Questions that assess external validity.

Table 6. Source of relevant articles by type of study design and outcome (n=171).

	Number of articles	Source
Study design		
Experimental	63	Reeves and Hammon (1946), Hurlbut (1950), Hurlbut (1951), Rosenberg *et al.* (1953), Morris, O'Connor, and Smadel (1955), Gresser *et al.* (1958), Buescher *et al.* (1959d), Scherer and Smith (1960), Gould, Barnett, and Suyemoto (1962), Gould, Byrne, and Hayes (1964), Sulkin, Sims, and Allen (1964), Sulkin, Allen, and Sims (1966), Doi, Shirasaka, and Sasa (1967), Kodama, Sasaki, and Inoue (1968), Nathanson and Cole (1970), Doi *et al.* (1970), Igarashi *et al.* (1972), Muangman *et al.* (1972), Takahashi (1976), Doi *et al.* (1977), Dhanda *et al.* (1977), Soman *et al.* (1977), Rosen *et al.* (1978), Rosen, Shroyer, and Lien (1980), Rosen (1981), Sasaki *et al.* (1982), Takahashi (1982), Boyle, Dickerman, and Marshall (1983), Oya *et al.* (1983), Banerjee, Ilkal, and Deshmukh (1984), Rosen and Shroyer (1985), Rosen *et al.* (1985), Leake and Johnson (1987), Yamamoto, Kimura, and Ohyama (1987), Hayakawa (1988), Rosen (1988), Ilkal *et al.* (1988), Takashima and Rosen (1989), Rosen *et al.* (1989), Ilkal *et al.* (1994), Weng *et al.* (1997), Samuel *et al.* (1998), Weng *et al.* (2000), Mwandawiro *et al.* (2000), Mourya and Mishra (2000), van den Hurk *et al.* (2003), Turell *et al.* (2006a), Turell *et al.* (2006b), van den Hurk *et al.* (2007), Johnson *et al.* (2009), van den Hurk *et al.* (2009), Kramer *et al.* (2011), Bosco-Lauth, Mason, and Bowen (2011), Nemeth *et al.* (2012), Huber *et al.* (2014), Nicholson, Ritchie, and van Den Hurk (2014), Sudeep *et al.* (2015), Huang *et al.* (2015), Mackenzie-Impoinvil *et al.* (2015), Ricklin *et al.* (2016), Do, Bui, and Phan (2016).
Observational	101	Hammon *et al.* (1958), Buescher *et al.* (1959a), Scherer, Buescher, and McClure (1959a), Buescher *et al.* (1959c), Scherer *et al.* (1959c), Konno *et al.* (1966), Pennington *et al.* (1968), Cates and Detels (1969), Dandawate *et al.* (1969), Wada *et al.* (1970), Yamada *et al.* (1971), Gould *et al.* (1973), Self *et al.* (1973), Okuno *et al.* (1973), Mitchell, Chen, and Boreham (1973), Johnsen *et al.* (1974), van Peenen and Joseph (1975), Fukumi *et al.* (1975), Wada *et al.* (1975), Wang (1975), Benenson *et al.* (1975), Chakravarty *et al.* (1975), Hayashi *et al.* (1975), Ura (1976), Simpson *et al.* (1976), Hsu, Huang, and Cross (1978), Khan and K. Banerjee (1980), Khan *et al.* (1981), Rodrigues, Guttikar, and Pinto (1981), Burke *et al.* (1985), Olson *et al.* (1985), Thein, Aung, and Sebastian (1988), Takashima *et al.* (1988), Rosen, Lien, and Lu (1989), Takashima *et al.* (1989), Somboon *et al.* (1989), Mourya *et al.* (1989), Dhanda *et al.* (1989), Mani *et al.* (1991), Reuben *et al.* (1992), Gingrich *et al.* (1992), Peiris *et al.* (1992), Mitchell *et al.* (1993), Peiris *et al.* (1993), Paul *et al.* (1993), Tan *et al.* (1993), Pant *et al.* (1994), Tadano *et al.* (1994), Bhattacharyya *et al.* (1995), Nga *et al.* (1995), Hanna *et al.* (1996), Khan *et al.* (1997), Vythilingam *et al.* (1997), Gajanana *et al.* (1997), Dhanda *et al.* (1997), Ritchie *et al.* (1997), Weng *et al.* (1999), Hanna *et al.* (1999), Victor *et al.* (2000), Johansen *et al.* (2000), Johansen *et al.* (2001), van den Hurk *et al.* (2001), van den Hurk *et al.* (2001), See *et al.* (2002), van den Hurk *et al.* (2003), Turell *et al.* (2003), Johansen *et al.* (2003), Bryant *et al.* (2005), Weng, Lien, and Ji (2005), Arunachalam *et al.* (2005), Das *et al.* (2005), Nitatpattana *et al.* (2005), Thenmozhi *et al.* (2006), van den Hurk *et al.* (2006), Hasegawa *et al.* (2008), Tewari *et al.* (2008), Samuel *et al.* (2008), Johansen, Power, Broom (2009), Sun *et al.* (2009), Arunachalam *et al.* (2009), Ohno *et al.* (2009), Nemeth *et al.* (2010), Konishi *et al.* (2010), Tiawsirisup and Nuchprayoon (2010), Kim *et al.* (2011), Nitatpattana *et al.* (2011), Chanyasanha *et al.* (2011), Li *et al.* (2011), Thakur *et al.* (2012), Feng *et al.* (2012), Upadhyayula *et al.* (2012), Hall-Mendelin *et al.* (2012), Tiawsirisup, Junpee, and Nuchprayoon (2012), Kumari *et al.* (2013), Seo *et al.* (2013), Liu *et al.* (2013), Borah *et al.* (2013), Lindahl *et al.* (2013), Su *et al.* (2014), Kim *et al.* (2015), Cha *et al.* (2015).

Both	7	Sabin (1947), Scherer, Moyer, and Izumi (1959), Buescher *et al.* (1959b), Hurlbut (1964), Doi *et al.* (1983), Chen *et al.* (2000), Saito *et al.* (2009).
Reporting of		
Vector competence	103	Reeves and Hammon (1946), Hurlbut (1950), Hurlbut (1951), Rosenberg *et al.* (1953), Buescher *et al.* (1959c), Gould, Barnett, and Suyemoto (1962), Hurlbut (1964), Konno *et al.* (1966), Doi, Shirasaka, and Sasa (1967), Cates and Detels (1969), Dandawate *et al.* (1969), Doi *et al.* (1970), Igarashi *et al.* (1972), Muangman *et al.* (1972), Mitchell, Chen, and Boreham (1973), Gould *et al.* (1973), Wang (1975), van Peenen and Joseph (1975), Fukumi *et al.* (1975), Wada *et al.* (1975), Hayashi *et al.* (1975), Takahashi (1976), Doi *et al.* (1977), Rosen *et al.* (1978), Hsu, Huang, and Cross (1978), Rosen, Shroyer, and Lien (1980), Rosen (1981), Khan *et al.* (1981), Takahashi (1982), Rosen and Shroyer (1985), Rosen *et al.* (1985), Burke *et al.* (1985), Olson *et al.* (1985), Leake and Johnson (1987), Yamamoto, Kimura, and Ohyama (1987), Hayakawa (1988), Rosen (1988), Takashima and Rosen (1989), Somboon *et al.* (1989), Rosen *et al.* (1989), Rosen, Lien, and Lu (1989), Takashima *et al.* (1989), Mourya *et al.* (1989), Dhanda *et al.* (1989), Gingrich *et al.* (1992), Peiris *et al.* (1992), Mitchell *et al.* (1993), Tan *et al.* (1993), Pant *et al.* (1994), Bhattacharyya *et al.* (1995), Hanna *et al.* (1996), Weng *et al.* (1997), Vythilingam *et al.* (1997), Gajanana *et al.* (1997), Dhanda *et al.* (1997), Ritchie *et al.* (1997), Samuel *et al.* (1998), Weng *et al.* (1999), Weng *et al.* (2000), Mwandawiro *et al.* (2000), Mourya and Mishra (2000), Victor *et al.* (2000), Johansen *et al.* (2000), Johansen *et al.* (2001), van den Hurk *et al.* (2001), van den Hurk *et al.* (2003), van den Hurk *et al.* (2003), Turell *et al.* (2003), Johansen *et al.* (2003), Bryant *et al.* (2005), Weng, Lien, and Ji (2005), Das *et al.* (2005), Nitatpattana *et al.* (2005), Thenmozhi *et al.* (2006), van den Hurk *et al.* (2006), Turell *et al.* (2006a), Turell *et al.* (2006b), van den Hurk *et al.* (2007), Samuel *et al.* (2008), Tewari *et al.* (2008), Johansen, Power, Broom (2009), Johnson *et al.* (2009), Sun *et al.* (2009), Arunachalam *et al.* (2009), Tiawsirisup and Nuchprayoon (2010), Kramer *et al.* (2011), Kim *et al.* (2011), Li *et al.* (2011), Feng *et al.* (2012), Upadhyayula *et al.* (2012), Tiawsirisup, Junpee, and Nuchprayoon (2012), Seo *et al.* (2013), Borah *et al.* (2013), Lindahl *et al.* (2013), Huber *et al.* (2014), Nicholson, Ritchie, and van Den Hurk (2014), Su *et al.* (2014), Sudeep *et al.* (2015), Huang *et al.* (2015), Mackenzie-Impoinvil *et al.* (2015), Kim *et al.* (2015).
Host competence	50	Morris, O'Connor, and Smadel (1955), Gresser *et al.* (1958), Hammon *et al.* (1958), Scherer, Buescher, and McClure (1959a), Buescher *et al.* (1959a), Scherer *et al.* (1959c), Buescher *et al.* (1959d), Scherer and Smith (1960), Sulkin, Sims, and Allen (1964), Sulkin, Allen, and Sims (1966), Kodama, Sasaki, and Inoue (1968), Pennington *et al.* (1968), Nathanson and Cole (1970), Self *et al.* (1973), Johnsen *et al.* (1974), Benenson *et al.* (1975), Simpson *et al.* (1976), Ura (1976), Dhanda *et al.* (1977), Soman *et al.* (1977), Khan and K. Banerjee (1980), Rodrigues, Guttikar, and Pinto (1981), Sasaki *et al.* (1982), Boyle, Dickerman, and Marshall (1983), Oya *et al.* (1983), Banerjee, Ilkal, and Deshmukh (1984), Ilkal *et al.* (1988), Thein, Aung, and Sebastian (1988), Takashima *et al.* (1988), Mani *et al.* (1991), Paul *et al.* (1993), Peiris *et al.* (1993), Ilkal *et al.* (1994), Tadano *et al.* (1994), Nga *et al.* (1995), Hanna *et al.* (1999), See *et al.* (2002), Hasegawa *et al.* (2008), Ohno *et al.* (2009), Nemeth *et al.* (2010), Konishi *et al.* (2010), Bosco-Lauth, Mason, and Bowen (2011), Nitatpattana *et al.* (2011), Chanyasanha *et al.* (2011), Nemeth *et al.* (2012), Thakur *et al.* (2012), Kumari *et al.* (2013), Liu *et al.* (2013), Cha *et al.* (2015), Ricklin *et al.* (2016).
More than one category	18	Sabin (1947), Buescher *et al.* (1959b), Scherer, Moyer, and Izumi (1959b), Gould, Byrne, and Hayes (1964), Wada *et al.* (1970), Yamada *et al.* (1971), Okuno *et al.* (1973), Chakravarty *et al.* (1975), Doi *et al.* (1983), Reuben *et al.* (1992), Khan *et al.* (1997), Chen *et al.* (2000), van den Hurk *et al.* (2001), Arunachalam *et al.* (2005), van den Hurk *et al.* (2009), Saito *et al.* (2009), Hall-Mendelin *et al.* (2012), Do, Bui, and Phan (2016).

Table 7. Proportion of JEV infection in positive mosquito pools (in observational studies only) by mosquito species (ordered alphabetically), and by author, year of publication, and country of origin, ordered from highest to lowest proportion of JEV infection [1].

Mosquito species	Author (year)	Country	Positive pools/Total pools tested[2]	Proportion positive pools (%)
Aedes (Cancraedes) sp.	Vythilingam *et al.* (1997)	Malaysia	1/19	5.26
Aedes albopictus	Weng *et al.* (1999)	Taiwan	20/39	51.28
	Su *et al.* (2014)	Taiwan	1/25	4.00
Aedes butleri	Vythilingam *et al.* (1997)	Malaysia	4/79	5.06
Aedes lineatopennis	Vythilingam *et al.* (1997)	Malaysia	1/6	16.67
Aedes scutellaris	van den Hurk *et al.* (2003)	India	1/1	100.00
Aedes vexans	Weng *et al.* (1999)	Taiwan	1/3	33.33
	Su *et al.* (2014)	Taiwan	3/32	9.38
Aedes vexans nipponii	Fukumi *et al.* (1975)	Japan	4/44,926	0.01
Aedes vexans nocturnus	Weng, Lien, and Ji (2005)	Taiwan	1/9	11.11
Anopheles annullaris	Olson *et al.* (1985)	Indonesia	1/28	3.57
Anopheles kochi	Tan *et al.* (1993)	Indonesia	2/28	7.14
Anopheles minimus	Su *et al.* (2014)	Taiwan	1/7	14.29
Anopheles peditaeniatus	Mourya *et al.* (1989)	India	1/133	0.75
Anopheles sinensis	Su *et al.* (2014)	Taiwan	6/419	1.43
	Feng *et al.* (2012)	China	3/14,170	0.02
Anopheles subpictus	Thenmozhi *et al.* (2006)	India	98/982	9.98
	Mourya *et al.* (1989)	India	1/87	1.15
	Dhanda *et al.* (1997)	India	1/163	0.61
Anopheles tessellatus	Su *et al.* (2014)	Taiwan	2/31	6.45
Anopheles vagus	Tan *et al.* (1993)	Indonesia	3/93	3.23
	Olson *et al.* (1985)	Indonesia	1/42	2.38
Armigeres subalbatus	Weng *et al.* (1999)	Taiwan	8/20	40.00
	Su *et al.* (2014)	Taiwan	3/30	10.00
	Tan *et al.* (1993)	Indonesia	3/114	2.63
	Chen *et al.* (2000)	Taiwan	1/123	0.81
	Feng *et al.* (2012)	China	2/394	0.51
	Fukumi *et al.* (1975)	Japan	1/11,666	0.01
Coquillettidia crassipes	van den Hurk *et al.* (2003)	India	2/3	66.67
Culex spp.	Tewari *et al.* (2008)	India	59/2,816	2.10
Culex annulirostris	van den Hurk *et al.* (2003)	India	2,368/3,197	74.07
	Ritchie *et al.* (1997)	Australia	8/134	5.97
	Hanna *et al.* (1996)	Australia	8/2,871	0.28
Culex annulus	Wang (1975)	Taiwan	220/223	98.65
	Weng *et al.* (1999)	Taiwan	1/3	33.33
	Su *et al.* (2014)	Taiwan	9/79	11.39
	Okuno *et al.* (1973)	Taiwan	6/91	6.59
	Hsu, Huang, and Cross (1978)	Taiwan	31/703	4.41
	Cates and Detels (1969)	Taiwan	3/174	1.72
Culex bitaeniorhynchus	van den Hurk *et al.* (2003)	India	7/10	70.00
	Seo *et al.* (2013)	South Korea	1/26	3.85
	Kim *et al.* (2011)	South Korea	1/45	2.22

	Tan *et al.* (1993)	Indonesia	1/85	1.18
Culex fuscanus	Weng *et al.* (1999)	Taiwan	1/2	50.00
Culex fuscocephala	Wang (1975)	Taiwan	353/359	98.33
	Su *et al.* (2014)	Taiwan	3/19	15.79
	Vythilingam *et al.* (1997)	Malaysia	2/76	2.63
	Gajanana *et al.* (1997)	India	6/305	1.97
	Dhanda *et al.* (1989)	India	1/85	1.18
	Mourya *et al.* (1989)	India	2/257	0.78
	Gould *et al.* (1973)	Thailand	2/142,375	<0.01
Culex fuscocephalus	van Peenen and Joseph (1975)	Indonesia	1/12	8.33
	Hsu, Huang, and Cross (1978)	Taiwan	19/282	6.74
	Tan *et al.* (1993)	Indonesia	3/185	1.62
Culex gelidus	Samuel *et al.* (2008)	India	56/64	87.50
	van den Hurk *et al.* (2003)	India	13/16	81.25
	van Peenen and Joseph (1975)	Indonesia	2/12	16.67
	Vythilingam *et al.* (1997)	Malaysia	12/224	5.36
	Mourya *et al.* (1989)	India	4/127	3.15
	Gajanana *et al.* (1997)	India	5/194	2.58
	Tewari *et al.* (2008)	India	4/177	2.26
	Upadhyayula *et al.* (2012)	India	12/590	2.03
	Arunachalam *et al.* (2009)	India	11/594	1.85
	Gould *et al.* (1973)	Thailand	3/11,495	0.03
	Peiris *et al.* (1992)	Sri Lanka	4/13,043	0.03
Culex orientalis	Kim *et al.* (2015)	South Korea	5/83	6.02
Culex palpalis	van den Hurk *et al.* (2003)	India	57/69	82.61
Culex pipiens	Seo *et al.* (2013)	South Korea	4/64	6.25
	Kim *et al.* (2015)	South Korea	1/264	0.38
	Buescher *et al.* (1959)	Australia	2/1,490	0.13
Culex pipiens fatigans	Wang (1975)	Taiwan	65/66	98.48
Culex pipiens pallens	Fukumi *et al.* (1975)	Japan	2/2,783	0.07
Culex pipiens quinquefasciatus	Tan *et al.* (1993)	Indonesia	10/333	3.00
Culex pseudovishnui	Dhanda *et al.* (1989)	India	3/81	3.70
	Borah *et al.* (2013)	India	3/107	2.80
	Mourya *et al.* (1989)	India	1/112	0.89
	Fukumi *et al.* (1975)	Japan	8/21,012	0.04
Culex quinquefasciatus	van den Hurk *et al.* (2003)	India	7/8	87.50
	Weng *et al.* (1999)	Taiwan	7/31	22.58
	Nitatpattana *et al.* (2005)	Thailand	2/25	8.00
	Mourya *et al.* (1989)	India	1/18	5.56
	Su *et al.* (2014)	Taiwan	2/74	2.70
	Vythilingam *et al.* (1997)	Malaysia	1/48	2.08
Culex rubithoracis	Weng, Lien, and Ji (2005)	Taiwan	4/22	18.18
Culex sitiens	Weng *et al.* (1999)	Taiwan	2/2	100.00
	van den Hurk *et al.* (2003)	India	3/8	37.50
	Weng, Lien, and Ji (2005)	Taiwan	1/34	2.94
	Johansen *et al.* (2001)	Australia	42/25,292	0.17
	Hall-Mendelin *et al.* (2012)	Australia	17/39,698	0.04
	van den Hurk *et al.* (2006)	Australia	1/22,833	<0.01

Culex tritaeniorhynchus	Wang (1975)	Taiwan	110/110	100.00
	Weng *et al.* (1999)	Taiwan	97/294	32.99
	Kim *et al.* (2011)	South Korea	50/207	24.15
	Seo *et al.* (2013)	South Korea	29/121	23.97
	Su *et al.* (2014)	Taiwan	468/2,242	20.87
	Victor *et al.* (2000)	India	2/10	20.00
	Buescher *et al.* (1959)	Australia	307/2,400	12.79
	Konno *et al.* (1966)	Japan	16/153	10.46
	Weng, Lien, and Ji (2005)	Taiwan	95/1,061	8.95
	Hayashi *et al.* (1975)	Japan	19/216	8.80
	Borah *et al.* (2013)	India	19/281	6.76
	Hsu, Huang, and Cross (1978)	Taiwan	18/267	6.74
	Das *et al.* (2005)	India	1/15	6.67
	Okuno *et al.* (1973)	Taiwan	6/91	6.59
	Dhanda *et al.* (1997)	India	7/163	4.29
	Vythilingam *et al.* (1997)	Malaysia	24/731	3.28
	van Peenen and Joseph (1975)	Indonesia	3/93	3.23
	Tewari *et al.* (2008)	India	13/429	3.03
	Dhanda *et al.* (1989)	India	3/117	2.56
	Arunachalam *et al.* (2009)	India	19/951	2.00
	Upadhyayula *et al.* (2012)	India	19/972	1.95
	Tan *et al.* (1993)	Indonesia	3/165	1.82
	Gajanana *et al.* (1997)	India	58/4,128	1.41
	Mourya *et al.* (1989)	India	3/272	1.10
	Li *et al.* (2011)	China	1/97	1.03
	Turell *et al.* (2003)	South Korea	14/4,281	0.33
	Olson *et al.* (1985)	Indonesia	1/596	0.17
	Pant *et al.* (1994)	India	1/753	0.13
	Sun *et al.* (2009)	China	12/14,840	0.08
	Fukumi *et al.* (1975)	Japan	435/598,434	0.07
	Feng *et al.* (2012)	China	15/37,119	0.04
	Rosen, Lien, and Lu (1989)	Taiwan	165/524,290	0.03
	Peiris *et al.* (1992)	Sri Lanka	4/17,436	0.02
	Gould *et al.* (1973)	Thailand	8/182,940	<0.01
Culex univittattus	Dhanda *et al.* (1989)	India	1/29	3.45
Culex vishnui	Borah *et al.* (2013)	India	7/198	3.54
	Gajanana *et al.* (1997)	India	22/1,080	2.04
	Tewari *et al.* (2008)	India	42/2,203	1.91
	Mourya *et al.* (1989)	India	2/290	0.69
	Dandawate *et al.* (1969)	India	2/5,553	0.04
Culex whitmorei	van den Hurk *et al.* (2003)	India	2/2	100.00
	Dhanda *et al.* (1989)	India	1/20	5.00
	Mourya *et al.* (1989)	India	1/132	0.76
	Peiris *et al.* (1992)	Sri Lanka	1/167	0.60
Mansonia indiana	Dhanda *et al.* (1997)	India	1/163	0.61
Mansonia septempunctata	van den Hurk *et al.* (2003)	India	4/14	28.57
Mansonia uniformis	van den Hurk *et al.* (2003)	India	11/11	100.00
	Su *et al.* (2014)	Taiwan	1/19	5.26

	Dhanda *et al.* (1997)	India	3/163	1.84
	Mourya *et al.* (1989)	India	2/281	0.71
Ochleratus normanensis	van den Hurk *et al.* (2003)	India	100/310	32.26
Ochleratus vigilax	van den Hurk *et al.* (2003)	India	3/3	100.00
Ochleratus vittiger	van den Hurk *et al.* (2003)	India	1/1	100.00
Ochlerotatus vigilax	Johansen *et al.* (2001)	Australia	1/3,073	0.03
Verrallina funerea	van den Hurk *et al.* (2003)	India	3/5	60.00

[1]A complete list of all mosquito species (n=149) across all observational studies (n=58) is available in Appendix B.
[2]Mosquito pools = 1 to 800 mosquitoes

Table 8. Minimum infection rates (MIR), standard errors (SE), and ranges reported across all observational studies (n=16) by author and year of publication and by mosquito species (ordered alphabetically).

Author (year)	Mosquito species[1]	MIR[2]	SE	MIR Range
Olson *et al.* (1985)	*Anopheles annullaris*	4.00	-	-
	Anopheles vagus	0.37	-	-
	Culex tritaeniorhynchus	0.01	-	-
Gingrich *et al.* (1992)	*Culex gelidus*	0.17 - 0.21	-	-
	Culex tritaeniorhynchus	0.09 - 0.1	-	-
Peiris *et al.* (1992)	*Aedes spp.*	0.23	-	-
	Culex fuscocephala	0.06 - 0.23	-	-
	Culex gelidus	0.06 - 0.23	-	-
	Culex pseudovishnui	0.06 - 0.23	-	-
	Culex tritaeniorhynchus	0.06 - 0.23	-	-
	Culex whitmorei	0.23	-	-
	Mansonia uniformis	0.23	-	-
	Mansonia uniformis	0.06	-	-
Dhanda *et al.* (1997)	*Culex tritaeniorhynchus*	0.00	-	-
Gajanana *et al.* (1997)	*Culex fuscocephala*	0.39	0.32	-
	Culex gelidus	0.52	0.46	-
	Culex tritaeniorhynchus	0.28	0.08	-
	Culex vishnui	0.41	0.18	-
Vythilingam *et al.* (1997)	*Culex tritaeniorhynchus*	0.1-5.6	-	-
Johansen *et al.* (2000)	*Culex sitiens subgroup*	0.01-0.02	-	-
Johansen *et al.* (2001)	*Culex sitiens group*	1.70	-	-
	Ochlerotatus vigilax	0.30	-	-
Turell *et al.* (2003)	*Culex tritaeniorhynchus*	2.6 - 13.2	-	-
Weng, Lien, and Ji (2005)	*Aedes albopictus*	0.00	-	-
	Aedes penghuensis	0.00	-	-
	Aedes subalbatus	0.00	-	-
	Aedes vexans nocturnus	16.40	-	-
	Anopheles sinensis	0.00	-	-
	Anopheles tessellatus	0.00	-	-
	Culex annulus	0.00	-	-
	Culex fuscanus	0.00	-	-
	Culex quinquefasciatus	0.00	-	-
	Culex rubithoracis	30.80	-	-
	Culex sitiens	1.70	-	-
	Culex tritaeniorhynchus	3.30	-	-
	Mansonia uniformis	0.00	-	-
	Mimomyia luzonensis	0.00	-	-
Thenmozhi *et al.* (2006)	*Anopheles subpictus*	0 - 12.5	-	-
Tewari *et al.* (2008)	*Culex spp.*	0 - 0.59	-	-
Feng *et al.* (2012)	*Anopheles sinensis*	0.21	-	-
	Armigeres subalbatus	5.08	-	-
Hall-Mendelin *et al.* (2012)	*Culex sitiens subgroup*	0.04 - 1.61	-	-
Upadhyayula *et al.* (2012)	*Culex gelidus*	0 - 333.3	-	-
	Culex tritaeniorhynchus	0 - 3.43	-	-

Borah *et al.* (2013)	*Culex pseudovishnui*	0.20	-	0.00-2.10
	Culex tritaeniorhynchus	0.90	-	0.00-4.30
	Culex vishnui	0.40	-	0.00-2.90

[1]Mosquito pools = 1 to 500 mosquitoes
[2]Minimum infection rates per 1,000 mosquitoes (presented as an average MIR or range of MIR values depending on how it was reported in the studies).
Minimum infection rate (MIR) is defined as the ratio of the number of positive mosquito pools to the total number of mosquitoes in the sample, assuming that only one infected individual is present in a positive pool (Bustamante and Lord, 2010).

Table 9. Maximum likelihood estimation (MLE) and 95% confidence intervals (CI) reported across all observational studies (n=6) by author, year of publication and by mosquito species (ordered alphabetically).

Author (year)	Mosquito species[1]	MLE[2]	95% CI
van den Hurk *et al.* (2006)	*Culex sitiens*	0.04	-
Arunachalam *et al.* (2009)	*Culex gelidus*	0.56	0.29-0.97
	Culex tritaeniorhynchus	0.63	0.34-1.07
Kim *et al.* (2011)	*Aedes vexans nipponii*	0.90	-
	Culex pipiens pallens	9.70	-
Lindahl *et al.* (2013)	*Culex quinquefasciatus*	1.30	0.10-6.30
		1.20	0.20-3.80
	Culex tritaeniorhynchus	1.60	0.40-4.40
		0.90	0.40-1.80
Seo *et al.* (2013)	*Culex bitaeniorhynchus*	2.80	-
	Culex pipiens	5.60	-
	Culex tritaeniorhynchus	11.80	-
Su *et al.* (2014)	*Aedes aegypti*	0.00	0.00-499.14
	Aedes albopictus	19.44	1.30-88.53
		0.00	0.00-22.75
	Aedes penghuiensis	0.00	0.00-10.46
	Aedes vexans	29.65	1.91-139.58
		9.75	1.79-32.33
	Anopheles ludlowae	0.00	0.00-793.45
	Anopheles minimus	53.78	3.38-230.59
	Anopheles sinensis	2.05	0.77-4.50
		5.33	0.34-25.44
	Anopheles tessellatus	4.81	0.33-23.37
		2.75	0.16-13.24
	Armigeres subalbatus	17.34	4.83-45.72
		0.00	0.00-56.26
	Coquillettidia crassipes	0.00	0.00-35.54
	Culex annulus	26.29	13.89-46.52
		1.41	0.08-6.76
	Culex bitaeniorhynchus	0.00	0.00-37.88
	Culex brevipalpis	0.00	0.00-793.45
	Culex fuscanus	0.00	0.00-499.14
		0.00	0.00-793.49
	Culex fuscocephala	7.77	2.17-20.82
	Culex mimeticus	0.00	0.00-793.45
	Culex murrelli	0.00	0.00-53.12
	Culex nigropunctatus	0.00	0.00-160.75
	Culex quinquefasciatus	1.64	0.30-5.38
		0.00	0.00-26.51
	Culex rubithoracis	0.00	0.00-42.44
	Culex sitiens	0.00	0.00-0.60
	Culex tritaeniorhynchus	7.68	6.97-8.45
		2.10	1.60-2.72
	Mansonia uniformis	18.14	1.06-90.71
		0.00	0.00-146.56
	Ochlerotatus albolateralis	0.00	0.00-793.45
	Ochlerotatus togoi	0.00	0.00-793.45

	Uranotenia macfarlanei	0.00	0.00-793.45

[1]Mosquito pools = 1 to 200 mosquitoes

[2]Maximum likelihood estimation per 1,000 mosquitoes.

Maximum likelihood estimation (MLE) represents the proportion of infected mosquitoes that maximizes the likelihood of the number of pools of a specific size to be virus positive, where the proportion is the parameter of a binomial distribution (Bustamante and Lord, 2010).

Table 10. JEV infection, dissemination, and transmission rates for 14 days post infection (DPI) or the closest to 14 DPI (most frequently reported incubation period) across all experimental studies reporting incubation period (n=30) by author, year of publication and by mosquito species (ordered alphabetically).

Author (year)	Mosquito Name	DPI*	Proportion infected[1]	Infection rate (%)[2]	Proportion disseminated[3]	Dissemination rate (%)[4]	Proportion transmitted[5]	Transmission rate (%)[6]	Mosquitoes/ pool
Reeves and Hammon (1946)	*Aedes dorsalis*	16	1/31	3.23	-	-	-	-	-
	Aedes nigromaculis	8-14	4/217	1.84	-	-	-	-	-
	Aedes varipalpus	6-14	0/153	0.00	-	-	-	-	-
	Aedes vexans	8-27	0/98	0.00	-	-	-	-	-
	Anopheles maculipennis freeborni	0-16	0/119	0.00	-	-	-	-	-
	Culex pipiens molestus	7-20	3/216	1.39	-	-	-	-	-
	Culex pipiens (pipiens)	20	2/15	13.33	-	-	-	-	-
	Culex quinquefasciatus	11-25	4/664	0.60	-	-	-	-	-
	Culex tarsalis	6-10	2/165	1.21	-	-	-	-	-
	Culiseta incidens	8-14	3/74	4.05	-	-	-	-	-
	Culiseta inornata	10-20	3/82	3.66	-	-	-	-	-
Hurlbut (1950)	*Culex quinquefasciatus*	6	5/5	100.00	-	-	-	-	-
Gresser *et al.* (1958)	*Culex tritaeniorhynchus*	18	7/8	87.50	-	-	-	-	-
Gould, Barnett, and Suyemoto (1962)	*Culex gelidus*	6 - 21	-	-	-	-	1/13	8.00	-
Gould, Byrne, and Hayes (1964)	*Culex gelidus*	6	-	-	-	-	0/13	0.00	-
	Culex tritaeniorhynchus	6	-	-	-	-	1/29	3.45	-
Hurlbut (1964)	*Aedes albopictus*	14	0/10	0.00	-	-	-	-	-
	Culex tritaeniorhynchus	14	4/26	15.38	-	-	-	-	-
Doi, Shirasaka, and Sasa (1967)	*Culex tritaeniorhynchus*	15	6/6	100.00	-	-	-	-	-
Doi *et al.* (1970)	*Culex pipiens*	14	2/5	40.00	-	-	-	-	-
	Culex tritaeniorhynchus	15	9/9	100.00	-	-	-	-	-
Muangman *et al.* (1972)	*Culex fuscocephala*	10	19/20	95.00	-	-	1/10	10.00	-
	Culex tritaeniorhynchus	10	20/20	100.00	-	-	0/10	0.00	-
Doi *et al.* (1977)	*Culex pipiens fatigans*	10-14	0/17	0.00	-	-	-	-	-
	Culex pipiens pallens	10-14	0/23	0.00	-	-	-	-	-
	Culex pseudovishnui	10-14	0/19	0.00	-	-	-	-	-
	Culex tritaeniorhynchus	10-14	8/9	88.90	-	-	-	-	-
Takahashi (1982)	*Culex tritaeniorhynchus*	10-14	19/20	95.00	-	-	19/19	100.00	-

Rosen and Shroyer (1985)	*Toxorhynchites amboinensis*	14	5/5	100.00	-	-	-	-	-
	Toxorhynchites brevipalpis	14	5/5	100.00	-	-	-	-	-
	Toxorhynchites rutilus	14	5/5	100.00	-	-	-	-	-
	Toxorhynchites Splendens	14	5/5	100.00	-	-	-	-	-
	Toxorhynchites Theobaldi	14	5/5	100.00	-	-	-	-	-
Rosen (1988)	*Aedes albopictus*	9-10	0/26	0.00	-	-	-	-	≤100
Takashima and Rosen (1989)	*Aedes japonicus*	1-20	18/20	90.00	-	-	3/4	75.00	-
	Aedes vexans nipponii	1-20	3/12	25.00	-	-	-	-	-
	Culex pipiens pallens	1-20	3/10	30.00	-	-	-	-	-
	Culex tritaeniorhynchus	1-20	15/15	100.00	-	-	6/6	100.00	-
Weng *et al.* (1997)	*Aedes albopictus*	14	-	-	-	-	5/13	38.46	-
Samuel *et al.* (1998)	*Culex tritaeniorhynchus*	12-14	17/26	65.40	-	-	3/19	15.79	-
Weng *et al.* (2000)	*Culex pipiens molestus*	7	-	-	-	-	3/3	100.00	-
	Culex tritaeniorhynchus	13	-	-	-	-	6/6	100.00	-
Chen *et al.* (2000)	*Aedes aegypti*	14	0/6	0.00	-	-	-	-	-
	Aedes albopictus	14	7/15	46.67	-	-	-	-	-
	Armigeres subalbatus	14	7/8	87.50	-	-	-	-	-
	Culex quinquefasciatus	14	2/5	40.00	-	-	-	-	-
Mourya and Mishra (2000)	*Culex pseudovishnui*	10	6/10	60.00	-	-	-	-	-
	Culex tritaeniorhynchus	10	8/10	80.00	-	-	-	-	-
	Culex vishnui	10	4/10	40.00	-	-	-	-	-
van den Hurk *et al.* (2003)	*Aedes aegypti*	14-15	16/60	26.67	0/60		15/60	25.00	-
	Coquillettidia xanthogaster	14-15	4/36	11.11	-	-	1/15	6.67	-
	Culex annulirostris	14	36/36	100.00	23/36	63.89	13/16	81.25	-
	Culex gelidus	14-15	4/4	100.00	-	-	1/1	100.00	-
	Culex quinquefasciatus	14-15	51/55	92.73	-	-	14/23	60.87	-
	Culex sitiens	14	33/36	92.00	4/36	11.11	10/15	66.67	-
	Mansonia septempunctata	9	16/24	66.67	0/24	0.00	13/24	54.17	-
	Mansonia uniformis	14-15	1/1	100.00	0/1	0.00	1/1	100.00	-
	Ochlerotatus kochi	14-15	6/28	21.43	-	-	0/8	0.00	-
	Ochlerotatus normanensis	14-15	0/1	0.00	0/1	0.00	0/1	0.00	-
	Ochlerotatus notoscriptus	13-14	13/48	27.00	4/48	8.33	3/11	27.27	-
	Ochlerotatus purpureus	14-15	2/2	100.00	0/2	0.00	2/2	100.00	-

	Ochlerotatus vigilax	14-15	1/9	11.11	-	-	1/8	12.50	-
	Verrallina carmenti	14-15	0/2	0.00	0/2	0.00	0/2	0.00	-
	Verrallina funerea	14-15	43/75	57.33	-	-	3/18	16.67	-
Turell *et al.* (2006a)	*Culex pipiens pallens*	12	0/40	0.00	-	-	-	-	-
	Culex tritaeniorhynchus	12	10/10	100.00	-	-	-	-	-
Turell *et al.* (2006b)	*Culex pipiens*	16-17	28/50	56.00	-	26.00	-	-	-
van den Hurk *et al.* (2007)	*Culex annulirostris Skuse*	13	22/23	95.65	-	-	-	96.00	-
	Culex gelidus	13	20/25	80.00	-	-	-	12.00	-
Johnson *et al.* (2009)	*Culex annulirostris*	12	20/25	80.00	14/56	25.00	3/12	25.00	-
	Culex gelidus	12	22/23	96.00	22/96	22.92	22/96	23.00	-
van den Hurk *et al.* (2009)	*Culex annulirostris*	5	1/4	25.00	-	-	-	-	-
Kramer *et al.* (2011)	*Aedes notoscriptus*	14	0/39	0.00	-	-	-	-	-
	Culex pipiens	14	5/50	10.00	2/5	40.00	0/5	0.00	-
	Culex quinquefasciatus	14	6/36	16.67	0/6	0.00	-	-	-
	Opifex fuscus	14	37/50	74.00	26/37	70.27	0/37	0.00	-
Huber *et al.* (2014)	*Aedes japonicus japonicus*	0-14	3/3	100.00	-	-	-	-	-
Nicholson, Ritchie, and van Den Hurk (2014)	*Aedes albopictus*	14	-	-	-	16.00	-	16.00	-
Huang *et al.* (2015)	*Culex quinquefasciatus*	14	22/26	84.60	7/14	50.00	-	-	-
Mackenzie-Impoinvil *et al.* (2015)	*Culex quinquefasciatus*	14	20/32	62.00	18/32	56.25	2/32	6.25	-
	Ochlerotatus detritus	14	25/32	78.00	29/32	90.63	1/32	3.13	-

[1] Proportion infected represents the number of positive infected mosquitoes divided by the total number of mosquitoes tested.
[2] Infection rate consists of an estimate of the prevalence of infection in a mosquito population (Bustamante and Lord, 2010).
[3] Proportion disseminated represents the number of positive mosquitoes with disseminated infection divided by the total number of mosquitoes tested.
[4] Dissemination rate refers to the proportion of mosquitoes containing virus in their legs, regardless of their infection status (Golnar *et al.*, 2015).
[5] Proportion transmitted consists of number of positive mosquitoes that transmit the virus divided by the total number of mosquitoes tested.
[6] Transmission rate is defined as the proportion of mosquitoes with a disseminated infection that transmit the virus after refeeding (Golnar *et al.*, 2015).
*When more than one trial on the same mosquito species and DPI was reported, the first result is shown (please refer to Appendix C for a complete list of results).

Table 11. Proportion of JEV infection in host species reported across all observational studies (n=33) and mosquito host preferences (all mosquito species) across all observational studies (n=16), by host species.

Proportion of JEV infection in host species				Mosquito host preferences	
Host species	**Author (year)**[1]	**Proportion infected**[2]	**Proportion positive (%)**	**Author (year)**[3]	**Bloodmeals**[4]
Bats	Hammon *et al.* (1958), Johnsen *et al.* (1974)	2/56	3.57	Reuben *et al.* (1992), Tiawsirisup, Junpee, and Nuchprayoon (2012)	13
Birds	Nemeth *et al.* (2010), Khan and Banerjee (1980), Takashima *et al.* (1989), Rodrigues, Guttikar, and Pinto (1981), Hammon *et al.* (1958), Paul *et al.* (1993), Buescher *et al.* (1959), Johnsen *et al.* (1974)	17/3,041	0.56	Johansen, Power, Broom (2009), Reuben *et al.* (1992), Hurlbut (1964), van den Hurk *et al.* (2003), Hall-Mendelin *et al.* (2012), Wang (1975), Mitchell, Chen, and Boreham (1973), van den Hurk *et al.* (2001)	74
Cattle	Peiris *et al.* (1993), Sabin (1947), Hammon *et al.* (1958), Johnsen *et al.* (1974)	573/644	88.98	Johansen, Power, Broom (2009), Reuben *et al.* (1992), Hurlbut (1964), Arunachalam *et al.* (2005), Gould *et al.* (1973), Self *et al.* (1973), van den Hurk *et al.* (2003), Samuel *et al.* (2008), Hall-Mendelin *et al.* (2012), Wang (1975), Somboon *et al.* (1989), Pennington and Phelps (1968), Mitchell, Chen, and Boreham (1973)	84
Chickens	Peiris *et al.* (1993), Khan and Banerjee (1980), Hanna *et al.* (1996), Mani *et al.* (1991), Sabin (1947), Hammon *et al.* (1958), Paul *et al.* (1993), Hayashi *et al.* (1975), Johnsen *et al.* (1974)	52/920	5.65	Reuben *et al.* (1992), Somboon *et al.* (1989), Pennington and Phelps (1968)	22
Ducks	Peiris *et al.* (1993), Khan and Banerjee (1980), Paul *et al.* (1993), Johnsen *et al.* (1974)	113/298	37.92	Reuben *et al.* (1992), Samuel *et al.* (2008)	13
Ardeid birds	Khan and Banerjee (1980), Rodrigues, Guttikar, and Pinto (1981), Buescher *et al.* (1959a), Buescher *et al.* (1959b), Scherer, Buescher, and McClure (1959)	891/3,001	29.69	Reuben *et al.* (1992)	12
Pigs	Thakur *et al.* (2012), Hurlbut (1964), Kumari *et al.* (2013), Cha *et al.* (2015), Konishi *et al.* (2010), Liu *et al.* (2013), Nitatpattana *et al.* (2011), Peiris *et al.* (1993), Self *et al.* (1973), Thein, Aung, and Sebastian (1988), Ura (1976), Tadano *et al.* (1994), Borah *et al.* (2013), Lindahl *et al.* (2013), Takashima *et al.* (1989), Chanyasanha *et al.* (2011), Burke *et al.* (1985), Hanna *et al.* (1996), Okuno *et al.* (1973), Li *et al.* (2011), Hanna *et al.* (1999), Peiris *et al.* (1992), Hammon *et al.* (1958), Paul *et al.* (1993), Hayashi *et al.* (1975), Johnsen *et al.* (1974), Konno *et al.* (1966), Nga *et al.* (1995), Scherer, Moyer, and Izumi (1959), Scherer *et al.* (1959), Simpson	4,281/20,942	20.44	Johansen, Power, Broom (2009), Reuben *et al.* (1992), Hurlbut (1964), Arunachalam *et al.* (2005), Gould *et al.* (1973), Self *et al.* (1973), van den Hurk *et al.* (2003), Samuel *et al.* (2008), Hall-Mendelin *et al.* (2012), Wang (1975), Somboon *et al.* (1989), Pennington and Phelps (1968), Mitchell, Chen, and Boreham (1973), van den Hurk *et al.* (2001)	84

	et al. (1976), Takashima *et al.* (1988), Yamada *et al.* (1971)				
Rabbits	Peiris *et al.* (1993)	0/69	0.00	Reuben *et al.* (1992)	12
Reptiles and amphibians	Hammon *et al.* (1958), Doi *et al.* (1983)	0/494	0.00	-	-
Wild Pigs	See *et al.* (2002), Ohno *et al.* (2009), Hayashi *et al.* (1975)	32/47	68.09	-	-
Cats and dogs	Peiris *et al.* (1993), Hanna *et al.* (1996), Hammon *et al.* (1958), Paul *et al.* (1993), Johnsen *et al.* (1974)	3/287	85.37	van den Hurk *et al.* (2003), Hall-Mendelin *et al.* (2012), Wang (1975), Pennington and Phelps (1968), Mitchell, Chen, and Boreham (1973), van den Hurk *et al.* (2001)	73
Sheep and Goats	Paul *et al.* (1993), Peiris *et al.* (1993), Sabin (1947), Hammon *et al.* (1958), Hayashi *et al.* (1975)	103/386	26.68	Samuel *et al.* (2008), Pennington and Phelps (1968)	8
Sylvatic mammals	Saito *et al.* (2009), Ohno *et al.* (2009)	239/1,183	20.20	-	-
Horses and donkeys	Hanna *et al.* (1996), Mani *et al.* (1991), Sabin (1947), Hammon *et al.* (1958)	34/54	62.96	Reuben *et al.* (1992), Self *et al.* (1973), van den Hurk *et al.* (2003), Hall-Mendelin *et al.* (2012), Pennington and Phelps (1968), van den Hurk *et al.* (2001)	57
Rats	Hammon *et al.* (1958)	0/26	0.00	Hall-Mendelin *et al.* (2012)	7

[1] Articles pertaining to JEV infection in hosts (observational studies reporting host competence).
[2] Proportion positive is the number of positive vertebrate hosts divided by the total number of vertebrate hosts tested (results combined from a total of 33 articles).
[3] Articles pertaining to mosquito host preferences (observational studies reporting vector competence).
[4] Number of blood meals taken from hosts by mosquitoes (mosquito host preferences) – results combined from 16 articles.

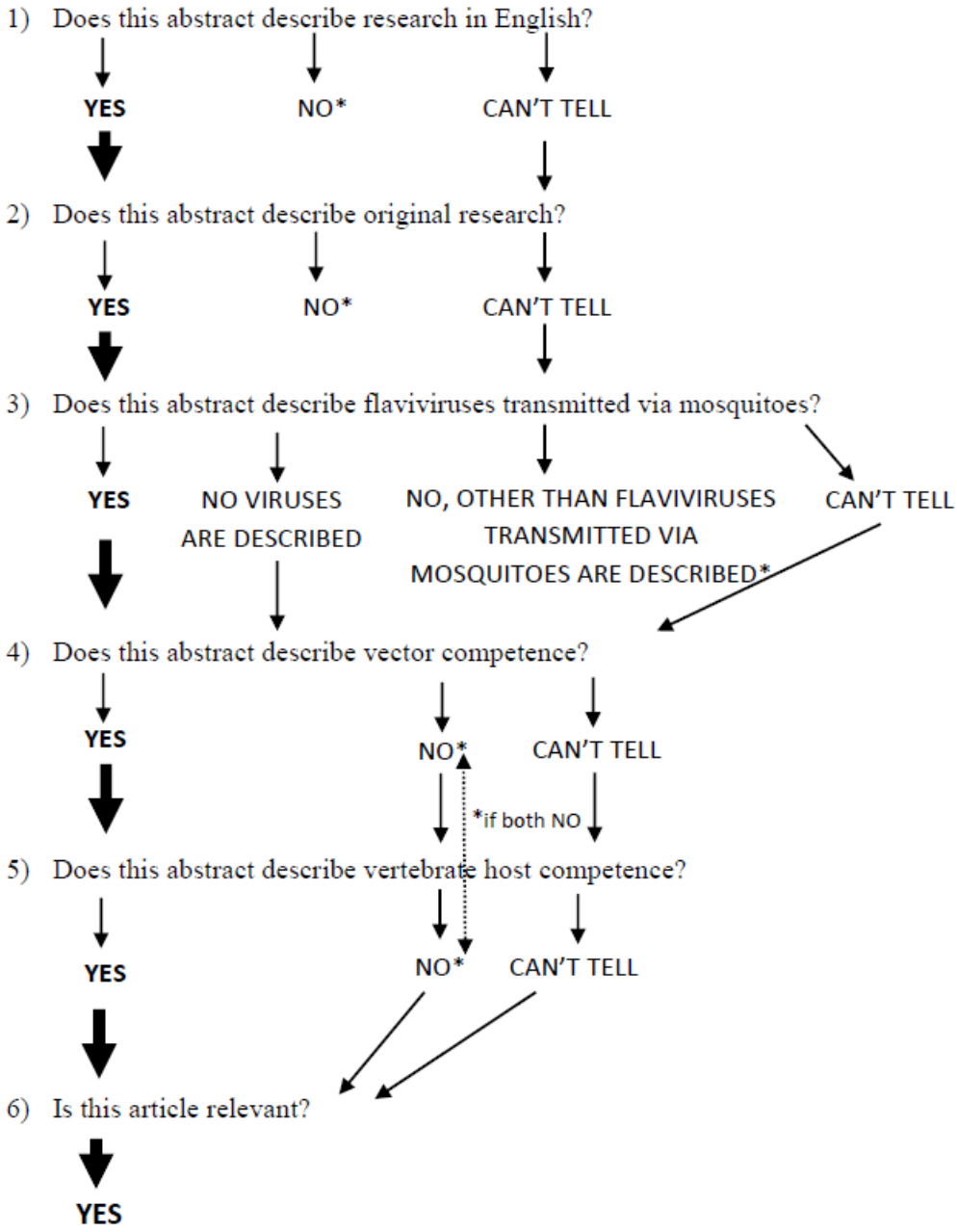

Figure 1. Flowchart depicting questions, and potential answers, for articles to be deemed relevant using the relevance screening tool.

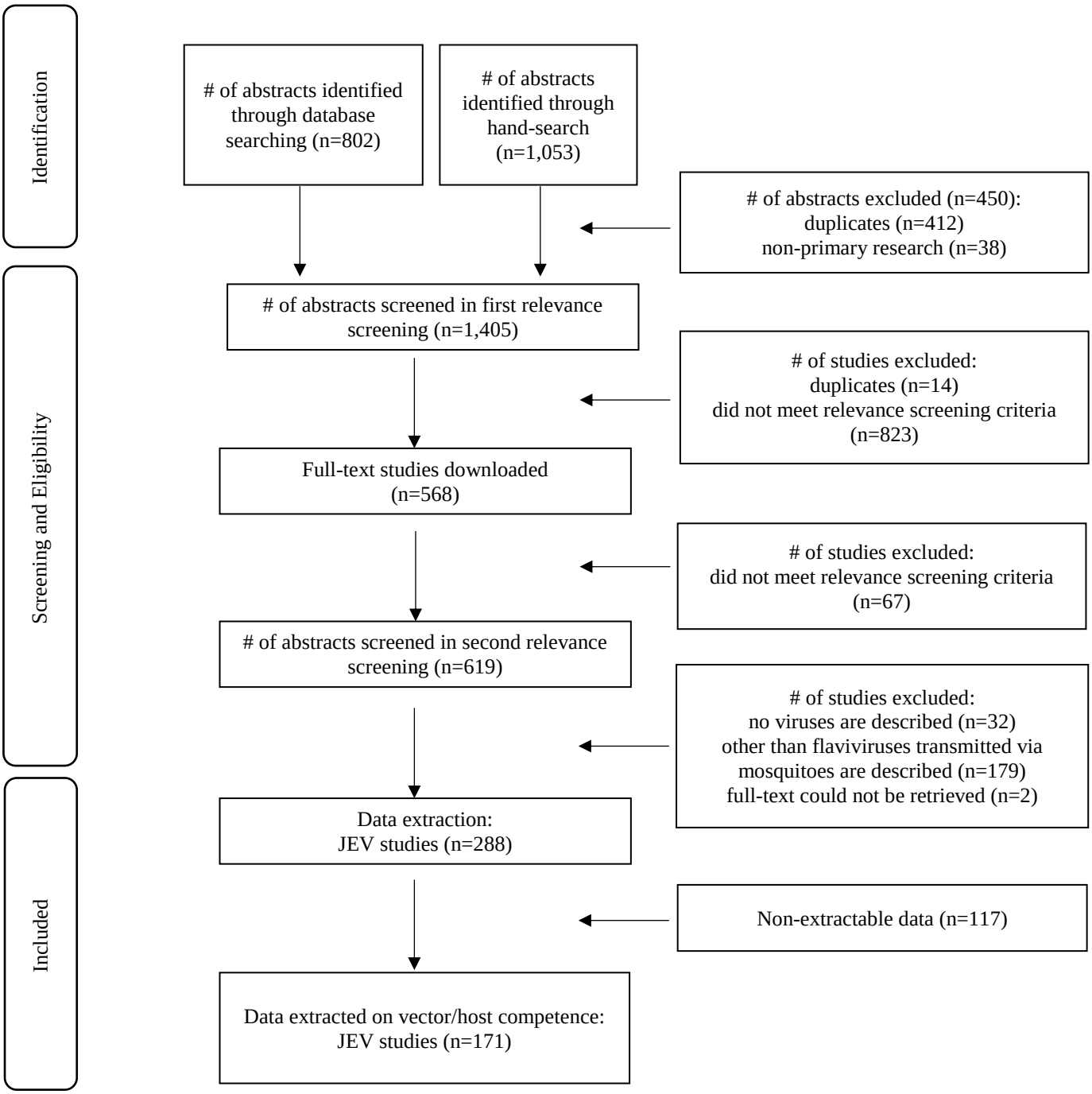

Figure 2. Flowchart of the articles identified, screened, and included for data extraction.

Chapter 3 - Meta-analyses of the proportion of Japanese encephalitis virus infection in vectors and vertebrate hosts

Summary

Japanese encephalitis (JE) is a vector-borne zoonosis in Southeast Asia transmitted by mosquitoes infected with the Japanese encephalitis virus (JEV) and considered an emerging exotic infectious disease with potential for introduction in currently JEV-free countries. Pigs and ardeid birds are reservoir hosts and play a major role on the transmission dynamics of the disease. The objective of the study was to quantitatively summarize the proportion of JEV infection in vectors and vertebrate hosts from data pertaining to observational studies obtained in a systematic review of the literature on vector and host competence for JEV, using meta-analyses.

Data gathered pertained to three outcomes: proportion of JEV infection in vectors, proportion of JEV infection in vertebrate hosts, and minimum infection rate (MIR) in vectors. Random-effects subgroup meta-analysis models were fitted by species (mosquito or vertebrate host species) to estimate pooled summary measures as well as to compute the variance between studies. Meta-regression models were fitted to assess the association between different predictors and the outcomes of interest and to identify sources of heterogeneity among studies. Predictors included in all models were mosquito/vertebrate host species, diagnostic methods, mosquito capture methods, season, country/region, age category, and number of mosquitos per pool.

Mosquito species, diagnostic method, country, and capture method represented important sources of heterogeneity associated with the proportion of JEV infection; host species and region were considered sources of heterogeneity associated with the proportion of JEV infection in hosts; and diagnostic and mosquito capture methods were deemed important contributors of heterogeneity for the MIR outcome.

Our findings provide reference pooled summary estimates of vector competence for JEV for some mosquito species, as well as of sources of variability for these outcomes. Moreover, this work provides useful guidelines when interpreting vector and host infection proportions or prevalence from observational studies, and contributes to further our understanding of vector and vertebrate host competence for JEV, elucidating information on the relative importance of vectors and hosts on JEV introduction and transmission.

Keywords: Japanese encephalitis virus, Japanese encephalitis, meta-analysis, vector, host, competence.

Introduction

Japanese encephalitis virus (JEV) is the causative agent of Japanese encephalitis (JE), the most important viral encephalitis occurring in humans, particularly in children aged 0 to 14 years, in Southeastern Asia (Campbell *et al.*, 2011). Japanese encephalitis is a mosquito-borne disease with JEV being transmitted by different species of mosquitoes, although the *Culex vishnui* subgroup, and particularly *Culex tritaeniorhynchus*, are considered the most relevant vectors (van den Hurk *et al.*, 2009).

The disease symptoms range from a nonspecific febrile illness to aseptic meningitis and severe encephalitis that may lead to irreversible neurological sequelae. Despite less than 1% of humans infected with JEV develop clinical disease, 20-30% of the cases are fatal, and about 30-50% of survivors experience neurological damage (Misra and Kalita, 2010; Campbell *et al.*, 2011).

Japanese encephalitis is more prevalent in rural and suburban areas, where both rice and pig production are present, as rice paddy fields provide the ideal conditions for mosquito breeding and pigs are considered the main amplifying reservoir for JEV (Campbell *et al.*, 2011; LeFlohic *et al.*, 2013).

There are two main JEV transmission cycles, a pig-associated rural domestic and a bird-associated wild cycle. Although humans and domestic animals other than pigs and birds may become infected via infected mosquito bites, they do not develop sufficient viremia to infect susceptible vectors and they are thus considered dead-end hosts (LeFlohic *et al.*, 2013).

Japanese encephalitis transmission is highly dynamic, usually occurring in epidemics, especially in the most temperate regions of Asia (higher latitudes), while in the tropics and subtropics the disease is endemic, peaking in the rainy season (Campbell *et al.*, 2011).

Japanese encephalitis virus has spread to new regions over the past decades, reaching Pakistan in the west and the Torres Strait in Australia in the southeast. The recent expansion is not fully understood, although they may include inadvertent transportation of infected vectors, human movement, bird migration (with climate change affecting migration patterns), and wind-blown mosquitoes (Mackenzie *et al.*, 2004; Erlanger *et al.*, 2009). Because expansion has occurred, there is a global concern related to the emergence of exotic vector-borne diseases, such as JE, in currently virus-free countries. Huang *et al.* (2014) reported that there is a considerable range of mosquito species that are susceptible to JEV and that, if competent vectors and vertebrate hosts are present in these regions, virus introduction is possible. Furthermore, other authors claimed that JEV viremia has been observed in more

than 90 wild and domestic birds, belonging to several avian families, and that JEV has been isolated in over 30 mosquito species (van den Hurk *et al.*, 2009; Huang *et al.*, 2014). Conversely, and despite many reviews and several references to the potential spread of JEV and the importance attributed to vector and vertebrate host competence in its introduction and transmission into new geographical areas, no quantification or thorough analysis of such parameters have been conducted so far (Nett *et al.*, 2009; van den Hurk *et al.*, 2009; Nemeth *et al.*, 2012; Huang *et al.*, 2014). An accurate evaluation of such parameters would further our understanding of the relative importance of vectors and vertebrate hosts on virus introduction and transmission, ultimately pointing to more effective prevention and mitigation strategies (Lord *et al.* 2015).

A systematic review of the literature is a tool that allows a rigorous and consistent identification, assessment, and summary of current scientific evidence, whereas a meta-analysis is a quantitative, statistical method that combines the results of the data gathered from the body of evidence, providing a more accurate estimate (i.e., a summary effect measure) of the outcomes of interest. Moreover, a meta-analysis allows exploring the sources of heterogeneity between results from different studies, thus considering possible sources of confounding and bias. A meta-analysis increases the power of a systematic review and provides valuable information to answer the research question posed and/or identifies potential knowledge gaps (Egger *et al.* 2001; Sutton *et al.*, 2001; Sargeant *et al.*, 2006; O'Connor *et al.*, 2014a; Sargeant and O'Connor, 2014a).

Hence, the objective of this study is to quantitatively summarize the proportion of viral infection in vectors and vertebrate hosts from data pertaining to observational studies obtained in a systematic review of the literature on vector and host competence for Japanese encephalitis virus, using meta-analyses.

Materials and Methods

Systematic review of the literature

The literature search was conducted in eight electronic databases and journal websites (Web of Science, Pubmed, Armed Forces Pest Management Board, The American Journal of Tropical Medicine and Hygiene, Journal of Medical Entomology, Journal of the American Mosquito Control Association, Vector Borne and Zoonotic Diseases, and Google Scholar) and the last day of search was April 25th, 2016. Additionally, a hand-search of the reference list of nine articles considered as key publications by the reviewers (Solomon *et al.*, 2000; Mackenzie *et al.*, 2004; Weaver and Barrett, 2004; Erlanger *et al.*, 2009; Nett *et al.*, 2009; van den Hurk *et al.*, 2009; Misra and Kalita, 2010; LeFlohic *et al.*, 2013; Huang *et al.*, 2014) was performed.

The identified articles were screened for relevance following a set of inclusion and exclusion criteria. To be considered relevant, the original research article (no literature searches or reviews were included) had to be written in English language and peer-reviewed. No restrictions regarding time of publication were imposed. Outcome measures of interest included vector transmission efficiency (infection, dissemination, and transmission rates), host preference of vectors, and susceptibility of infection (minimum infection rates, maximum likelihood estimation for vectors, and proportion of JEV infection for both vectors and host species). Relevance screening was performed by two reviewers working independently and conflicts were resolved by consensus or by consulting a third reviewer whenever consensus could not be reached.

Data pertaining to the outcome measures previously identified were then retrieved to an Excel® (Microsoft Corp., Redmond WA, 2013) spreadsheet, using a pre-designed template. Information on internal and external validity of the articles (assessment of the risk of bias) was evaluated by two reviewers working independently in a set of 10 articles, with all remaining articles being assessed by one reviewer. Assessment of the risk of bias was based on a set of criteria related to the study question, study population, inclusion and exclusion criteria, study period, study area, exposures, outcomes, and bias, for observational studies; and study question, study population, intervention, experimental conditions, experimental setting, randomization, blinding, and outcomes, for experimental studies.

We followed the protocols described by Sargeant and O'Connor (2014a; 2014b), and O'Connor *et al.* (2014a; 2014b) for performing systematic reviews in veterinary medicine,

and the Cochrane Review Handbook guidelines (Higgins and Green, 2011) for the risk of bias assessment.

Information regarding all steps of the SR, summary of search results, inclusion and exclusion criteria, outcomes, and identification of key domains for risk of bias assessment in observational and experimental studies) are available elsewhere (Oliveira *et al.*, 2017, publication under review).

Data analysis

Meta-analysis

To assess vector and host competence for JEV, we performed meta-analyses for three outcomes whose data we gathered from observational studies: proportion of JEV infection in vectors, proportion of JEV infection in vertebrate hosts, and minimum infection rate (MIR) in vectors. We did not carry out a meta-analysis for maximum likelihood estimation (MLE), though it was an outcome included in the systematic review, as the data pertained to very few articles (n = 6).

The definition of the outcomes of interest is available in Table 12. Proportion of JEV infection was computed as the number of positive units (mosquito pools or vertebrate hosts) divided by the total number of sampled units. Minimum infection rate was defined as the ratio of the number of positive mosquito pools to the total number of mosquitoes in the sample, assuming that only one infected individual is present in a positive pool (Bustamante and Lord, 2010).

Articles reporting only the total percentage of infection (or MIR), without specifying the numerator or denominator, were not considered in this meta-analysis. Similarly, articles reporting only the number of positive samples or the total number of samples were not considered.

For the meta-analysis of the proportion of JEV infection in vectors, we only included articles reporting mosquito species with more than 1% infection and more than 1,000 individual mosquitoes. We did include, however, articles that did not specify the number of mosquitoes per pool, as we could not tell apart the ones including more than 1,000 mosquitoes from those which did not. For the purposes of this study, we assumed these pools contained at least 1,000 individual mosquitoes. For the outcomes proportion of JEV infection in vertebrate hosts and MIR in vectors, all observations pertaining to all host and mosquito species reported were included.

Proportions and MIR reported were first logit-transformed and standard errors (S.E.) of the logit of the proportion or MIR were then computed, according to the following formulae (Sanchez *et al.*, 2007):

$$\text{logit proportion} = \ln\left(\frac{p}{1-p}\right) \qquad \text{S.E.} = \sqrt{\frac{1}{n \times p \times (1-p)}}$$

where p is the proportion of JEV infection or MIR and n is the sample size (i.e., total number of sampled units – mosquito pools, vertebrate hosts, or individual mosquitoes).
For interpretation, the pooled logit estimates and their 95% confidence intervals were back-transformed (Lambert *et al.*, 2015), as follows:

$$p = \frac{e^{logit}}{e^{logit} + 1}$$

We assumed that substantial heterogeneity existed among the studies, hence, we decided a priori to fit a random-effects meta-analysis using the method of DerSimonian and Laird (1986) to estimate the variance between studies, using a restricted maximum likelihood (REML) algorithm. Moreover, subgroup meta-analyses by species (mosquito species or vertebrate host) were performed by running independent models for the three different outcomes using the *metan* command in Stata-SE 12.0 (StataCorp., College Station TX, USA).

Meta-regression

Meta-regression models were fitted to identify sources of heterogeneity among studies and to assess the association between different predictors and the outcomes of interest: proportion of JEV infection in vectors, proportion of JEV infection in vertebrate hosts and MIR (defined above).
Random effects meta-regression models using the restricted maximum likelihood method (*metareg*, Stata-SE 12.0 (StataCorp., College Station TX, USA)) were performed following the formula:

$$\text{logit proportion}_j = \beta_0 + \beta X_j + \mu_j + \varepsilon_j$$

where β_0 is the intercept, βX_j is the coefficient for the jth predictor, μ_j is the effect of study j, and ε_j is the error term, i.e., the differences between studies due to sampling variation. Meta-regression models were performed using logit transformed outcomes and within-study standard errors.

We quantified heterogeneity using the I^2 value, which represents the proportion of total variability in pooled estimates that can be attributed to heterogeneity (O'Connor *et al.* 2014b), and followed the recommendations for interpretation given by O'Connor *et al.* (2014b): I^2 values of 0 – 40%: unimportant heterogeneity; 30 – 60%: moderate heterogeneity; 50 – 90%: substantial heterogeneity; and 75 – 100%: considerable heterogeneity.

To evaluate predictors that may have contributed to the variation in results across studies, we fitted univariable meta-regression models (testing one predictor at a time) followed by a multivariable model (testing multiple predictors simultaneously). Univariable analyses between predictors of interest (i.e., mosquito or vertebrate host species) and outcomes were performed and their significance assessed using a partial *F*-test: *P*-values < 0.1 were deemed significant and used to determine the inclusion of the predictors in the multivariable main effects model.

Predictors of interest for the outcome pertaining to the proportion of JEV infection in vectors included mosquito species, diagnostic method, country, capture method, season, and number of mosquitoes per pool. For the outcome related to the proportion of JEV infection in hosts, predictors included host species, region, season, age category, and diagnostic method. Predictors for the MIR outcome included mosquito species, diagnostic method, capture method, season, and country. A detailed description of each predictor is available in the next section and in Table 13.

Confounding was assessed by including each predictor, considered as a priori confounder based on causal diagrams, in the model at a time (bivariable analysis) and checking for changes in the coefficients, both in magnitude (>30%) and direction, and changes in *P*-values of the main predictors of interest.

If there was evidence of confounding, the confounder was kept in the model. If there was no evidence of confounding and the predictor was no longer significant (*P*-value > 0.1), it was removed from the final model.

Whenever there were concerns of overfitting the model, which can affect the precision of the parameter estimates and test statistics, we present the results from univariable analyses. The dataset had to contain a minimum of $10(k+1)$ observations, where k is the number of predictors in the model, in order to adequately fit the model, following Hosmer and Lemeshow's (2000) recommendations (Dohoo *et al.*, 2009).

For the outcome proportion of JEV infection in vectors, a second model was performed including only the mosquito species represented in more than 10 articles. The same

procedures, regarding univariable and multivariable analyses and assessment of confounding, were followed.

Predictors and outcomes

Table 13 provides a detailed description of the predictors included in the meta-regression analyses, which were selected for inclusion based on biological importance and completeness of observations. Mosquito species included different genera and/or species. The variable pertaining to vertebrate hosts was categorized as follows: pigs, birds, sylvatic mammals, cattle, sheep and goats, cats and dogs, chickens, ducks, rabbits, herons, horses and donkeys, wild pigs, bats, rats, reptiles, and amphibians. Season was categorized into trimesters, so that trimester 1 included the months of December to February, trimester 2 included the months of March to May, trimester 3 June to August, and trimester 4 September to November. Two other categories were created, one referring to studies performed across the year (all year round), and the other when more than one trimester was recorded (more than one trimester). The variable corresponding to diagnostic methods used for diagnosis of JEV differed between vectors and vertebrate hosts. For vectors, diagnostic methods were classified into: a) virus isolation methods (using cell culture techniques or insect bioassays and virus identification by serotype identification with antibodies, such as indirect immunofluorescence assay (IFA)), b) antigen-capture enzyme assays (detection of antigens by antigen-capture enzyme-linked immunosorbent assay (ELISA) or enzyme immunoassays (EIA) (alone or in combination with virus isolation)), and c) PCR (PCR or RT-PCR alone or in combination with antigen-capture enzyme assays, virus isolation, or both). For vertebrate hosts, diagnostic methods were categorized as: a) ELISA or immunochromatography (detection of antibodies by ELISA or immunochromatography only, or in combination with other methods, such as hemagglutination inhibition tests (HAI), virus isolation, and neutralization tests), b) hemagglutination inhibition tests (HAI) (HAI only, or in combination with virus isolation and neutralization tests), and c) neutralization tests (including plaque reduction neutralization test (PRNT), only, or in combination with virus isolation, and virus isolation only).
Mosquito capture methods were classified as: a) manual passive (aspirations), b) manual active (use of sweep nets or drop nets), c) mechanical visual (use of visual attractants like UV (black light) or white light), d) mechanical olfactory (use of olfactory attractants like CO_2 and other lures, such as octanol), e) mechanical visual and olfactory (use of both visual and

olfactory attractants), and f) manual and mechanical (any combination of manual and mechanical capture methods).

The variable pertaining to country of origin differed according to the outcome of interest and included one or more countries based on number of observations and geographical proximity. For outcomes related to vectors, categories for country included: a) Australasia (including Australia, Papua New Guinea, Indonesia, and Saipan (Mariana Islands)), b) India (including India, Sri Lanka, and Bangladesh), c) China and Taiwan, Japan and South Korea, and d) Thailand (Thailand, Malaysia, and Vietnam). The rationale used for determining these categories was geographical proximity of the countries reported in the articles and number of observations each contained. For outcomes related to vertebrate hosts, we considered two categories for region/country of origin: a) North (including China, Japan, and South Korea) and b) South (including Nepal, Taiwan, India, South Korea, USA, Japan, China, Thailand, Sri Lanka, Myanmar, Vietnam, Australia, Singapore, Guam (US), and Saipan (US)). The division of countries into North and South was based on the climate map proposed by Schuh *et al.* (2013).

Age categories of vertebrate hosts consisted of young, adult, and both. Young cattle were defined as animals aged up to 24 months, pigs up to seven months old, and sheep and goats up to 14 months old (Akers and Denbow, 2008). All remaining host species categories were reported as young or adults, as the data extracted in the systematic review did not include age specification.

When fitting meta-regression models, referent categories of predictors were selected, according to biological plausibility or number of observations (Tables 17, 18, and 19).

Results

Systematic review of the literature

From 1,855 articles initially identified, 171 were selected as relevant and subjected to data extraction and risk of bias assessment. Fifty nine percent (n = 101) of the articles were observational studies, 37% (n = 63) were experimental studies, and 4% (n = 7) had an experimental and an observational component. About 60% of the articles reported vector competence, contrasting with host competence (29%) and more than one category (11%). Regarding the risk of bias assessment, all observational studies had a low risk of bias, defined as plausible bias that is unlikely to seriously alter the results, and all experimental studies had a high risk of bias, defined as plausible bias that seriously weakens confidence in the results (Higgins and Green, 2011).

Sixty-seven observational studies were considered for the meta-analysis models, 18 reporting proportion of JEV infection in vectors with more than 1% infection and more than 1,000 individual mosquitoes; 33 reporting proportion of JEV infection in hosts; and 16 reporting MIR. The remaining 104 articles pertained to other outcome measures that are out of the scope of this manuscript.

Meta-analyses

Given the difference in magnitude of the outcomes of interest across vectors, a subgroup analysis by mosquito species was performed. Summary effect measures (pooled estimates) and their 95% confidence intervals, both the logit estimates and the back-transformed proportions, are presented by mosquito species in Tables 14 to 16. Weights, by mosquito species, are computed using a variation of the inverse-variance approach (DerSimonian and Laird method), and are presented as percentage of the overall total.

Subgroup analysis showed large differences (range = 0.10 in *Aedes butleri* to 0.98 in *Culex pipiens fatigans*) between the subgroup overall estimates of the proportion of JEV infection. The lowest proportion of infection was 0.10, meaning that 10% of the total number of *Aedes butleri* pools tested in the 18 articles included in this meta-analysis were infected with JEV. On the other hand, 98% of the *Culex pipiens fatigans* pools were reported to be JEV positive across studies. Although pooled estimates of the proportion of JEV infection in vectors showed some variability, this variability was considered unimportant for mosquito species *Anopheles subpictus* and *Ochleratus normanens* (I^2 = 36.5% and I^2 = 37.0%, respectively), and moderate for *Culex tritaeniorhynchus* and *Culex palpalis* (I^2 = 58.2% and I^2 = 52.8%,

respectively). There was evidence of considerable heterogeneity (I^2 > 85%) for estimates of the proportion of JEV infection in all remaining mosquito species (Table 14).

Subgroup meta-analysis of studies reporting the proportion of JEV infection grouped by vertebrate hosts also ranged greatly, with horses and donkeys showing the highest proportion of JEV infection (0.65) and bats showing the lowest (0.04). Overall, the proportion of JEV infection across all vertebrate host species was 35%. Results of pooled estimates are listed in Table 15.

Furthermore, in these models the variation in the pooled estimates was substantial to considerable: in pigs, birds, and sylvatic mammals, 97% of the variability in the effect size was due to heterogeneity, while in cattle and wild pigs it was 92%. Point estimates in ducks and herons also had considerable heterogeneity (I^2 = 93.8% and 90.7%, respectively) and chickens and cats and dogs had substantial heterogeneity (I^2 = 60.0% and 76.2%).

Summary effect measures of reported minimum infection rates (MIR) are presented in Table 16 and ranged from 0.14 in *Mansonia uniformis* to 0.72 in *Anopheles subpictus*. Pooled estimates of MIR showed considerable heterogeneity across studies in all mosquito species, with I^2 varying from 80.7% in *Anopheles subpictus* to 100% in the *Culex sitiens* subgroup.

It is important to emphasize that because of the high heterogeneity in some of the point estimates obtained from meta-analyses models, pooled estimates were provided for reference only.

Meta-regression

Univariable and multivariable meta-regression models

Results of the univariable meta-regression models of the study results on predictors that can further explain variation in effects between studies for the proportion of JEV infection in vectors are presented in Table 17. Predictor variables including mosquito species, diagnostic method, country, and capture method were significant in the univariable screen (*P*-value < 0.1). Predictors pertaining to season and mosquitoes/pool were not significantly associated (*P*-value > 0.1) with the outcome.

When compared to *Culex tritaeniorhynchus*, a higher proportion of JEV infection was reported in the following species: *Coquillettidia crassipes, Culex annulirostris, Culex annulus*, *Culex bitaeniorhynchus*, *Culex fuscocephala*, *Culex palpalis,* and *Culex pipiens fatigans* (Table 17).

Proportion of JEV infection among mosquitoes was lower when either PCR or antigen-capture enzyme assays were used for diagnosis compared to virus isolation.

The proportion of JEV infection in vectors reported in articles from China and Taiwan was higher than from the Australasia region, whereas other countries (India, Japan and South Korea, and Thailand) reported lower proportion of JEV infection than Australasia.

Articles reporting the use of the manual active method of mosquito capture revealed greater proportion of JEV infection in vectors compared to articles reporting the use of the mechanical visual and olfactory method. The remaining capture methods (manual and mechanical, manual passive, mechanical olfactory, and mechanical visual) had lower reported proportions of JEV infection (Table 17).

Mosquito species, capture method, and country were significant in the univariable screen and thus, were included in a multivariable model. In addition, there was evidence that capture method acted as a confounder of the association between mosquito species, our main predictor of interest, and the outcome. The final model for the proportion of JEV infection in vectors however, consisted of 57 observations (thus $<10(k + 1)$), which prevented us from fitting a multivariable model. Therefore, only the results from the univariable analysis meta-regression are provided (Table 17).

A univariable meta-regression screen was performed to determine associations between each of the predictors of interest (host species, region, season, age category, and diagnostic method) and the proportion of JEV infection in vertebrate hosts (data not shown). Vertebrate host species, region, and season were significantly associated (P-value < 0.1) with the outcome.

The proportion of JEV infection in wild pigs, horses and donkeys, cats and dogs, and cattle was greater compared to domestic pigs. Conversely, all other species (bats, birds, chickens, ducks, herons, reptiles and amphibians, sheep and goats, and sylvatic mammals) had lower proportion of JEV infection than pigs. Proportion of infection in vertebrate hosts was greater in the Southern region compared to the Northern region, and greater in all season categories (all year round, more than one trimester, and trimesters 1, 2, and 4) compared to the third trimester (June to August).

The multivariable meta-regression model of proportion of JEV infection in vertebrate hosts is available in Table 18. Host species and region were significantly associated (P-value <0.1) with the outcome and thus considered in the multivariable meta-regression model. Proportion of JEV infection in wild pigs, horses and donkeys, and cats and dogs was greater compared to

domestic pigs. Bats, birds, cattle, chickens, ducks, herons, reptiles and amphibians, sheep and goats, and sylvatic mammals had lower proportion of JEV infection than pigs. Moreover, the proportion of infection in vertebrate hosts was greater in the Southern region compared to the North (Table 18).

Results of the univariable meta-regression models of the proportion of MIR in vectors are presented in Table 19. Diagnostic method and capture method were significantly associated (*P*-value < 0.1) with the MIR outcome. Minimum infection rates in mosquitoes were greater in articles reporting the use of PCR and lower in those reporting the use of virus isolation compared to the articles reporting the use of antigen-capture enzyme assays as the method of diagnosis. Lastly, MIR in mosquitoes were greater when the method of capture reported was a combination of manual and mechanical, compared to manual passive, and lower when the method used was either mechanical visual or mechanical olfactory (Table 19). A multivariable meta-regression model of MIR could not be built due to the low number of observations (n = 46), hence, the results of univariable models are presented (Table 19).

Discussion

This was the first study that has been performed to quantitatively summarize vector and host competence outcomes pertaining to the proportion of JEV infection in vectors and vertebrate hosts, as well as to evaluate sources of heterogeneity, using a meta-analysis approach. Furthermore, we explored study characteristics thought to influence the effect size as sources of heterogeneity using meta-regression models.

Although pooled estimates did not appropriately summarize the proportion of JEV infection in most mosquito species and in all vertebrate host species assessed, as evidenced by the presence of substantial heterogeneity (O'Connor *et al.,* 2014), results of pooled estimates are mainly presented (Tables 14, 15, and 16) for reporting purposes. The statistical assessment of heterogeneity reflects artifactual and real sources of variability, with the former being explained by differences in study design issues as well as other differences across studies (Dooho *et al.*, 2009). Similarly, it is important to note there is inheritably high clinical heterogeneity in animal studies, and specifically in entomological studies, where real differences in response between populations are expected due to the diversity in biological, ecological, and geographical factors, among others, arising from the study of multiple and diverse species. Regardless of the source, evaluation and quantification of causes of heterogeneity allows us to better interpret these pooled mean estimates and their range.

The highest proportions of JEV infection in vectors were reported in *Culex pipiens fatigans* (98%), *Culex annulus* (79%), *Culex fuscocephala* (77%), *Culex palpalis* (75%), and *Culex annulirostris* (74%), which aligns with our current knowledge of the *Culex* genus being reported as important JEV vectors (Misra and Kalita, 2010). Despite overall high heterogeneity among vector species for this outcome, *Anopheles subpictus* and *Ochleratus normanens*, and *Culex tritaeniorhynchus* and *Culex palpalis* presented unimportant and moderate heterogeneity, respectively, allowing us to accurately summarize and report those pooled estimates. For instance, proportion of JEV infection in *Culex tritaeniorhynchus* (26%) could be used in a risk assessment model to evaluate the risk of introduction of JEV via infected *Culex tritaeniorhynchus* in a JEV-free region. Other factors contributing to the heterogeneity observed, besides mosquito species, included the type of diagnostic method used to quantify mosquito infection, country where data were collected, and mosquito capture method used. Surprisingly, articles reporting data on *Culex tritaeniorhynchus*, which is considered the most significant JEV vector in Southeastern Asia (Solomon, 2000; Mackenzie *et al.*, 2004; Weaver and Barrett, 2004; van den Hurk *et al.*, 2009; Le Flohic *et al.*, 2013), did

not show the highest estimates of proportion of JEV infection. However, it is important to highlight that other studies have pointed to the fact that infection in mosquitoes is not always a direct indicator of risk, mainly because vector abundance, density, age, and climate play a major role on arbovirus transmission (Bustamante and Lord, 2010). In any case, *Culex tritaeniorhynchus* presented moderate heterogeneity among studies, contrasting with the considerable heterogeneity reported in most mosquito species. Lower heterogeneity could nevertheless be related to the fact that *Culex tritaeniorhynchus* was represented in more articles (n = 10) than any other mosquito species, thus increasing the precision of the estimate.

The method of mosquito capture was also an important source of heterogeneity, with the manual active method, which includes the use of sweep or drop nets to catch mosquitoes, being associated with a higher proportion of JEV infection in vectors than the mechanical visual and olfactory method, which use attractants to aid in mosquito trapping. Mosquito capture method as a source of heterogeneity is consistent with previous research, as Lord *et al.* (2015) suggest that estimation of the parameters involved in vector competence may differ due to ecological heterogeneity and may be affected by method bias, which translates into a mosquito capture method favoring one species over another (Lord *et al.*, 2015). An example of method bias is given by Kilpatrick *et al.* (2005) when referring to the underrepresentation of some mosquito species, such as *Ochleratus trivittatus* when using light traps (mechanical visual method) and *Culex pipiens* compared to *Aedes vexans* when using CO_2-baited light traps (mechanical visual and olfactory method). Moreover, Lord *et al.* (2016) also proposed that sampling design of JEV studies tend to be based on capturing mosquitoes from around cattle sheds at dusk, which influence the observed dominance of *Culex tritaeniorhynchus* as the primary JEV vector reported in the literature. Hence, different capture methods may enhance the collection of mosquito species with different competence for JEV (manual active method may favor the collection of species with a higher proportion of JEV infection), thus contributing to the heterogeneity reported.

Regarding pooled estimates of proportion of JEV infection in vertebrate hosts, articles report horses and donkeys (65%), cats and dogs (64%), and wild pigs (53%) among the host species with the highest proportions of infection, and not domestic pigs, as expected due to their role as main JEV reservoir hosts. Nevertheless, the species reported, excluding wild pigs, are dead-end hosts and thus do not play a relevant role on the transmission dynamics of JE and JEV. Wild pigs, on the other hand, are amplifying hosts that do contribute to JEV

transmission. According to Nett *et al.* (2009), the northern and central coast of California have a significant wild pig population, which could contribute to transmission and establishment of JEV in the United States, should it be introduced in the country. The same would apply to any region potentially at risk that has a considerable population of wild pigs, even if not having an intense pig production or not having been traditionally associated with backyard pig raising.

When exploring potential sources of heterogeneity of point estimates pertaining to the proportion of JEV in hosts, countries were divided into two main geographical regions (North and South) to reduce the number of categories being analyzed, as opposed to considering each country or group of countries as a unique category, similar to what was done for the outcome pertaining to the proportion of JEV in vectors, in which we grouped countries according to geographical proximity. Schuh *et al.* (2013) demonstrated that there is an association between JEV genotype and climate, further dividing the countries where JEV is present into a Northern and a Southern region. The geographical distribution suggested by Schuh *et al.* (2013) was thus followed in this study. Proportion of infection in vertebrate hosts was greater in the South compared to the North, which may be related to the fact that Southern countries have an endemic pattern of JEV transmission, as opposed to the epidemic pattern found in the temperate regions of Northern Asia. Thus, because JEV transmission is present all year round in the Southern countries of Asia, there are increased opportunities for host infection, which may lead to the higher proportion of infection reported in vertebrate hosts.

In addition to region, host species also represented an important source of heterogeneity among studies for the outcome proportion of JEV infection in vertebrate hosts, based on a multivariable meta-regression model.

Though considered the main reservoir host for JEV, pigs were not among the vertebrate host species with the highest proportion of JEV infection. Wild pigs, horses and donkeys, herons, and cats and dogs had higher proportion of JEV infection compared to domestic pigs. This may be due to an intensification of industrial pig farming across Asia (Erlanger *et al.,* 2009), which led to a decrease in backyard pig farming, coupled with an increase in biosecurity measures. This is a controversial hypothesis, as previous literature suggests that the industrialization of pig farming did, in fact, enhance the risk of JEV transmission (Le Flohic *et al.,* 2013). However, other studies support that JEV transmission is possible without the intervention of pigs (Weaver and Barrett, 2004) and that JE also occurs in regions of

Bangladesh and India, where pig farming is low compared to other livestock, mainly due to differences in religious practices, as Muslims usually do not eat pork (Lord *et al.*, 2015). Moreover, van den Hurk *et al.* (2008), determined that pig relocation did not decrease the risk for JEV transmission to humans in northern Australia, further dismissing the importance of pigs as the main JEV amplifying host in specific regions.

Although the highest proportion of JEV infection in vectors were reported in species of the *Culex* genus, the highest MIR, however, was not reported in the same mosquito species (70% in *Culex sitiens* subgroup and 72% in *Anopheles subpictus*). Furthermore, MIR pooled estimates were only available for one mosquito species for which high proportion of JEV infection had been reported (*Culex fuscocephala*), and the values were not comparable (77% for the proportion of JEV infection outcome *versus* 19% for the MIR outcome). Minimum infection rate (MIR) is one of the methods, along with maximum likelihood estimation (MLE), available to estimate the proportion of infected mosquitoes from pooled samples. Maximum likelihood estimation is defined by Bustamante and Lord (2010) as the proportion (proportion being a parameter for a binomial distribution) of infected mosquitoes that maximizes the likelihood of *n* pools to be infected, as opposed to MIR, which is the ratio of positive mosquito pools to the total number of pools in the sample. While MIR assumes that there is only one infected individual present in a positive pool, MLE uses an algorithm to consider variations in pool size. In other words, MIR represents the proportion of mosquitoes carrying a particular virus, and in comparison with the MLE method, estimates the lower bound of the infection rate (Gu *et al.*, 2003). Potential disparities between mosquito species deemed to have higher proportion of JEV infection but lower MIR can be explained by the true infection rate, number of pool tested and pool sizes. Disparities in the sample size (number of articles included) of each meta-analysis could also play a role in the differences observed. When infection in the mosquito populations are at high levels, during periods of high transmission, or when pool sizes are large, using MIR underestimates mosquito infections (Gu *et al.*, 2003; Bustamante and Lord, 2010). Therefore, MLE data would be important to more accurately assess infection in mosquito populations.

However, although MLE estimation is considered more robust and accurate than MIR, MIR has been more widely adopted for reporting infection rate (n = 16) than MLE. In fact, although gathered in the data extraction step of our systematic review, MLE results were not subjected to a meta-analysis because data resulted from a limited number of articles (n = 6) and the analysis could not be carried out. Because of the importance of estimating infection

rates of mosquito-borne diseases in disease transmission and in surveillance programs, the body of evidence will benefit from studies reporting infection rates using the MLE method. Sources of heterogeneity between studies for MIR point estimates included the diagnostic method used and the method of mosquito capture. The fact that other predictors tested were not deemed significant to explain heterogeneity may be due to the small number of studies (*n* = 16) included in the meta-regression analysis for this specific outcome.

The approach used in this study allowed us to obtain estimates of variability of proportion of JEV infection in vectors and vertebrate hosts among the studies from which data were retrieved. Pooled estimates of mosquito species presenting unimportant (*Anopheles subpictus* and *Ochleratus normanens*) or moderate (*Culex palpalis*) heterogeneity could be due to a smaller number of articles reporting JEV infection for those species. As mentioned above, however, *Culex tritaeniorhynchus*, was the most represented species across studies, making its estimates useful for using as input parameters in risk assessment models assessing the potential introduction of JEV into currently virus-free regions, including the United States. More studies addressing vector competence in underrepresented mosquito species would therefore improve the precision of estimates, granting more accurate data to be incorporated in such predictive models.

Furthermore, our approach to explore heterogeneity is relevant to understand the sources of variability associated with the predictors and outcomes of interest. Our findings provide useful guidelines when interpreting vector and host infection proportions or prevalence, especially when comparing results from studies that use different study designs. We concluded that mosquito and vertebrate host species, diagnostic method, mosquito capture method, and country were the predictors explaining most of the heterogeneity among studies. More specifically, this study led to a better understanding of the influence of certain predictors, such as mosquito capture method or species, in the interpretation of the outcomes. Regarding mosquito species, proportion of JEV infection should be cautiously interpreted, as it does not directly translate into higher transmission risk, as suggested by previous literature (Bustamante and Lord, 2010). Again, JEV transmission results from a complex interplay of factors, such as environmental and ecological characteristics, as suggested by the high heterogeneity found in this meta-analysis, which should be taken into consideration when interpreting infection proportions for each species.

Another important predictor to consider is mosquito capture method, as different methods may attract different mosquito species, thus biasing infection data towards an over or

underrepresentation of certain mosquito species (Lord *et al.*, 2015). Mosquitoes may also belong to different stages of development, as oviposition traps collect older mosquitoes that have already blood fed and laid eggs, while light traps or manual aspiration methods usually collect host seeking mosquitoes. This difference in developmental stages of mosquitoes may also impact the results of mosquito collection.

Lastly, although meta-regression models allowed us to investigate whether specific predictors explained any of the heterogeneity of effects between studies, it is important to note that a post hoc selection of characteristics or predictors that might explain heterogeneity can lead to false positive conclusions (Thompson and Higgins, 2002). Although no specific protocol was in place to identify appropriate covariates, we believe there was a strong rationale for including diagnostic methodologies and study descriptors as covariates of interest. Granting there may be additional confounders that were not accounted for, the small sample size (number of articles) of most of our models limited our ability to fit multivariable models.

A limitation of this study is related to the large variability reported in the outcome measures of interest that translated into the heterogeneity found across articles and demonstrated in the meta-analysis models. These are related to differences in study methodology, data collection, data reporting, and results presentation, as well as geographical distribution and environmental factors inherent to those regions. When initially posing the research question for the systematic review, we aimed at investigating vector competence in North America. Because only a few articles could be retrieved from the databases and journal websites, the research question was expanded to include worldwide estimates, thus leading to high levels of variation described in this study. Similarly, while not imposing any restrictions on study design specifications (including year of publication), neither on the predictors analyzed (mosquito capture method, species, and diagnostic method), allowed us to retrieve large amounts of data, it also led to the heterogeneity observed. Variability regarding diagnostic methods in particular were related to the large span of years comprised in all articles retrieved (from 1946 to 2016), which reflects on the technical and scientific improvements that occurred over the 70 years covered in the literature search. Moreover, the grouping of predictors pertaining to study characteristics into meaningful and representative categories was challenging due to the large diversity in methodology observed across studies.

Despite the challenges posed by the large variability among studies, this meta-analysis provides a quantitative summary of results of multiple studies evaluating JEV infection in mosquitoes and hosts. This quantitative approach to vector and vertebrate host competence

expands our understanding of the relative importance of vectors and vertebrate hosts on JEV introduction and transmission, addressing an important knowledge gap identified in the beginning of our study, and thus providing useful data to be used in risk assessment models. These models have application in decision-making processes related to the implementation of strategies aiming at preventing the introduction of emerging vector-borne zoonoses in susceptible regions, such as the United States (Lord *et al.*, 2015).

Future studies should focus on vector competence of underrepresented mosquito species and countries where data are not available, particularly in regions where JE cases have not been reported but that are flagged as potentially at risk. Lastly, though not easily achievable in observational studies, a higher degree of standardization regarding mosquito trapping and JEV diagnostic methods should be aimed at, as it would help obtaining a more accurate quantification of outcomes, such as the ones assessed in the current study.

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Tables

Table 12. Outcome measures quantified in the meta-analyses.

	Vector competence	Host competence
Susceptibility to infection	Proportion of JEV infection[1]	Proportion of JEV infection[2]
	Minimum infection rate[3]	-
	Maximum likelihood estimation[4]	-

[1] Proportion of JEV infection is the sum of positive mosquito pools divided by the total number of pools tested in observational studies.
[2] Proportion of positive vertebrate hosts equals the sum of positive samples divided by the sum of samples tested.
[3] Minimum infection rate (MIR) is defined as the ratio of the number of positive mosquito pools to the total number of mosquitoes in the sample, assuming that only one infected individual is present in a positive pool (Bustamante and Lord, 2010).
[4] Maximum likelihood estimation (MLE) represents the proportion of infected mosquitoes that maximizes the likelihood of the number of pools of a specific size to be virus positive, where the proportion is the parameter of a binomial distribution (Bustamante and Lord, 2010).

Table 13. Predictors pertaining to study characteristics included in the meta-analyses of proportion of JEV infection in vectors and vertebrate hosts, and minimum infection rates (MIR).

Variable	Description	Categories
Species	Mosquito/vertebrate host species or genera.	Vectors: several species (n = 24); Hosts: Pigs, birds, sylvatic mammals, cattle, sheep and goats, cats and dogs, chickens, ducks, rabbits, herons, horses and donkeys, wild pigs, bats, rats, reptiles and amphibians
Season	Trimester of the year during which the study was conducted.	Trimester 1 (December-February), trimester 2 (March-May), trimester 3 (June-August), trimester 4 (September-November), all year-round
Diagnostic method	Diagnostic method used for detecting JEV.	Vectors: virus isolation, antigen-capture enzyme assays, PCR [1]; Hosts: ELISA or immunochromatography, hemagglutination inhibition tests, neutralization tests [2]
Capture method	Capture method used for capturing mosquitoes.	Manual passive, manual active, mechanical visual, mechanical olfactory [3]
Mosquitoes/pool	Number of mosquitoes included in each pool.	-
Country category	Country category where the study was conducted.	Vectors: Australasia, India, China and Taiwan, Japan and South Korea, Thailand [4]; Hosts: North and South [5]
Age	Age of vertebrate host.	Young and adult

[1] Virus isolation may use cell culture techniques or insect bioassays and virus identification by serotype identification with antibodies, such as indirect immunofluorescence assay (IFA).
Antigen-capture enzyme assays include the detection of antigens by antigen-capture enzyme-linked immunosorbent assay (ELISA) or enzyme immunoassays (EIA), alone or in combination with virus isolation.
PCR or RT-PCR was used alone or in combination with antigen-capture enzyme assays, virus isolation, or both.
[2] ELISA or immunochromatography includes the detection of antibodies by ELISA or immunochromatography only, or in combination with other methods, such as hemagglutination inhibition tests (HAI), virus isolation, and neutralization tests.
Hemagglutination inhibition tests (HAI) may have been used alone or in combination with virus isolation and neutralization tests.
Neutralization tests, including PRNT, may have been used alone or in combination with virus isolation. Virus isolation only is also included in this category.
[3] Manual passive method includes aspirations; manual active uses sweep or drop nets; mechanical visual uses visual attractants, such as UV (black light) or white light; mechanical olfactory uses olfactory attractants, such as octanol.
[4] Australasia includes Australia, Papua New Guinea, Indonesia, and Saipan (Mariana islands); India includes India, Sri Lanka, and Bangladesh; Thailand includes Thailand, Malaysia, and Vietnam.
[5] North includes the following countries: China, Japan, and South Korea.
South includes the following countries: Australia, Guam (US), India, Myanmar, Nepal, Saipan (US), Singapore, Sri Lanka, Taiwan, Thailand, USA, and Vietnam (Schuh *et al.*, 2013).

Table 14. Subgroup meta-analysis[‡] of studies reporting the proportion of JEV infection in vectors grouped by mosquito species. Each effect size* represents pooled estimates (effect size) of the outcome for each mosquito species, and the overall represents the overall pooled estimate across all mosquito species.

Mosquito species	Effect size (logit)	95% CI (logit)	Proportion of JEV infection[†]	95% CI (proportion)	% Weight
Aedes vexans	-1.79	-3.91, 0.33	0.14	0.02, 0.58	1.29
Anopheles minimus	-1.79	-3.91, 0.33	0.14	0.02, 0.58	1.29
Anopheles tessellatus	-1.79	-3.91, 0.33	0.14	0.02, 0.58	1.29
Armigeres subalbatus	-1.85	-3.07, -0.64	0.14	0.04, 0.35	1.78
Culex annulus	1.35	-4.39, 7.10	0.79	0.01, 1.00	3.83
Culex fuscocephala	1.22	-4.41, 6.85	0.77	0.01, 1.00	3.76
Culex tritaeniorhynchus	-1.04	-1.21, -0.88	0.26	0.23, 0.29	28.59
Culex gelidus	-0.04	-3.06, 2.98	0.49	0.04, 0.95	3.37
Anopheles subpictus	-1.46	-1.80, -1.13	0.19	0.14, 0.24	23.67
Aedes butleri	-2.17	-3.21, -1.13	0.10	0.04, 0.24	1.87
Coquilettidia crassipes	0.69	-1.70, 3.08	0.67	0.15, 0.96	1.15
Culex annulirostris	1.05	0.97, 1.13	0.74	0.73, 0.76	2.19
Culex bitaeniorhynchus	0.85	-0.50, 2.20	0.70	0.38, 0.90	1.70
Culex palpalis	1.08	-0.07, 2.24	0.75	0.48, 0.90	3.65
Culex quinquefasciatus	-0.13	-4.20, 3.94	0.47	0.01, 0.98	2.61
Culex sitiens	-0.51	-1.94, 0.92	0.38	0.13, 0.72	1.66
Culex whitmorei	-1.63	-3.16, -0.10	0.16	0.04, 0.47	2.52
Mansonia septempunctata	-0.92	-2.08, 0.24	0.28	0.11, 0.56	1.81
Ochleratus normanensis	-0.82	-1.27, -0.37	0.31	0.22, 0.41	4.17
Verrallina funerea	0.00	-1.96, 1.96	0.50	0.12, 0.88	1.37
Culex pipiens fatigans	4.17	2.19, 6.15	0.98	0.90, 1.00	1.36
Aedes albopictus	0.17	-0.50, 0.84	0.54	0.38, 0.70	2.05
Culex pipiens	-1.39	-3.59, 0.81	0.20	0.03, 0.69	1.25
Culex pipiens quinquefasciatus	-2.04	-3.24, -0.84	0.12	0.04, 0.30	1.79
Overall	**-0.70**	**-1.07, -0.33**	**0.33**	**0.26, 0.42**	**100.00**

[‡] Random-effects meta-analysis using the method of DerSimonian and Laird (1986) to estimate the variance between studies, using a restricted maximum likelihood (REML) algorithm. I^2 range: 36.5% (*P*-value=0.00) (*Anopheles subpictus*) - 98.6% (*P*-value=0.64) (*Culex annulus*)

[*] Computed for the group of studies reporting proportion of JEV in each mosquito species.

[†] $p = (e^{logit} / (e^{logit} + 1))$

CI: confidence interval

Table 15. Subgroup meta-analysis[‡] of studies reporting the proportion of JEV infection in vertebrate hosts grouped by host species. Each effect size* represents pooled estimates (effect size) of the outcome for each host species, and the overall represents the overall pooled estimate across all vertebrate host species.

Vertebrate host species	Effect size (logit)	95% CI (logit)	Proportion of JEV infection[†]	95% CI (proportion)	% Weight
Pigs	-0.36	-0.64, -0.08	0.41	0.35, 0.48	59.11
Birds	-2.05	-3.25, -0.84	0.11	0.04, 0.30	6.12
Sylvatic mammals	-0.95	-1.90, 0.01	0.28	0.13, 0.50	4.37
Cattle	-0.25	-1.17, 0.67	0.44	0.24, 0.66	3.16
Sheep and Goats	-0.77	-1.01, -0.53	0.32	0.27, 0.37	2.91
Cats and dogs	0.58	-0.40, 1.56	0.64	0.40, 0.83	2.38
Chickens	-2.47	-2.94, -2.01	0.08	0.05, 0.12	2.67
Ducks	-0.67	-2.60, 1.26	0.34	0.07, 0.78	2.02
Herons	-0.94	-1.25, -0.63	0.28	0.22, 0.35	13.97
Horses and donkeys	0.62	-0.24, 1.49	0.65	0.44, 0.82	0.95
Wild Pigs	0.12	-2.93, 3.17	0.53	0.05, 0.96	0.96
Bats	-3.26	-4.67, -1.85	0.04	0.01, 0.14	0.45
Reptiles and amphibians	-1.20	-2.12, -0.29	0.23	0.11, 0.43	0.92
Overall	**-0.62**	**-0.83, -0.41**	**0.35**	**0.30, 0.40**	**100.00**

[‡] Random-effects meta-analysis using the method of DerSimonian and Laird (1986) to estimate the variance between studies, using a restricted maximum likelihood (REML) algorithm. I^2 range: 60.00% (*P*-value=0.00) (chickens) - 96.8% (*P*-value=0.64) (pigs)

[*] Computed for the group of studies reporting proportion of JEV in each vertebrate host species.

[†] $p = (e^{logit} / (e^{logit} + 1))$

Table 16. Subgroup meta-analysis[‡] of studies reporting proportion of minimum infection rates (MIR) in vectors grouped by mosquito species. Each effect size* represents pooled estimates (effect size) of the outcome for each mosquito species, and the overall represents the overall pooled estimate across all mosquito species.

Mosquito species	Effect size (logit)	95% CI (logit)	MIR[†]	95% CI (proportion)	% Weight
Anopheles sinensis	-1.32	-1.36, -1.28	0.21	0.20, 0.22	2.44
Culex tritaeniorhynchus	-1.19	-1.70, -0.68	0.23	0.15, 0.34	21.22
Mansonia uniformis	-1.85	-3.34, -0.36	0.14	0.03, 0.41	4.02
Anopheles subpictus	0.93	-0.03, 1.89	0.72	0.49, 0.87	4.38
Culex gelidus	-1.01	-1.80, -0.22	0.27	0.14, 0.44	15.36
Culex fuscocephala	-1.47	-2.53, -0.41	0.19	0.07, 0.40	7.23
Culex vishnui	-0.37	-0.48, -0.26	0.41	0.38, 0.44	4.80
Culex spp.	-0.34	-1.07, 0.38	0.41	0.26, 0.59	18.55
Culex pseudovishnui	-1.32	-1.65, -1.00	0.21	0.16, 0.27	5.42
Culex sitiens subgroup	0.84	-2.67, 4.34	0.70	0.07, 0.99	7.26
Ochlerotatus vigilax	-0.85	-0.93, -0.77	0.30	0.28, 0.32	2.43
Anopheles vagus	-0.53	-1.16, 0.10	0.37	0.24, 0.52	2.16
Aedes spp.	-1.21	-1.43, -0.99	0.23	0.19, 0.27	2.40
Culex whitmorei	-1.21	-1.56, -0.86	0.23	0.17, 0.30	2.34
Overall	**-0.79**	**-1.06, -0.51**	**0.31**	**0.26, 0.37**	**100.00**

[‡] Random-effects meta-analysis using the method of DerSimonian and Laird (1986) to estimate the variance between studies, using a restricted maximum likelihood (REML) algorithm. I^2 range: 80.7% (*P*-value=0.06) (*Anopheles subpictus*) – 100.00% (*P*-value=0.64) (*Culex sitiens subgroup*)

[*] Computed for the group of studies reporting proportion of JEV in each mosquito species.

[†] $p = (e^{logit} / (e^{logit} + 1))$

Table 17. Coefficients, *P*-values, and 95% Confidence Intervals of the association between predictors of interest with the proportion of JEV infection in vectors (from univariable meta-regression models‡) *n* = 18 studies.

Predictor	*n*	Coefficient (logit)	Standard Error (logit)	95% CI (logit)	*P*-value	Overall *P*-value
Mosquito species						0.08
Culex tritaeniorhynchus	10	Reference				
Aedes albopictus	1	1.27	1.25	-1.27, 3.81	0.32	
Aedes butleri	1	-1.07	1.32	-3.75, 1.61	0.42	
Aedes vexans	1	-0.69	1.62	-4.00, 2.61	0.67	
Anopheles minimus	1	-0.69	1.62	-4.00, 2.61	0.67	
Anopheles subpictus	1	-0.25	0.48	-1.23, 0.74	0.61	
Anopheles tessellatus	1	-0.69	1.62	-4.00, 2.61	0.67	
Armigeres subalbatus	1	-0.75	1.36	-3.51, 2.01	0.58	
Coquillettidia crassipes	1	1.79	1.72	-1.72, 5.29	0.31	
Culex annulirostris	1	2.15	1.20	-0.30, 4.59	0.08	
Culex annulus	2	2.29	0.95	0.37, 4.22	0.02	
Culex bitaeniorhynchus	1	1.95	1.39	-0.88, 4.78	0.17	
Culex fuscocephala	2	2.51	0.96	0.56, 4.45	0.01	
Culex gelidus	2	1.11	1.01	-0.96, 3.17	0.28	
Culex palpalis	1	2.09	0.97	0.11, 4.06	0.04	
Culex pipiens	1	-0.29	1.65	-3.65, 3.07	0.86	
Culex pipiens fatigans	1	5.27	1.58	2.06, 8.48	0.00	
Culex pipiens quinquefasciatus	1	-0.94	1.35	-3.69, 1.81	0.49	
Culex quinquefasciatus	2	0.96	1.16	-1.41, 3.32	0.42	
Culex sitiens	1	0.59	1.41	-2.28, 3.46	0.68	
Culex whitmorei	3	-0.51	1.18	-2.92, 1.90	0.67	
Mansonia septempunctata	1	0.18	1.34	-2.55, 2.91	0.90	
Ochleratus normanensis	1	0.16	0.90	-1.68, 2.00	0.86	
Verralina funerea	1	1.10	1.57	-2.10, 4.30	0.49	
Intercept		-1.10	0.32	-1.76, -0.44	0.00	
Diagnostic method						0.01
Virus isolation	9	Reference				
Not reported	2	0.41	0.50	-0.60, 1.43	0.42	
PCR	4	-1.24	0.55	-2.34, -0.15	0.03	
Antigen-capture enzyme assays	3	-0.98	0.48	-1.95, -0.01	0.05	
Intercept		-0.31	0.32	-0.96, 0.33	0.34	
Country						0.01
Australasia	3	Reference				
China and Taiwan	3	0.07	0.55	-1.03, 1.16	0.90	
India	5	-1.34	0.50	-2.35, -0.33	0.01	
Japan and South Korea	5	-1.20	0.53	-2.27, -0.13	0.03	
Thailand	2	-2.20	1.09	-4.40, -0.01	0.05	
Intercept		0.02	0.37	-0.73, 0.77	0.96	
Capture method						<0.01
Mechanical visual and olfactory	4	Reference				

Not reported	2	-0.81	0.99	-2.80, 1.18	0.42
Manual active	1	4.46	0.60	3.25, 5.67	0.00
Manual and mechanical	3	-0.76	0.32	-1.41, -0.11	0.02
Manual passive	4	-1.12	0.33	-1.79, -0.46	0.00
Mechanical olfactory	3	-1.01	0.54	-2.10, 0.08	0.07
Mechanical visual	1	-1.32	1.08	-3.50, 0.86	0.23
Intercept		-0.29	0.23	-0.76, 0.18	0.22

‡ Random effects meta-regression models using the restricted maximum likelihood method (REML).

Table 18. Coefficients, *P*-values, and 95% Confidence Intervals of the association between predictors of interest with the proportion of JEV infection in vertebrate hosts (from a multivariable meta-regression model‡) *n* = 33 studies.

Predictor	*n*	Coefficient (logit)	Standard Error (logit)	95% CI (logit)	*P*-value	Overall *P*-value
Host species						<0.01
Pigs	21	Reference				
Bats	2	-3.78	1.72	-7.17, -0.40	0.03	
Birds	7	-2.49	0.52	-3.51, -1.46	0.00	
Cats and dogs	5	0.07	0.78	-1.47, 1.61	0.93	
Cattle	4	-0.77	0.69	-2.14, 0.59	0.26	
Chickens	9	-2.82	0.73	-4.26, -1.37	0.00	
Ducks	4	-1.18	0.84	-2.84, 0.49	0.16	
Herons	5	-0.33	0.35	-1.01, 0.36	0.35	
Horses and donkeys	4	0.13	1.20	-2.23, 2.50	0.91	
Reptiles and amphibians	2	-0.39	1.21	-2.77, 1.99	0.75	
Sheep and goats	5	-0.91	0.70	-2.30, 0.48	0.20	
Sylvatic mammals	2	-0.10	0.58	-1.24, 1.05	0.87	
Wild pigs	2	1.02	1.19	-1.32, 3.36	0.39	
Region						<0.01
North	16	Reference				
South	17	1.37	4.77	0.80, 1.93	0.00	
Intercept		-0.85	-4.67	-1.20, -0.49	0.00	

‡ Random effects meta-regression models using the restricted maximum likelihood method (REML).

Table 19. Coefficients, *P*-values, and 95% Confidence Intervals of the association between predictors of interest with minimum infection rates (MIR) in vectors (from univariable meta-regression models‡) *n* = 16 studies.

Predictor	*n*	Coefficient (logit)	Standard Error (logit)	95% CI (logit)	*P*-value	Overall *P*-value
Diagnostic method						0.02
Antigen-capture enzyme assays	8	Reference				
PCR	5	0.08	0.78	-1.50, 1.65	0.92	
Virus isolation	3	-1.50	0.53	-2.56, -0.43	0.01	
Intercept		-0.34	0.32	-1.00, 0.31	0.29	
Capture method						0.07
Manual passive	4	Reference				
Not reported	1	-0.07	0.78	-1.64, 1.49	0.93	
Manual and mechanical	3	0.23	0.90	-1.58, 2.05	0.80	
Mechanical olfactory	4	-0.44	0.78	-2.01, 1.13	0.58	
Mechanical visual	2	-1.66	0.68	-3.04, -0.28	0.02	
Intercept		-0.25	0.52	-1.31, 0.81	0.63	

‡ Random effects meta-regression models using the restricted maximum likelihood method (REML).

Chapter 4 - Meta-analyses of Japanese encephalitis virus infection, dissemination, and transmission rates in vectors

Summary

Japanese encephalitis (JE), a mosquito-borne disease with an incidence of 1.8 per 100,000 people in Southeast Asia and the Pacific islands, is considered the most important cause of human encephalitis in that region. Japanese encephalitis is transmitted by mosquitoes infected with the Japanese encephalitis virus (JEV), and its transmission cycle involves pigs and ardeid birds as reservoir hosts. The objective of this study was to summarize and quantify JEV infection, dissemination, and transmission rates in mosquitoes, using a meta-analysis approach using data from experimental studies, gathered by means of a systematic review of the literature.

Random-effects subgroup meta-analysis models by mosquito species were performed to estimate pooled estimates and to calculate the variance between studies for the three outcomes of interest (JEV infection, dissemination, and transmission rates in mosquitoes). To identify sources of heterogeneity among studies and to assess the association between different predictors (mosquito species, virus administration route, incubation period, and diagnostic method) with the outcomes of interest, we fitted meta-regression models.

Mosquito species and administration route represented the main sources of heterogeneity associated with JEV infection rate in vectors. Due to a small number of observations or lack of evidence of statistically significant conditional associations between predictors and the other outcomes (dissemination and transmission rates), no multivariable meta-regression models were fitted.

This study provided summary effect size estimates to be used as reference when assessing transmission efficiency of vectors and explored sources of variability for JEV infection rate in vectors. Because transmission efficiency, as part of vector competence assessment, is an important parameter when studying the relative contribution of vectors to JEV transmission, our findings contribute to further our knowledge, potentially moving us towards more informed and targeted actions to prevent and control JEV in both affected and susceptible regions worldwide.

Keywords: Japanese encephalitis virus, Japanese encephalitis, meta-analysis, vector, competence, experimental

Introduction

Japanese encephalitis virus (JEV) is a *Flavivirus* responsible for approximately 67,900 annual cases of Japanese encephalitis (JE) in Southeastern Asia and the Pacific Rim, and it is considered the most important cause of viral encephalitis worldwide. Most of China, Southeast Asia, and the Indian subcontinent experience JE outbreaks and, overall, the reported incidence is of 1.8 per 100,000 people. Moreover, about 75% of JE cases occur in children up to 14 years old, with clinical disease varying from flu-like to severe neuropsychiatric symptoms (Solomon *et al.*, 2000; Campbell *et al.*, 2011).

JEV is transmitted by mosquitoes, mainly from the *Culex* genus, and its epidemiology is complex and dynamic. There are two main transmission patterns, a wild cycle maintained by birds, especially ardeid birds such as egrets and herons, and a domestic cycle associated with pigs. Humans do not contribute to JEV transmission, as they are incidental hosts who may become infected but are not able to transmit the virus. Viral genetic determinants appear to contribute only partially to the epidemiological pattern of JEV, with environmental, ecological, and immunological factors playing a paramount role on the dynamics of the enzootic cycle of JE and JEV (LeFlohic *et al.*, 2013).

The emergence or reemergence of arboviruses is a global concern, with JEV being among the viruses considered to be a public health threat, due to its changing epidemiology and geographic expansion over the past decades (Gubler, 2002). Furthermore, the wide range of susceptible vector species for JEV and the current scientific evidence pointing to the possibility of JEV introduction into new geographic regions, given the widespread presence of competent mosquito and vertebrate host species, calls for an accurate assessment of the different parameters taking part in the epidemiology of JEV. Among these parameters, vector competence is considered crucial, as flavivirus-mosquito interactions are central to the epidemiology of JEV and its epidemic potential (Nett *et al.*, 2009; van den Hurk *et al.*, 2009; Nemeth *et al.*, 2012; Huang *et al.*, 2014).

Transmission experiments allow the identification of the mechanisms of infection, dissemination, and transmission of JEV in mosquitoes, demonstrating which factors are determinant for the mosquito's ability to acquire, maintain, and transmit the virus, i.e., virus competence (Huang *et al.*, 2014). Reports of such experiments are found in the literature but no comprehensive assessment of vector competence in all different mosquito species tested to date, has been performed.

A systematic review is a methodology used to gather information from the literature, providing a systematic, repeatable, and robust framework for its compilation and evaluation. A further quantitative summary of the data extracted from the systematic review is provided by a meta-analysis, which is a statistical method that combines results from studies with the purpose of estimating a summary effect measure. When data are too heterogeneous to allow for such estimation, a meta-regression may be performed to explore the sources of heterogeneity and thus further our understanding on the research question being studied (Egger *et al.* 2001; Sutton *et al.*, 2001; Sargeant *et al.*, 2006; O'Connor *et al.*, 2014a; Sargeant and O'Connor, 2014a). In order to thoroughly assess infection, dissemination, and transmission rates of mosquitoes, we carried out a systematic review and meta-analysis with the objective of quantitatively assessing vector competence from experimental studies.

Materials and Methods

Systematic review of the literature

The literature search was performed in the English language, with no restrictions as to year of publication, and the last day of search was April 25th, 2016. A combination of different keywords was used when searching the databases and journal websites and is available elsewhere (Oliveira *et al.*, 2017, publication under review). We searched eight electronic databases and journal websites, as follows: Web of Science, Pubmed, Armed Forces Pest Management Board, The American Journal of Tropical Medicine and Hygiene, Journal of Medical Entomology, Journal of the American Mosquito Control Association, Vector Borne and Zoonotic Diseases, and Google Scholar). A hand-search of the reference list of nine JEV reviews considered by the authors as key publications was also performed (Solomon *et al.*, 2000; Mackenzie *et al.*, 2004; Weaver and Barrett, 2004; Erlanger *et al.*, 2009; Nett *et al.*, 2009; van den Hurk *et al.*, 2009; Misra and Kalita, 2010; LeFlohic *et al.*, 2013; Huang *et al.*, 2014).

The articles identified in the literature search were screened for relevance to select the articles from which data were to be extracted and assessed. The relevance screening process was achieved by using a set of inclusion and exclusion criteria that included peer-reviewed articles in the English language, without limitations regarding time, and excluded literature reviews. Two reviewers worked independently in the relevance screening process and conflicts were resolved by consensus or by consulting a third reviewer whenever consensus could not be reached. Outcome measures related to the inclusion criteria were vector transmission efficiency (infection, dissemination, and transmission rates), host preference of vectors, and susceptibility of infection (minimum infection rates, maximum likelihood estimation for vectors, and proportion of JEV infection for both vectors and host species). Data were extracted using a pre-designed template and the risk of bias assessment was then performed to evaluate internal and external validity. When pre-testing our data extraction and risk of bias assessment tools, data were extracted by two reviewers in a set of five relevant articles and assessed for their risk of bias in a set of 10 articles. All remaining articles were then assessed by one reviewer. Criteria for assessing the risk of bias pertained to the study question, study population, inclusion and exclusion criteria, study period, study area, exposures, outcomes, and bias, for observational studies; and study question, study population, intervention, experimental conditions, experimental setting, randomization, blinding, and outcomes, for experimental studies.

Guidelines described by Sargeant and O'Connor (2014a; 2014b) and O'Connor *et al.* (2014a; 2014b) were followed to perform the systematic review, and the Cochrane Review Handbook guidelines (Higgins and Green, 2011) were followed when conducting the risk of bias assessment.

All steps of the systematic review, including a summary of search results, inclusion and exclusion criteria, outcomes, and identification of key domains for risk of bias assessment in observational and experimental studies) are provided elsewhere (Oliveira *et al.*, 2017, publication under review).

Data analysis

Meta-analysis

To quantitatively assess vector competence from experimental studies gathered in the systematic review, three independent meta-analysis models were carried out for outcomes of interest pertaining to vector transmission efficiency, specifically: JEV infection rates in vectors, JEV dissemination rates in vectors, and JEV transmission rates in vectors. Infection rate is defined as the sum of individual mosquitoes that test positive for JEV (or pools of mosquitoes, if applicable) divided by the total number of mosquitoes (or pools) tested. Dissemination rate, as defined by Golnar *et al.* (2015), is the proportion of mosquitoes that contain virus in their legs, irrespective of their infection status, whereas transmission rate is defined as the proportion of mosquitoes with a disseminated infection that transmit the virus upon refeeding (Golnar *et al.*, 2015).

All observations pertaining to all mosquito species were included in the assessment of our outcomes of interest.

Although the outcomes are referred to as rates, it is important to highlight that infection, dissemination, and transmission rates are actually proportions, as a rate is a ratio with the denominator representing the number of subject-time units at risk (in this case mosquito-time units at risk, or mosquito lifespan), and no time component is present in these measures (Dohoo *et al.*, 2009). Nonetheless, because these are the terms more commonly used and recognized among entomologists, we kept their usage, bearing in mind their application within the context.

Infection, dissemination, and transmission rates reported were first logit-transformed and standard errors (S.E.) of the logit of the rates were computed, following the formulae provided by Sanchez *et al.* (2007):

$$\text{logit proportion} = \ln\left(\frac{p}{1-p}\right) \qquad \text{S.E.} = \sqrt{\frac{1}{n \times p \times (1-p)}}$$

where p is the proportion of infection, dissemination, or transmission and n is the sample size (i.e., total number of mosquitoes).

Pooled logit estimates and their 95% confidence intervals were back-transformed for interpretation purposes (Lambert *et al.*, 2015), using the following formula:

$$p = \frac{e^{logit}}{e^{logit} + 1}$$

Because we assumed *a priori* that there was substantial heterogeneity among the studies, we fitted random-effects meta-analysis. We used the method of DerSimonian and Laird (1986) to estimate the variance between studies, using a restricted maximum likelihood (REML) algorithm. Using the *metan* command in Stata-SE 12.0 (StataCorp., College Station TX, USA), we ran independent models for the three different outcomes and performed subgroup meta-analyses by mosquito species.

Meta-regression

Meta-regression models were fitted to determine the association between predictors of interest with infection, dissemination, and transmission rates in mosquitoes, as well as to identify sources of heterogeneity among the studies.

Random effects meta-regression models were performed using the restricted maximum likelihood (REML) method, the logit transformed outcomes, and the within-study standard errors. The *metareg* command in Stata-SE 12.0 (StataCorp., College Station TX, USA) was used according to the formula:

$$\text{logit proportion}_j = \beta_0 + \beta X_j + \mu_j + \varepsilon_j$$

where β_0 is the intercept, βX_j is the coefficient for the jth predictor, μ_j is the effect of study j, and ε_j is the error term (differences between studies due to sampling variation).

For the quantification of heterogeneity, we used *I*-squared (I^2), which depicts the proportion of total variability in point estimates that can be attributed to heterogeneity (O'Connor *et al.*, 2014b). *I*-squared values were interpreted following the recommendations by O'Connor *et al.*

(2014b): I^2 values of 0 – 40%: unimportant heterogeneity; 30 – 60%: moderate heterogeneity; 50 – 90%: substantial heterogeneity; and 75 – 100%: considerable heterogeneity.
Univariable meta-regression models were fitted to assess the contribution of each predictor to the variation reported in the results across all studies. After that, a multivariable model was carried out by testing conditional associations among multiple predictors.
Partial *F*-tests were used to assess the significance of the association between the predictors and outcomes of interest and *P*-values < 0.1 were considered statistically significant, determining the inclusion of the predictors in the multivariable meta-regression models.
Predictors of interest for all three outcomes (JEV infection, dissemination, and transmission rates) included mosquito species, administration route, incubation period (in days), and diagnostic method. Table 20 provides a detailed description of predictors, which are also defined in the next section.
Confounders were considered *a priori* based on causal diagrams and assessment of confounding was performed by carrying out bivariable analysis including each predictor in the model at a time and checking for changes in the coefficients, both in magnitude (>30%) and direction, and changes in *P*-values of our main predictor of interest. If there was evidence of a confounding effect, the confounder was kept in the model.
Because overfitting of the model may affect the precision of the parameter estimates and test statistics, results from univariable analyses are presented when fewer than 10(k+1) observations were available in the dataset, where k is the number of predictors in the model, as recommended by Hosmer and Lemeshow (2000) (cited by Dohoo *et al.*, 2009).

Predictors and outcomes

Predictors of interest were selected for model testing inclusion based on biological importance and completeness of observations, and their definition is given in Table 20.
Our main predictor of interest was mosquito species, which included 50 genera or species (as reported in the articles).
The variable corresponding to administration route was categorized into three categories: oral feeding (on pledgets/membranes, on a host, or both), intrathoracic inoculation, and vertical transmission (achieved by infecting the parent mosquito intrathoracically or by oral feeding).
Diagnostic method was categorized into the following: PCR, which could include real-time RT-PCR, RT-PCR alone or in combination with antigen-capture enzyme assays (e.g., ELISA) or virus isolation; virus isolation (cell culture techniques or insect bioassays); and

virus isolation (with immunofluorescence, hemagglutination inhibition tests (HAI), or neutralization tests), which referred to virus identification by serotype identification with antibodies (indirect immunofluorescence assay – e.g. IFA), hemagglutination inhibition tests, or neutralization tests.

Incubation period was recorded in days and corresponded to the period between experimental infection and testing, which was given as a mean value whenever a range of days was reported.

Referent categories of predictors in the meta-regression models were selected based on biological plausibility or highest frequency of observations (Table 5).

Results

Systematic review of the literature

A total of 171 out of the 1,855 articles initially identified in the literature search were considered relevant and were included in the data extraction and risk of bias assessment of the systematic review.

Fifty nine percent of the articles reported observational studies, 37% experimental studies, and 4% (seven articles) included both components. Vector competence was reported in 60% of the articles, while host competence was reported in 29%, and more than one category was reported in 11% of the articles. All observational studies had a low risk of bias, defined as plausible bias that is unlikely to seriously alter the results, and all experimental studies had a high risk of bias, which is defined as plausible bias that seriously weakens confidence in the results (Higgins and Green, 2011).

Thirty-three experimental studies reported JEV infection, dissemination, and transmission rates in vectors and were thus considered in this meta-analysis. The remaining 138 articles pertained to other outcome measures that are out of the scope of this manuscript.

Meta-analyses

A subgroup analysis by mosquito species was performed for the three outcomes of interest. Tables 21 to 23 display the pooled estimates for each outcome and their 95% confidence intervals (logit and back-transformed).

The magnitude of the pooled estimates across studies largely differed across all mosquito species.

When reporting JEV infection in vectors, pooled estimates ranged between 2% in *Aedes nigromaculis* and 96% in *Culex annulirostris* across the 29 studies included for this outcome. JEV infection rate in *Culex tritaeniorhynchus* was 52%, whereas the overall pooled estimate of JEV infection rate across all mosquito species was 39% (Table 21).

Heterogeneity was considered unimportant for articles reporting *Aedes togoi* (I^2 = 11.9%) and *Ochlerotatus vigilax* (I^2 = 28.6%), moderate (I^2 values between 50 and 60%) for *Culex pipiens*, *Culex annulirostris*, and *Culex annulus*, substantial (I^2 above 60%) for studies reporting *Aedes albopictus*, *Culex pseudovishnui*, and *Ochlerotatus detritus* and considerable heterogeneity (I^2 above 80%) for *Culex gelidus*, *Culex pipiens molestus*, *Culex tritaeniorhynchus*, *Culex pipiens pallens*, and *Culex pipiens fatigans*.

Subgroup analysis for JEV dissemination rate produced pooled estimates ranging from 8% in *Ochlerotatus notoscriptus* to 76% in *Ochlerotatus detritus*. The overall pooled estimate of JEV dissemination rate across all mosquito species was 42% (Table 22).

Pooled estimates of JEV dissemination rate showed considerable heterogeneity (I^2 > 80%) in *Culex quinquefasciatus* and *Culex annulirostris*, and moderate to substantial heterogeneity in *Culex sitiens, Ochlerotatus vigilax*, and *Ochlerotatus detritus* (I^2 =50-60%) across studies.

Pooled estimates from subgroup meta-analysis of studies reporting JEV transmission rates varied between 0% in *Aedes albopictus* and 80% in *Culex pipiens molestus* (overall estimate = 33%) (Table 23). Pooled estimates of JEV transmission rate showed considerable heterogeneity across studies in *Culex gelidus* (I^2 = 92.4%) and *Culex sitiens* (I^2 = 83.9%); substantial heterogeneity (I^2 above 70%) in *Culex annulirostris, Culex quinquefasciatus*, and *Culex tritaeniorhynchus*; and moderate heterogeneity in *Ochlerotatus detritus* (I^2 = 54.6%).

Because of the high heterogeneity found in the meta-analyses models for the three outcomes reported, pooled estimates were provided for reference only, and meta-regression models were fitted to explore their sources of heterogeneity.

Meta-regression

Mosquito species and administration route were significantly associated (*P*-value < 0.1) with JEV infection in vectors in the univariable screen (Table 24).

Aedes japonicus, Culex annulirostris, Culex annulirostris Skuse, Culex fuscocephala, Culex gelidus, Culex sitiens, Mansonia septempunctata, Ochlerotatus detritus, Opifex fuscus, and *Verrallina funerea* showed higher proportion of JEV infection rates compared to *Culex tritaeniorhynchus*, considered the most relevant vector species for JEV.

Furthermore, higher JEV infection rates were reported across studies in which intrathoracic inoculation was used, compared to oral feeding. Conversely, compared to oral feeding, vertical transmission was associated with lower infection rates (Table 24).

Associations between incubation period and diagnostic method with dissemination rate were not statistically significant (*P*-value > 0.1). However, we could not fit univariable meta-regression models to evaluate associations between administration route or mosquito species with dissemination rate due to an insufficient number of articles reporting values for these predictors. Similarly, univariable meta-regression models could not be carried out to investigate the association between administration route with JEV transmission rate.

Associations between mosquito species, incubation period, and diagnostic method with JEV transmission rate were not statistically significant (*P*-value > 0.1).

Multivariable meta-regression models were not fitted for any of the outcomes due to insufficient number of observations or lack of evidence of statistically significant conditional associations between predictors and outcomes. Thus, estimates from univariable meta-regression models are presented (Table 24).

Discussion

To date, this is the first study aiming at summarizing information from experiments related to JEV infection, dissemination, and transmission rates in vectors using a meta-analysis methodology and by combining the results from multiple research articles gathered using a systematic review of the literature. Moreover, by performing meta-regression models, we explored the sources of heterogeneity that could explain the variation reported in pooled estimates from the meta-analysis models.

Highest JEV infection rates were reported in *Culex annulirostris*, *Culex sitiens*, and *Culex fuscocephala*, which supports previous research claiming that mosquito species with importance as JEV vectors belong to the *Culex* genus (Misra and Kalita, 2010). *Aedes japonicus*, however, was also among the species with the highest JEV infection rates (90%), reflecting the wide range of mosquito species that may become infected with JEV, as pointed by previous research (van den Hurk *et al.*, 2009; Huang *et al.*, 2014).

Nonetheless, and though infection rates usually provide an estimate of prevalence of viral infection in a mosquito population, it is not always a direct indicator of risk for reasons discussed by Bustamante and Lord (2010). The proportion of mosquitoes that are capable of virus transmission (i.e., infectious mosquitoes) is not a constant fraction of the number of infected mosquitoes. Also, mosquito sampling, pooling, and virus testing tend to underestimate infection rates in mosquito populations. Thus, the risk of arbovirus transmission to humans and animals is not always directly proportional to higher infection rates. For this reason, when estimating risk of arbovirus transmission, infection rates should always be taken into account along with other parameters, such as mosquito abundance (including abundance of parous females and changes in the relative abundance of total mosquitoes), age, climate and other environmental factors (temperature, humidity, and rainfall patterns), and previous data records that compare baseline transmission patterns with those occurring in periods of epizootics and epidemics (Bustamante and Lord, 2010).

Dissemination and transmission rates, defined by Golnar *et al.* (2015) as the proportion of mosquitoes containing virus in their legs, regardless of their infection status, and the proportion of mosquitoes with a disseminated infection that transmits the virus after refeeding, respectively, provide us with more information regarding mosquito infectiousness, as opposed to mosquito infection. Mosquito species with the highest dissemination rates included *Ochlerotatus detritus* (76%) and *Opifex fuscus* (70%), none of which were among the species with the highest infection rates, actually supporting the hypothesis provided by

Bustamante and Lord (2010). The highest JEV transmission rates were reported in *Aedes japonicus* (75%), a mosquito species with one of the highest infection rates reported in the meta-analysis model for that outcome. According to the European Centre for Disease Prevention and Control (ECDC), *Aedes japonicus* has become the third invasive mosquito species reported in Europe, mainly due to international trade in used tires. Similarly, this mosquito species has also been reported in the United States (Darsie and Ward, 2005), thus making *Aedes japonicus* a potential JEV vector in North America, should all other transmission conditions be met.

Nevertheless, it is important to point out that mosquito species with high infection, dissemination, and transmission rates pertain to very few studies (with the exception of *Culex annulirostris*, which is represented in three articles, all other mosquito species mentioned as having high infection, dissemination, and transmission rates pertain to one article). The limited number of studies used in the meta-analyses models affects the precision of the estimates and warrants the need of future research focusing on dissemination and transmission experiments on mosquito species which have been previously identified as competent for JEV.

Studies pertaining to *Culex tritaeniorhynchus*, which is considered the most significant JEV vector in Asia (Solomon, 2000; Mackenzie *et al.*, 2004; Weaver and Barrett, 2004; van den Hurk *et al.*, 2009; Le Flohic *et al.*, 2013) and whose competence for JEV has been demonstrated in laboratory experiments (Gresser *et al.*, 1958), reported a pooled estimate of JEV transmission rate of 36%, which is lower than many other mosquito species not commonly associated with JEV infection and transmission, such as *Aedes japonicus* (though transmission results for *Culex tritaeniorhynchus* pertained to six experimental studies, as opposed to one for *Aedes japonicus*).

The low number of articles included in the models, especially for the outcomes JEV dissemination (n = 7) and JEV transmission rates (n = 15), prevented us from building multivariable meta-regression models to explore concurrent sources of heterogeneity. Univariable meta-regression models could, nonetheless, be fitted for the JEV infection rate outcome. Factors contributing to the heterogeneity observed were mosquito species and administration route. Several mosquito species (*Aedes japonicus, Culex annulirostris, Culex fuscocephala, Culex gelidus, Culex sitiens, Mansonia septempunctata, Ochlerotatus detritus, Opifex fuscus,* and *Verrallina funereal*) reported higher proportion of JEV infection rates compared to *Culex tritaeniorhynchus*. Pooled estimates for JEV infection showed

unimportant heterogeneity for *Aedes togoi* and *Ochlerotatus vigilax*, and moderate for *Culex pipiens fatigans*, *Culex pipiens*, and *Culex annulirostris*. Similarly, *Ochlerotatus detritus* showed moderate heterogeneity in the JEV transmission outcome. Therefore, those pooled estimates could be used as input parameters in risk assessment models that aim at estimating risk profiles of JEV introduction in susceptible regions. However, except for *Culex pipiens* and *Culex annulirostris*, all other mosquito species pertained to one article, which might explain the lower values of I^2 observed, limiting their usefulness.

Administration route was also an important source of heterogeneity, with higher JEV infection rates being reported across studies in which intrathoracic inoculation was the administration route employed, compared to oral feeding. Intrathoracic inoculation is considered a more direct method of experimental mosquito infection, as the virus is directly inoculated into the thorax of mosquitoes, which may explain the higher rates reported. Oral feeding, on the other hand, as a method of inoculation has higher external validity, as it resembles the actual infection process occurring in nature, where mosquitoes feed orally on infected hosts before they become infected. Because JEV must pass the mosquito's midgut barrier, not all infected mosquitoes become infectious (Bustamante and Lord, 2010), which aligns with the lower rates found in articles reporting this inoculation route. Vertical transmission, which occurs when an infected female mosquito passes the virus to its offspring, either by transovarial transmission or during oviposition in the fully formed egg (Lequime and Lambrechts, 2014), is associated with lower JEV infection rates across mosquito species. Considered as a strategy by which JEV survives the cold season in temperate regions in Asia, the lower infection rates reported in articles where vertical transmission occurred is not surprising, as the virus has more barriers to cross (the ovaries of the female parent mosquito or the egg), other than the midgut, before reaching the salivary glands, where it is readily available for infecting a host.

Additionally, although temperature was recorded in the datasets, it was not considered as a predictor in the meta-regression models because we assumed it was causally related to the outcomes only through incubation period, which was considered as a predictor of interest. Based on our causal diagrams, temperature was considered a simple antecedent variable, and its inclusion in the meta-regression models would have not changed the incubation period-vector competence outcomes, and any association with the outcomes would be contained within the association explained by incubation period (Dohoo *et al.*, 2009). Nonetheless, incubation period was not a significant source of heterogeneity explaining the variation

among studies for any of the outcomes. This could be related to the limited number of studies included, which prevented us from finding a significant statistical association.

The meta-analyses performed in the current study allowed us to recognize the large variability among experimental studies reporting JEV infection, dissemination, and transmission rates in vectors, making results challenging to contrast and synthesize. Regardless, we provide a quantitative summary of the results of multiple articles reporting JEV infection, dissemination, and transmission rates in vectors, expanding our knowledge on transmission efficiency of vectors, thus leading to a better understanding of vector competence and the relative importance of vectors in JEV transmission.

As suggested by Lord *et al.* (2015), assessing the ability of mosquito species to become infected and subsequently transmit JEV is an important step that leads to the accurate quantification of the role different vectors play in JEV transmission. The relative roles of potential vector species in JEV transmission are useful parameters to be inputted in different models, such as mathematical models that study the transmission patterns of arboviruses (Lord *et al.*, 2015). Furthermore, the relative importance of different vectors is considered as a surrogate measure for direct estimates of vectorial capacity (i.e., daily rate at which future inoculations arise from an infective case), which are highly demanding of data and thus impractical to assess (Dye, 1992).

Because JEV competence experiments, particularly transmission efficiency experiments, improve our understanding of which vector species contribute to JEV transmission, they aid in the assessment of the potential for JEV to spread to new geographical areas globally. By advancing our knowledge on transmission risk in space and time, better decisions regarding mitigation strategies, including vaccination programs directed towards the populations and regions at higher risk, may be achieved and more informed efforts targeted (Lord *et al.*, 2015).

Due to the limited number of studies available for the JEV dissemination and transmission outcomes, sources of heterogeneity could not be explored. Therefore, more studies on JEV dissemination and transmission in vectors should be carried out to address this gap and provide more data to help further our knowledge and to increase the precision of estimates for the different mosquito species, in order to use them as input parameters in risk assessment models aiming at studying risk profiles of JEV introduction in currently JEV-free regions.

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Tables

Table 20. Predictors pertaining to study characteristics included in the meta-analyses of infection, dissemination, and transmission rates.

Variable	Description	Categories
Mosquito species	Mosquito species or genera.	Several species (n = 50).
Administration route	Administration route used to experimentally infect mosquitoes.	Oral feeding, intrathoracic inoculation, vertical transmission [1]
Diagnostic method	Diagnostic method used for detecting JEV.	PCR, virus isolation (cell culture techniques or insect bioassays), virus isolation (with immunofluorescence, hemagglutination inhibition tests, or neutralization tests) [2]
Incubation period	Period (in days) between experimental infection and testing [3]	-

[1] Oral feeding comprises feeding on pledgets/membranes, on a host, or both.
Intrathoracic inoculation pertains to virus inoculation in the thoracic region of the mosquito.
Vertical transmission includes parents infected intrathoracically or by oral feeding.
[2] PCR includes real-time RT-PCR, RT-PCR alone or in combination with antigen-capture enzyme assays (e.g. ELISA) or virus isolation.
Virus isolation (cell culture techniques or insect bioassays) may use cell culture techniques or insect bioassays.
Virus isolation (with immunofluorescence, hemagglutination inhibition tests or neutralization tests) refers to virus identification by serotype identification with antibodies (indirect immunofluorescence assay), hemagglutination inhibition tests, or neutralization tests.
[3] A mean value of incubation period was calculated whenever a range of days was reported, otherwise the actual incubation period, in days, was presented.

Table 21. Subgroup meta-analysis[‡] of studies reporting JEV infection rates in vectors by mosquito species. Each effect size* represents pooled estimates (effect size) of the outcome for each mosquito species, and the overall represents the overall pooled estimate across all mosquito species.

Mosquito species	Effect size (logit)	95% CI (logit)	JEV infection rates [†]	95% CI (rates)	% Weight
Aedes albopictus	-1.37	-1.84, -0.90	0.20	0.14, 0.29	5.74
Culex pipiens	-0.35	-1.11, 0.42	0.41	0.25, 0.60	3.82
Culex quinquefasciatus	-0.67	-1.66, 0.31	0.34	0.16, 0.58	7.93
Opifex fuscus	1.05	0.42, 1.68	0.74	0.60, 0.84	0.50
Culex annulirostris	0.91	0.05, 1.78	0.71	0.51, 0.86	2.51
Culex gelidus	1.17	-0.65, 2.98	0.76	0.34, 0.95	1.45
Culex annulirostris	3.18	1.10, 5.26	0.96	0.75, 0.99	0.28
Aedes aegypti	-1.01	-1.58, -0.44	0.27	0.17, 0.39	0.51
Coquillettidia xanth	-2.08	-3.12, -1.04	0.11	0.04, 0.26	0.44
Culex sitiens	1.93	1.29, 2.56	0.87	0.78, 0.93	1.60
Mansonia septempunctata	0.69	-0.15, 1.53	0.67	0.46, 0.82	0.47
Ochlerotatus kochi	-1.30	-2.20, -0.40	0.21	0.10, 0.40	0.46
Ochlerotatus notoscr	-1.02	-1.62, -0.42	0.27	0.16, 0.40	0.76
Ochlerotatus vigilax	-1.21	-1.94, -0.48	0.23	0.13, 0.38	1.20
Verrallina funerea	0.30	-0.15, 0.75	0.57	0.46, 0.68	0.52
Culex pipiens molestus	-3.04	-5.39, -0.69	0.05	0.00, 0.33	0.88
Culex tritaeniorhynchus	0.09	-0.17, 0.35	0.52	0.46, 0.59	27.22
Armigeres subalbatus	-0.13	-1.08, 0.82	0.47	0.25, 0.69	2.18
Culex pipiens pallen	-1.73	-3.15, -0.32	0.15	0.04, 0.42	2.27
Toxorhynchites amboinensis	-0.25	-1.04, 0.55	0.44	0.26, 0.63	1.59
Toxorhynchites brevipalpis	-0.80	-2.18, 0.59	0.31	0.10, 0.64	0.58
Toxorhynchites rutilus	-0.41	-2.19, 1.37	0.40	0.10, 0.80	0.32
Toxorhynchites Theobaldi	-0.41	-2.19, 1.37	0.40	0.10, 0.80	0.32
Aedes japonicus	2.20	0.73, 3.67	0.90	0.67, 0.98	0.37
Aedes vexans nipponi	-0.20	-1.08, 0.68	0.45	0.25, 0.66	0.46
Culex pipiens fatiga	-1.66	-4.24, 0.92	0.16	0.01, 0.72	0.75
Culex pseudovishnui	-0.74	-1.05, -0.42	0.32	0.26, 0.40	13.90
Ochlerotatus detritus	0.25	-0.74, 1.25	0.56	0.32, 0.78	1.72
Culex vishnui	-0.94	-1.23, -0.65	0.28	0.23, 0.34	12.52
Culex fuscocephala	2.79	1.36, 4.23	0.94	0.80, 0.99	0.57
Aedes dorsalis	-3.40	-5.40, -1.40	0.03	0.00, 0.20	0.29
Aedes nigromaculis	-3.97	-4.95, -2.99	0.02	0.01, 0.05	0.45
Aedes vexans	-3.14	-5.14, -1.14	0.04	0.01, 0.24	0.29
Culex pipiens (pipiens)	-1.87	-3.36, -0.38	0.13	0.03, 0.41	0.36
Culex tarsalis	-4.40	-5.79, -3.01	0.01	0.00, 0.05	0.38
Culiseta incidens	-3.16	-4.32, -2.00	0.04	0.01, 0.12	0.42
Culiseta inornata	-3.27	-4.43, -2.11	0.04	0.01, 0.11	0.42
Aedes togoi	-0.24	-0.91, 0.45	0.44	0.29, 0.61	1.50
Aedes alcasidi	-0.92	-2.57, 0.73	0.28	0.07, 0.67	0.34
Armigeres flavus	-2.64	-4.68, -0.60	0.07	0.01, 0.35	0.28
Culex annulus	-0.30	-1.70, 1.10	0.43	0.15, 0.75	1.44
Overall	**-0.43**	**-0.59, -0.28**	**0.39**	**0.36, 0.43**	**100.00**

[‡] Random-effects meta-analysis using the method of DerSimonian and Laird (1986) to estimate the variance between studies, using a restricted maximum likelihood (REML) algorithm. I^2 range: 11.9% (*Aedes togoi*, *P*-value = 0.50) – 93.6% (*Culex quinquefasciatus*, *P*-value = 0.18)

[*] Computed for the group of studies reporting JEV infection rates in each mosquito species.

[†] $p = (e^{logit} / (e^{logit} + 1))$

Table 22. Subgroup meta-analysis[‡] of studies reporting JEV dissemination rates in vectors grouped by mosquito species. Each effect size* represents pooled estimates (effect size) of the outcome for each mosquito species, and the overall represents the overall pooled estimate across all mosquito species.

Mosquito species	Effect size (logit)	95% CI (logit)	JEV dissemination rates [†]	95% CI (rates)	% Weight
Culex pipiens	-0.41	-2.19, 1.37	0.40	0.10, 0.80	3.20
Culex quinquefasciatus	0.42	-0.57, 1.41	0.60	0.36, 0.80	22.26
Opifex fuscus	0.85	0.14, 1.56	0.70	0.54, 0.83	4.96
Culex annulirostris	-0.40	-1.48, 0.68	0.40	0.19, 0.66	21.90
Culex gelidus	-1.21	-1.68, -0.74	0.23	0.16, 0.32	5.26
Culex sitiens	-1.44	-2.28, -0.60	0.19	0.09, 0.35	16.41
Ochlerotatus notoscriptus	-2.44	-3.48, -1.40	0.08	0.03, 0.20	4.44
Ochlerotatus vigilax	-1.09	-2.12, -0.06	0.25	0.11, 0.49	9.35
Ochlerotatus detritus	1.18	-0.11, 2.46	0.76	0.47, 0.92	12.21
Overall	**-0.33**	**-0.83, 0.16**	**0.42**	**0.30, 0.54**	**100.00**

[‡] Random-effects meta-analysis using the method of DerSimonian and Laird (1986) to estimate the variance between studies, using a restricted maximum likelihood (REML) algorithm. I^2 range: 62.1% (*Ochlerotatus vigilax*, *P*-value = 0.04) – 85.4% (*Culex annulirostris*, *P*-value = 0.47)

[*] Computed for the group of studies reporting JEV dissemination rates in each mosquito species.

[†] $p = (e^{logit} / (e^{logit} + 1))$

Table 23. Subgroup meta-analysis[‡] of studies reporting JEV transmission rates in vectors grouped by mosquito species. Each effect size* represents pooled estimates (effect size) of the outcome for each mosquito species, and the overall represents the overall pooled estimate across all mosquito species.

Mosquito species	Effect size (logit)	95% CI (logit)	JEV transmission rates [†]	95% CI (rates)	% Weight
Aedes albopictus	-5.57	-11.02, -0.13	0.00	0.00, 0.47	0.32
Culex pipiens	-2.44	-3.62, -1.26	0.08	0.03, 0.22	3.12
Culex quinquefasciatus	-0.23	-1.21, 0.75	0.44	0.23, 0.68	12.20
Culex annulirostris	-0.13	-1.31, 1.05	0.47	0.21, 0.74	8.27
Culex gelidus	-0.71	-1.64, 0.23	0.33	0.16, 0.56	12.25
Aedes aegypti	-1.10	-1.69, -0.51	0.25	0.16, 0.37	2.66
Coquillettidia xanthogaster	-2.64	-4.68, -0.60	0.07	0.01, 0.35	1.31
Culex sitiens	-1.15	-3.30, 0.99	0.24	0.04, 0.73	5.28
Mansonia septempunctata	0.17	-0.63, 0.97	0.54	0.35, 0.73	2.46
Ochlerotatus notoscriptus	-1.10	-2.24, 0.04	0.25	0.10, 0.51	3.12
Ochlerotatus vigilax	-1.95	-4.05, 0.15	0.12	0.02, 0.54	1.27
Verrallina funerea	-1.61	-2.85, -0.38	0.17	0.05, 0.41	2.02
Culex pipiens molestus	1.39	-0.81, 3.59	0.80	0.31, 0.97	1.20
Culex tritaeniorhynchus	-0.56	-1.25, 0.14	0.36	0.22, 0.53	25.40
Aedes japonicus	1.10	-1.15, 3.35	0.75	0.24, 0.97	1.16
Aedes vexans nipponi	-0.69	-2.40, 1.02	0.33	0.08, 0.73	1.57
Ochlerotatus detritus	-0.70	-1.56, 0.17	0.33	0.17, 0.54	10.44
Culex fuscocephala	-1.64	-2.52, -0.76	0.16	0.07, 0.32	5.96
Overall	**-0.71**	**-1.02, -0.40**	**0.33**	**0.26, 0.40**	**100.00**

[‡] Random-effects meta-analysis using the method of DerSimonian and Laird (1986) to estimate the variance between studies, using a restricted maximum likelihood (REML) algorithm. I^2 range: 54.6% (*Ochlerotatus detritus*, *P*-value = 0.11) – 92.4% (*Culex gelidus*, *P*-value = 0.14)

[*] Computed for the group of studies reporting JEV transmission rates in each mosquito species.

[†] $p = (e^{logit} / (e^{logit} + 1))$

Table 24. Coefficients, *P*-values, and 95% Confidence Intervals of the association of predictors of interest on JEV infection rates in vectors (from univariable meta-regression models[‡]) *n* = 29 studies.

Variable	*N*	Coefficient (logit)	Standard Error (logit)	95% CI (logit)	*P*-value	Overall *P*-value
Mosquito species						<0.01
Culex tritaeniorhynchus	13	Reference				
Aedes aegypti	2	-1.15	1.21	-3.54, 1.23	0.34	
Aedes albopictus	5	-1.57	0.39	-2.33, -0.80	0.00	
Aedes alcasidi	1	-1.06	1.45	-3.92, 1.79	0.46	
Aedes dorsalis	1	-3.54	1.56	-6.62, -0.47	0.02	
Aedes japonicus	1	2.06	1.40	-0.70, 4.81	0.14	
Aedes nigromaculis	1	-4.11	1.28	-6.63, -1.60	0.00	
Aedes togoi	1	-0.20	0.71	-1.60, 1.19	0.77	
Aedes vexans	2	-3.28	1.56	-6.36, -0.21	0.04	
Aedes vexans nipponii	1	-0.34	1.26	-2.82, 2.14	0.79	
Armigeres flavus	1	-2.78	1.57	-5.89, 0.32	0.08	
Armigeres subalbatus	2	-0.27	0.59	-1.44, 0.90	0.65	
Coquilletiidia xanthogaster	1	-2.22	1.29	-4.76, 0.32	0.09	
Culex annulirostris	3	0.70	0.55	-0.38, 1.79	0.20	
Culex annulus	1	-0.44	0.71	-1.84, 0.97	0.54	
Culex fuscocephala	1	2.65	1.12	0.45, 4.85	0.02	
Culex gelidus	3	0.92	0.72	-0.49, 2.34	0.20	
Culex pipiens	3	-0.48	0.46	-1.38, 0.42	0.30	
Culex pipiens (pipiens)	1	-2.01	1.40	-4.78, 0.75	0.15	
Culex pipiens fatigans	1	-1.67	0.99	-3.62, 0.27	0.09	
Culex pipiens molestus	2	-3.16	0.92	-4.97, -1.35	0.00	
Culex pipiens pallens	4	-1.79	0.58	-2.94, -0.65	0.00	
Culex pseudovishnui	2	-0.82	0.28	-1.37, -0.27	0.00	
Culex quinquefasciatus	8	-0.77	0.34	-1.44, -0.09	0.03	
Culex sitiens	1	1.78	0.69	0.43, 3.14	0.01	
Culex tarsalis	1	-4.54	1.37	-7.25, -1.83	0.00	
Culex vishnui	1	-1.00	0.29	-1.57, -0.43	0.00	
Culiseta incidens	1	-3.30	1.32	-5.90, -0.71	0.01	
Culiseta inornata	1	-3.41	1.32	-6.01, -0.82	0.01	
Mansonia septempunctata	1	0.55	1.25	-1.92, 3.01	0.66	
Ochlerotatus detritus	1	0.10	0.67	-1.21, 1.41	0.88	
Ochlerotatus kochi	1	-1.44	1.26	-3.93, 1.04	0.25	
Ochlerotatus notoscriptus	1	-1.28	0.98	-3.21, 0.66	0.20	
Ochlerotatus vigilax	1	-1.39	0.79	-2.94, 0.16	0.08	
Opifex fuscus	1	0.91	1.22	-1.49, 3.30	0.46	
Toxorrhynchites Theobaldi	1	-0.55	1.49	-3.49, 2.38	0.71	
Toxorrhynchites amboinensis	1	-0.39	0.68	-1.73, 0.96	0.57	
Toxorrhynchites brevipalpis	1	-1.00	1.11	-3.18, 1.18	0.37	
Toxorrhynchites rutilus	1	-0.55	1.49	-3.49, 2.38	0.71	
Verrallina funerea	1	0.16	1.20	-2.20, 2.51	0.90	
Intercept		0.14	0.16	-0.18, 0.46	0.38	
Administration route						
Oral feeding	24	Reference				<0.01
Intrathoracic inoculation	3	0.14	0.44	-0.73, 1.00	0.75	

Vertical transmission	4	-1.05	0.27	-1.58, -0.52	0.00
Intercept		-0.27	0.11	-0.48, -0.06	0.01

‡ Random effects meta-regression models using the restricted maximum likelihood method (REML).

Chapter 5 - Discussion and Conclusion

Discussion

The three previous chapters evaluated vector and host competence for JEV, compiling the information gathered from the current body of evidence. The vector and vertebrate host competence outcome measures assessed pertained to vector transmission efficiency and host preference, and vector and host susceptibility to JEV infection. Whereas Chapter 2 provided a comprehensive resource of competent vectors and hosts in JEV affected countries, along with their reported JEV infection, using a systematic review of the literature methodology, Chapters 3 and 4 provided a quantitative summary of vector competence in terms of proportion of JEV infection in vectors and vertebrate hosts (Chapter 3), and JEV infection, dissemination, and transmission rates in vectors (Chapter 4), using a meta-analysis approach. Furthermore, these chapters explored study characteristics as sources of heterogeneity explaining the variation reported among studies for the different outcomes studied.

This study demonstrated that several mosquito species of medical and veterinary importance, including known disease vectors of other arboviruses, are competent to JEV, further elucidating on their geographical distribution worldwide.

The most abundantly reported outcome measure was proportion of JEV infection from observational studies and infection rates from experimental studies. Dissemination and transmission rates, as well as maximum likelihood estimation (MLE) values and minimum infection rates (MIR) in vectors were the least reported outcomes. Regarding host infection, there were only a few studies reporting results on some vertebrate host species, such as reptiles and amphibians. Most of the issues when extracting data were related to lack of standardization and consistency of the methods used for assessing the different study characteristics, particularly mosquito trapping and diagnostic methods. However, when compiling the different methods reported into categories, the standardization issue was resolved.

Major data gaps were related to the lack of studies in JEV-free countries, especially studies focusing on vector competence. A greater impact on JEV transmission knowledge would be achieved if studies on proportion of JEV infection in the most abundant mosquito species of potentially at risk areas, such as North and South America, were performed. This type of information would improve our knowledge regarding the presence or absence of infected mosquitoes in these regions, despite no JE cases have occurred so far, with direct impact on potential surveillance actions and outbreak preparedness and response. Additionally,

transmission experiments reporting infection, dissemination, and transmission rates in mosquito species not reported in this SR but abundant in such regions and whose JEV competence status is unknown, also represents an important data gap that would benefit from future research.

Pooled estimates of proportion of JEV infection for several mosquito species were deemed unimportant or moderate, constituting more accurate estimates than those with substantial and considerable heterogeneity. Such mosquito species included species from the *Culex* genus, which are considered important JEV vectors (Misra and Kalita, 2010). Of these, *Culex tritaeniorhynchus* and *Culex pipiens* are present in the US (Darsie and Ward, 2005). However, lower heterogeneity in *Culex tritaeniorhynchus* could be related to the greater number of articles in which it was represented (n = 10), compared to other mosquito species, which increases the precision of the estimate.

Moreover, in our study, trials reporting on *Aedes japonicus*, a potential JEV vector in the US and other regions, showed high JEV proportion (ECDC, 2017; Darsie and Ward, 2005). Nevertheless, Bustamante and Lord (2010) have claimed that infection in vectors is not a straightforward indicator of risk, as other parameters are also involved in the complex dynamics of JEV's transmission cycle. These parameters include vector abundance, age, climate, other environmental conditions, and availability of hosts, and should be taken into consideration when assessing JEV transmission.

Regardless, all outcomes reported overall high variability across mosquito species, which is consistent with the high heterogeneity obtained in the meta-analyses models. The broadness of the research question posed in the systematic review led to the retrieval of large amounts of data, and contributed to the high variability observed. Likewise, heterogeneity may be related to differences in study methodology, data collection, reporting, and presentation, as well as differences in detection methods, geographical distribution, and environmental factors of the regions represented. Specifically, mosquito capture method was found to be an important predictor responsible for the heterogeneity observed and because different mosquito trapping methods may attract different mosquito species, as suggested by Lord *et al.* (2015), JEV transmission and infection outcomes should be interpreted bearing in mind potential biases towards an over or underrepresentation of certain mosquito species, depending on the trapping method used.

Other sources of heterogeneity included diagnostic method, which could be attributed to the large span of years represented in the articles gathered in the SR, which ranged from 1946 to 2016, thus reflecting scientific and technical improvements related to diagnostic techniques. Geographical region was also identified as a source of heterogeneity, with Southern regions having higher proportions of JEV, which could be explained by the fact that JEV transmission occurs all year round, and therefore increased opportunities for host infection take place.

In experimental studies, the administration route used to experimentally infect mosquitoes was attributed as a source of heterogeneity for JEV infection rates. This source was biologically coherent, with intrathoracic inoculation being significantly associated with higher infection rates, as expected. Using this administration route implies that JEV does not have to cross the midgut barrier and thus, infection is directly achieved.

Lastly, the predictor vertebrate host species was also found to be a source of heterogeneity. The role vertebrate hosts play on JEV transmission point to horses and donkeys, cats and dogs, and wild pigs as the species with the highest JEV infection. Interestingly, wild pigs are amplifying and reservoir hosts for JEV, which leads us to argument that any JEV-free region having considerable population of wild pigs, regardless of the presence of domestic pigs or pig farming, may be potentially at risk, as wild pigs may act as the amplifying vertebrate host necessary for JEV transmission. In fact, the wild pig population of northern and central California may pose a risk to the transmission and establishment of JEV in the US (Nett *et al.*, 2009). As far as the remaining species referred to as having high JEV proportion of infection (horses, donkeys, cats and dogs) are concerned, they do not constitute a risk to JEV transmission or human infection, as they are dead-end hosts, not contributing to the virus maintenance cycle.

Conclusion

The information gathered and quantitatively assessed in the different chapters of this thesis substantially contributed to further our understanding regarding the role of different vectors and vertebrate hosts in the epidemiology and transmission of JEV. The research presented in this thesis comprise useful guidelines for interpreting vector and host competence, advancing our knowledge on the relative importance of vectors and vertebrate hosts on JEV introduction and transmission.

Moreover, transmission studies, such as the ones included in Chapter 4 of this thesis, have a paramount role on the understanding of the mechanisms under which mosquitoes become infected, disseminate infection, and then transmit the virus to vertebrate hosts, i.e., vector competence.

The relative roles of potential mosquito species in JEV transmission are useful parameters that can be used as inputs in different models, such as mathematical models that study the transmission patterns of arboviruses (Lord *et al.*, 2015) or risk assessment models that investigate risk profiles for JEV introduction in regions potentially at risk.

A risk assessment model that investigates the probability of introduction, transmission, and establishment of JEV in the US is currently under study. Within the risk assessment framework, we will be using simulations that incorporate input data variability and uncertainty, aiming at generating probability distributions of JEV risk of introduction in the US, for which the estimates identified and synthesized in the current work will prove useful. Regarding the probability of introduction, several potential pathways of entry have been identified and include: a) entry through infected vectors (by aircraft, vessel, tires or wind), b) importation of infected viremic animals, c) importation of infected animal products, d) importation of infected biological materials, e) entry of viremic migratory birds, and f) entry of infected humans. The information gathered in the SR and summarized in the meta-analyses is useful for populating some of these pathways, particularly the proportion of JEV infection in vectors, which provide estimates of mosquito infection probabilities from different regions in Asia. These are then taken into account when estimating the probability of at least one infected mosquito being introduced via aircraft, vessel, tires, or wind.

These models have direct application when implementing mitigation and control strategies aiming at countering the introduction of JEV and other emerging vector-borne zoonoses in susceptible countries, aiding in decision-making processes, such as vaccination efforts (Lord *et al.*, 2015).

Future studies

Future research should aim at obtaining vector and host abundance data in countries not represented in this study, particularly those that are at risk of JEV introduction and establishment. Maximum likelihood estimation values of mosquito infection would also comprise an important source of evidence to assess infection in mosquito populations, as they represent more accurate estimates of mosquito infection, compared to minimum infection

rates, especially during periods of high transmission or when pool sizes are large (Gu *et al.*, 2003; Bustamante and Lord, 2010).

Furthermore, more studies, such as JEV transmission experiments, should be conducted to address vector (and vertebrate host) competence in underrepresented species, thus improving the precision of estimates to be inputted in the aforementioned predictive models.

Particularly, more research on vertebrate hosts, such as reptiles and amphibians, which are thought of as having a role in the overwintering mechanism of JEV, are needed.

A higher level of standardization and consistency of the methods used for assessing the different study characteristics, especially mosquito capture and diagnostic methods, would benefit future assessments of JEV vector and host competence.

A quantitative risk assessment of the risk of introduction of JEV in the US is the next step comprising the efforts targeted in this thesis, with the objective of evaluating the likelihood of introduction of the virus in the continental US territory, and subsequent spread and consequences.

The need of such assessment is strengthened by the presence of several mosquito species that are competent to JEV, as pointed out in Chapter 1, as well as vertebrate hosts, with special relevance to the feral pig population in California (Nett *et al.*, 2009).

The fact that the US has competent vectors and hosts for JEV but no JE cases have been reported to date may be due to other factors intervening in the complex dynamics of JEV transmission. Factors such as climate, including temperature and rainfall patterns, and farming practices could play a role on the absence of JEV transmission in the US. Moreover, cross-protection with other flaviviruses, such as West Nile virus and St. Louis encephalitis virus (both present in the US), poor resistance of JEV in the environment (a very labile virus that is sensitive to UV light), and the relatively short duration of viremia in both pigs and birds (three to four days) may also explain the current JEV-free status of the US.

Nevertheless, availability of vectors and hosts, and favorable environmental conditions (Nett *et al.*, 2009) put the US at risk of an inadvertent JEV introduction, thus calling for action in what regards putting in place surveillance mechanisms.

Research efforts should ultimately aim at preventing the introduction of JEV or counter the effects of a potential JEV introduction into currently JEV-free countries, including the US. This type of information has applicability to other arbovirus of animal and veterinary importance, with the results obtained being equally useful to include in other models investigating other vector-borne diseases.

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Appendix A - Complete list of search terms and different combinations used for searching the selected databases and journals.

Web of Science

1. TOPIC: (Japanese) AND TOPIC: (Encephalitis) OR TOPIC: (viral encephalitis) OR TOPIC: (JE) OR TOPIC: (JEV) AND TOPIC: (vector competence) AND TOPIC: (mosquito) AND TOPIC: (host competence) AND TOPIC: (United States) OR TOPIC: (US) OR TOPIC: (USA) OR TOPIC: (North America)
Timespan: All years.
Search language=English
Results: 3,122,242
2. TITLE: (((((((japanese AND encephalitis) OR viral encephalitis) OR JE) OR ((((JEV AND vector competence) AND mosquito) AND host competence) AND United States)) OR USA) OR US) OR North America)
Timespan: All years.
Search language=English
Results: 346,820
3. TOPIC: ((japanese encephalitis OR viral encephalitis OR JE OR JEV) AND vector competence AND mosquito AND host competence AND (United States OR USA OR US OR North America))
Timespan: All years.
Search language=English
Results: 45
4. TOPIC: (japanese encephalitis OR viral encephalitis OR JE OR JEV AND vector competence AND mosquito AND host competence AND United States OR USA OR US OR North America)
Timespan: All years.
Search language=Auto
Results: 3,128,582
5. TOPIC: (((japanese AND encephalitis) OR viral encephalitis OR JE OR JEV) AND vector competence AND mosquito AND host competence AND (United States OR USA OR US OR North America))
Timespan: All years.
Search language=Auto
Results: 45
6. Search 3 without region. TS: ((Japanese encephalitis OR viral encephalitis OR JE OR JEV) AND vector competence AND mosquito AND host competence)
Timespan: All years.
Search language=English
Results: 112

PubMed

1. All Fields: ((japanese AND encephalitis) OR viral encephalitis OR JE OR JEV) AND vector competence AND mosquito AND host competence AND (United States OR USA OR US OR North America)
Results: 22
2. All Fields: (Japanese AND encephalitis AND (United States OR US OR USA OR North America) AND vector competence AND mosquitoes AND vector competence)
Results: 0

3. All Fields: (Japanese AND encephalitis AND (United States OR US OR USA OR North America) AND mosquitoes)
Results: 129
4. ((Japanese AND encephalitis) OR (viral AND encephalitis) OR JE OR JEV) AND (United States OR US OR USA OR North America) AND mosquito
Results: 1460
5. Search 1 without Region. All Fields: ((Japanese AND encephalitis) OR viral encephalitis OR JE OR JEV) AND vector competence AND mosquito AND host competence)
Results: 25
6. All Fields: ((Japanese encephalitis virus) OR JE OR JEV) AND vector competence AND mosquito AND host competence)
Results: 1
7. All Fields: ((Japanese encephalitis) OR JE OR JEV) AND vector competence AND mosquito AND host competence)
Results: 1

Armed Forces Pest Management Board

1. Find results in AFPMB website with all the words: Japanese Encephalitis
Result: 68
http://www.afpmb.org/content/search-afpmborg
2. Find results in AFPMB website with all the words: Japanese Encephalitis United States mosquito
Result: 49
http://www.afpmb.org/content/search-afpmborg
3. Find results in DWFP publications with all the words: Japanese Encephalitis
Result: 17
http://www.afpmb.org/content/dwfp-publication-search
4. Find results in AFPMB website with all the words: Japanese Encephalitis mosquito vector host competence
Result: 14
http://www.afpmb.org/content/search-afpmborg

Google Scholar

1. Find article with all of the worlds in title: Japanese encephalitis
And with at least one of the words in the title: United States US USA North America
Results: 61
http://scholar.google.com/scholar?as_q=Japanese+Encephalitis&as_epq=&as_oq=United+States+USA+US+North+America&as_eq=&as_occt=title&as_sauthors=&as_publication=&as_ylo=&as_yhi=&btnG=&hl=en&as_sdt=1%2C5
2. Find article with all of the worlds in the article: Japanese encephalitis mosquito vector competence host
And with at least one of the words in the article: United States US USA North America
Results: 4400
http://scholar.google.com/scholar?as_q=Japanese+Encephalitis+mosquito+vector+competence+host&as_epq=&as_oq=United+States+USA+US+North+America&as_eq=&as_occt=any&as_sauthors=&as_publication=&as_ylo=&as_yhi=&btnG=&hl=en&as_sdt=1%2C5
3. Find article with all of the worlds in title: Japanese Encephalitis
And with at least one of the words in the title: mosquito, vector, competence, host.
Without patents and without citations
1970-2016
Results: 179
http://scholar.google.com/scholar?q=allintitle%3A+Japanese+Encephalitis+mosquito+OR+vector+OR+competence+OR+host&hl=en&as_sdt=0%2C5&as_vis=1&as_ylo=1970&as_yhi=2016

The American journal of tropical medicine and hygiene

1. Searching journal content for Japanese Encephalitis (all words) in title, United States US USA (any words) in title or abstract, and mosquito vector competence host (any words) in full text.
Results: 11
http://www.ajtmh.org/search?submit=yes&pubdate_year=&volume=&firstpage=&doi=&author1=&author2=&title=Japanese+Encephalitis&andorexacttitle=and&titleabstract=United+states+US+USA&andorexacttitleabs=or&fulltext=mosquito+vector+competence+host&andorexactfulltext=or&fmonth=&fyear=&tmonth=&tyear=&format=standard&hits=10&sortspec=relevance&submit=yes&submit=Submit
2. Searching journal content for Japanese Encephalitis viral (any words) in title, United States mosquito (all words) in title or abstract, and vector competence host US USA (any words) in full text.
Results: 7
http://www.ajtmh.org/search?submit=yes&pubdate_year=&volume=&firstpage=&doi=&author1=&author2=&title=Japanese+Encephalitis+viral+&andorexacttitle=or&titleabstract=United+States+mosquito&andorexacttitleabs=and&fulltext=vector+competence+host+US+USA&andorexactfulltext=or&fmonth=&fyear=&tmonth=&tyear=&format=standard&hits=10&sortspec=relevance&submit=yes&submit=Submit
3. Searching journal content for Japanese Encephalitis (all words) in title and vector competence host mosquito (any words) in title or abstract.
Results: 33
http://www.ajtmh.org/search?submit=yes&pubdate_year=&volume=&firstpage=&doi=&author1=&author2=&title=Japanese+Encephalitis&andorexacttitle=and&titleabstract=vector+competence+host+mosquito&andorexacttitleabs=or&fulltext=&andorexactfulltext=and&fmonth=&fyear=&tmonth=&tyear=&format=standard&hits=10&sortspec=relevance&submit=yes&submit=Submit

Journal of Medical Entomology

1. For title "Japanese encephalitis viral JE JEV" (match any words) and abstract or title "United States US USA" (match any words) and full text or abstract or title "mosquito vector competence host" (match whole all)
Results: 9
http://jme.oxfordjournals.org/search/title%3AJapanese%2Bencephalitis%2Bviral%2BJE%2BJEV%20title_flags%3Amatch-any%20abstract_title%3AUnited%2BStates%2BUS%2BUSA%20abstract_title_flags%3Amatch-any%20text_abstract_title%3Amosquito%2Bvector%2Bcompetence%2Bhost%20text_abstract_title_flags%3Amatch-all%20numresults%3A10%20sort%3Arelevance-rank%20format_result%3Astandard%20jcode%3Ajmedent
2. For title "Japanese encephalitis" (match all words) and abstract or title "United States US USA" (match any words) and full text or abstract or title "mosquito vector competence host" (match whole all)
Results: 1
http://jme.oxfordjournals.org/search/title%3AJapanese%2Bencephalitis%20title_flags%3Amatch-all%20abstract_title%3AUnited%2BStates%2BUS%2BUSA%20abstract_title_flags%3Amatch-any%20text_abstract_title%3Amosquito%2Bvector%2Bcompetence%2Bhost%20text_abstract_title_flags%3Amatch-all%20numresults%3A10%20sort%3Arelevance-rank%20format_result%3Astandard%20jcode%3Ajmedent
3. For title "Japanese encephalitis JE JEV" (match any words) and abstract or title "mosquito vector competence host" (match any words)
Results: 126
http://jme.oxfordjournals.org/search/title%3AJapanese%2Bencephalitis%2BJE%2BJEV%20title_flags%3Amatch-any%20abstract_title%3Amosquito%2Bvector%2Bcompetence%2Bhost%20abstract_title_flags%3A

match-any%20numresults%3A10%20sort%3Arelevance-rank%20format_result%3Astandard%20jcode%3Ajmedent
4. For title "Japanese encephalitis" (match all words) and abstract or title "mosquito vector competence host JE JEV" (match any words)
Results: 30
http://jme.oxfordjournals.org/search/title%3AJapanese%2Bencephalitis%20title_flags%3Amatch-all%20abstract_title%3Amosquito%2Bvector%2Bcompetence%2Bhost%2BJE%2BJEV%20abstract_title_flags%3Amatch-any%20numresults%3A10%20sort%3Arelevance-rank%20format_result%3Astandard%20jcode%3Ajmedent

Journal of the American Mosquito Control Association

1. ti(Japanese AND encephalitis) OR ti((viral and encephalitis OR JE OR JEV)) AND (vector competence OR host competence) AND (United States OR US OR USA OR North America)
Results: 1454
http://search.proquest.com/results/D8BCEE4BD8C447DEPQ/1?accountid=11789
2. ti(japanese encephalitis OR viral encephalitis OR JE OR JEV) AND ti((United States OR US OR USA OR North America)) AND ab((Vector competence host OR mosquito))
Results: 3
http://search.proquest.com/results/E56B1C3F6F204756PQ/1?accountid=11789
3. ti(japanese encephalitis OR viral encephalitis OR JE OR JEV) AND ti((United States OR US OR USA OR North America)) AND (Vector competence host OR mosquito)
Results: 10
http://search.proquest.com/results/6D28E55E06494409PQ/1?accountid=11789
4. ti((Japanese AND encephalitis) OR (viral AND encephalitis) OR JE OR JEV) AND ab((vector OR host)) AND all(mosquito)
http://search.proquest.com/results/9F0CB20EA7E44588PQ/1?accountid=11789
Results: 149

Vector borne and zoonotic diseases

1. You searched for: [Article title: japanese] AND [[Article title: encephalitis] OR [Article title: viral] OR [Article title: je] OR [Article title: jev]] AND [Article title: united] AND [[Article title: sates] OR [Article title: us] OR [Article title: usa] OR [Article title: north]] AND [Article title: america] AND [All: mosquitos]
Results: 0
2. You searched for: [All: japanese encephalitis] AND [All: united] AND [[All: sates] OR [All: us] OR [All: usa] OR [All: north]] AND [All: america] AND [All: mosquitos]
Results: 77
http://online.liebertpub.com/action/doSearch?field1=AllField&text1=Japanese+Encephalitis+&logicalOpe1=AND&field2=AllField&text2=United+Sates+OR+US+OR+USA+OR+North+America&logicalOpe2=AND&field3=AllField&text3=mosquitos+&search=&history=&AfterYear=&BeforeYear=&sortBy=relevancy&displaySummary=false&pageSize=100
3. You searched for: [Article title: japanese encephalitis] AND [Abstract: united] AND [[Abstract: sates] OR [Abstract: us] OR [Abstract: usa] OR [Abstract: north]] AND [Abstract: america] AND [Abstract: mosquitos]
Results: 1
http://online.liebertpub.com/action/doSearch?field1=Title&text1=Japanese+Encephalitis&logicalOpe1=AND&field2=Abstract&text2=United+Sates+OR+US+OR+USA+OR+North+America&logicalOpe2=AND&field3=Abstract&text3=mosquitos&search=&history=&AfterYear=&BeforeYear=&sortBy=relevancy&displaySummary=false&pageSize=100
4. You searched for: [Article title: japanese encephalitis] AND [[Abstract: mosquito] OR [Abstract: vector] OR [Abstract: host]]
Results: 15

http://online.liebertpub.com/action/doSearch?field1=Title&text1=Japanese+encephalitis&logicalOpe1=AND&field2=Abstract&text2=mosquito+OR+vector+OR+host&logicalOpe2=AND&field3=Abstract&text3=&search=&history=&AfterYear=&BeforeYear=&sortBy=relevancy&displaySummary=false&pageSize=100

Appendix B - Proportion of JEV infection in all mosquito species (n=149) and all observational studies (n=58) by mosquito species (ordered alphabetically), and by author, year of publication, and country of origin, ordered from oldest to most recent year of publication.

Mosquito species[1]	Author (year)	Country	Positive pools/Total pools tested[2]	Proportion positive (%)
Aedes spp.	Dandawate *et al.* (1969)	India	0/47	0.00
	Peiris *et al.* (1992)	Sri Lanka	0/503	0.00
	Vythilingam *et al.* (1997)	Malaysia	0/4	0.00
	Bryant *et al.* (2005)	Vietnam	0/27	0.00
Aedeomyia catasticta	Dhanda *et al.* (1989)	India	0/4	0.00
	van den Hurk *et al.* (2001)	Australia	0/19	0.00
	Johansen *et al.* (2003)	Australia	0/5	0.00
Aedes (Cancraedes) sp.	Vythilingam *et al.* (1997)	Malaysia	1/19	5.26
Aedes aegypti	Dandawate *et al.* (1969)	India	0/12	0.00
	Tan *et al.* (1993)	Indonesia	0/87	0.00
	Bryant *et al.* (2005)	Vietnam	0/9	0.00
	Su *et al.* (2014)	Taiwan	0/2	0.00
Aedes albopictus	Dandawate *et al.* (1969)	India	0/21	0.00
	Mitchell *et al.* (1993)	Saipan (Mariana islands)	0/3	0.00
	Tan *et al.* (1993)	Indonesia	0/15	0.00
	Vythilingam *et al.* (1997)	Malaysia	0/10	0.00
	Weng *et al.* (1999)	Taiwan	20/39	51.28
	Bryant *et al.* (2005)	Vietnam	0/10	0.00
	Weng, Lien, and Ji (2005)	Taiwan	0/12	0.00
	Kim *et al.* (2011)	South Korea	0/2	0.00
	Tiawsirisup, Junpee, and Nuchprayoon (2012)	Thailand	0/6	0.00
	Seo *et al.* (2013)	South Korea	0/15	0.00
	Su *et al.* (2014)	Taiwan	1/25	4.00
	Kim *et al.* (2015)	South Korea	0/64	0.00
Aedes alterans	van den Hurk *et al.* (2001)	Australia	0/1	0.00
Aedes andamanesis	Bryant *et al.* (2005)	Vietnam	0/1	0.00
Aedes bekkui	Kim *et al.* (2011)	South Korea	0/3	0.00
Aedes butleri	Vythilingam *et al.* (1997)	Malaysia	4/79	5.06
Aedes caecus	Tan *et al.* (1993)	Indonesia	0/9	0.00
Aedes carmenti	van den Hurk *et al.* (2001)	Australia	0/2	0.00
Aedes culiciformis	Ritchie *et al.* (1997)	Australia	0/142	0.00
	van den Hurk *et al.* (2001)	Australia	0/357	0.00
Aedes desmotes	Weng *et al.* (1999)	Taiwan	0/1	0.00
Aedes funereus	Ritchie *et al.* (1997)	Australia	0/1	0.00

Aedes jamesi	Dandawate *et al.* (1969)	India	0/2	0.00
Aedes japonicus	Takashima *et al.* (1989)	Japan	0/912	0.00
Aedes kochi	Ritchie *et al.* (1997)	Australia	0/294	0.00
	van den Hurk *et al.* (2001)	Australia	0/178	0.00
Aedes lineatopennis	Vythilingam *et al.* (1997)	Malaysia	1/6	16.67
	van den Hurk *et al.* (2001)	Australia	0/227	0.00
	Johansen *et al.* (2003)	Australia	0/1,785	0.00
	Kim *et al.* (2011)	South Korea	0/11	0.00
	Seo *et al.* (2013)	South Korea	0/1	0.00
Aedes lineatus	van den Hurk *et al.* (2001)	Australia	0/10	0.00
Aedes littlechildi	van den Hurk *et al.* (2001)	Australia	0/66	0.00
Aedes normanensis	van den Hurk *et al.* (2001)	Australia	0/6,403	0.00
Aedes notoscriptus	Ritchie *et al.* (1997)	Australia	0/1	0.00
	van den Hurk *et al.* (2001)	Australia	0/2	0.00
Aedes oakleyi	Mitchell *et al.* (1993)	Saipan (Mariana islands)	0/5	0.00
Aedes palmarum	van den Hurk *et al.* (2001)	Australia	0/1	0.00
Aedes penghuensis	Weng, Lien, and Ji (2005)	Taiwan	0/2	0.00
	Su *et al.* (2014)	Taiwan	0/10	0.00
Aedes poicillius	Olson *et al.* (1985)	Indonesia	0/8	0.00
	Tan *et al.* (1993)	Indonesia	0/14	0.00
Aedes pseudoalbopictus	Weng *et al.* (1999)	Taiwan	0/1	0.00
Aedes purpureus	van den Hurk *et al.* (2001)	Australia	0/57	0.00
Aedes saipanensis	Mitchell *et al.* (1993)	Saipan (Mariana islands)	0/2	0.00
Aedes scutellaris	van den Hurk *et al.* (2001)	Australia	0/2	0.00
	van den Hurk *et al.* (2003)	India	1/1	100.00
Aedes stoneorum	Ritchie *et al.* (1997)	Australia	0/1	0.00
	van den Hurk *et al.* (2001)	Australia	0/5	0.00
Aedes subalbatus	Weng, Lien, and Ji (2005)	Taiwan	0/1	0.00
Aedes tremulus	Ritchie *et al.* (1997)	Australia	0/1	0.00
Aedes vexans	Dandawate *et al.* (1969)	India	0/23	0.00
	Olson *et al.* (1985)	Indonesia	0/16	0.00
	Mourya *et al.* (1989)	India	0/1	0.00
	Takashima *et al.* (1989)	Japan	0/383	0.00
	Vythilingam *et al.* (1997)	Malaysia	0/10	0.00
	Weng *et al.* (1999)	Taiwan	1/3	33.33
	Turell *et al.* (2003)	South Korea	0/4,334	0.00
	Su *et al.* (2014)	Taiwan	3/32	9.38
	Kim *et al.* (2015)	South Korea	/168	0.00
Aedes vexans nipponii	Fukumi *et al.* (1975)	Japan	4/44,926	0.01
	Kim *et al.* (2011)	South Korea	0/325	0.00
	Seo *et al.* (2013)	South Korea	0/106	0.00
Aedes vexans nocturnus	Hsu, Huang, and Cross (1978)	Taiwan	0/19	0.00
	Mitchell *et al.* (1993)	Saipan (Mariana islands)	0/16	0.00
	Weng, Lien, and Ji (2005)	Taiwan	1/9	11.11
Aedes vigilax	Ritchie *et al.* (1997)	Australia	0/5	0.00
	van den Hurk *et al.* (2001)	Australia	0/1,761	0.00

Aedes vittatus	Dhanda *et al.* (1989)	India	0/5	0.00
Anopheles spp.	Takashima *et al.* (1989)	Japan	0/1,093	0.00
	Bryant *et al.* (2005)	Vietnam	0/48	0.00
Anopheles aconitus	Tan *et al.* (1993)	Indonesia	0/1	0.00
Anopheles amictus	Johansen *et al.* (2003)	Australia	0/372	0.00
Anopheles annulipes	van den Hurk *et al.* (2001)	Australia	0/1	0.00
	Johansen *et al.* (2003)	Australia	0/104	0.00
Anopheles annullaris	Khan *et al.* (1981)	Bangladesh	0/1	0.00
	Olson *et al.* (1985)	Indonesia	1/28	3.57
	Tan *et al.* (1993)	Indonesia	0/35	0.00
Anopheles bancroftiii	van den Hurk *et al.* (2001)	Australia	0/156	0.00
	Johansen *et al.* (2003)	Australia	0/71,084	0.00
Anopheles barbirostris	Dandawate *et al.* (1969)	India	0/17	0.00
	Olson *et al.* (1985)	Indonesia	0/27	0.00
	Dhanda *et al.* (1989)	India	0/2	0.00
	Tan *et al.* (1993)	Indonesia	0/9	0.00
Anopheles culicifacies	Dandawate *et al.* (1969)	India	0/6	0.00
	Dhanda *et al.* (1989)	India	0/3	0.00
	Mourya *et al.* (1989)	India	0/1	0.00
Anopheles farauti	Ritchie *et al.* (1997)	Australia	0/6	0.00
	van den Hurk *et al.* (2001)	Australia	0/168	0.00
	Johansen *et al.* (2003)	Australia	0/8,600	0.00
Anopheles hilli	van den Hurk *et al.* (2001)	Australia	0/1	0.00
Anopheles hyrcanus	Dandawate *et al.* (1969)	India	0/91	0.00
	Bryant *et al.* (2005)	Vietnam	0/11	0.00
Anopheles indefinitus	Tan *et al.* (1993)	Indonesia	0/5	0.00
Anopheles kochi	Olson *et al.* (1985)	Indonesia	0/15	0.00
	Tan *et al.* (1993)	Indonesia	2/28	7.14
Anopheles lineatopennis	Olson *et al.* (1985)	Indonesia	0/24	0.00
Anopheles ludlowae	Su *et al.* (2014)	Taiwan	0/1	0.00
Anopheles maculatus	Tan *et al.* (1993)	Indonesia	0/54	0.00
Anopheles meraukensis	Johansen *et al.* (2003)	Australia	0/5	0.00
Anopheles minimus	Su *et al.* (2014)	Taiwan	1/7	14.29
Anopheles nigerrimus	Dhanda *et al.* (1989)	India	0/1	0.00
Anopheles pallidus	Dandawate *et al.* (1969)	India	0/73	0.00
	Dhanda *et al.* (1989)	India	0/8	0.00
Anopheles peditaeniatus	Khan *et al.* (1981)	Bangladesh	0/1	0.00
	Dhanda *et al.* (1989)	India	0/25	0.00
	Mourya *et al.* (1989)	India	1/133	0.75
Anopheles sinensis	Fukumi *et al.* (1975)	Japan	/37,798	0.00
	Hsu, Huang, and Cross (1978)	Taiwan	0/165	0.00
	Weng *et al.* (1999)	Taiwan	0/9	0.00
	Bryant *et al.* (2005)	Vietnam	0/387	0.00
	Weng, Lien, and Ji (2005)	Taiwan	0/18	0.00
	Sun *et al.* (2009)	China	0/3,853	0.00
	Feng *et al.* (2012)	China	3/14,170	0.02
	Su *et al.* (2014)	Taiwan	6/419	1.43
Anopheles sineroides	Fukumi *et al.* (1975)	Japan	/164	0.00

Anopheles stephensi	Tiawsirisup, Junpee, and Nuchprayoon (2012)	Thailand	0/3	0.00
Anopheles subpictus	Dandawate *et al.* (1969)	India	0/425	0.00
	Dhanda *et al.* (1989)	India	0/22	0.00
	Mourya *et al.* (1989)	India	1/87	1.15
	Dhanda *et al.* (1997)	India	1/163	0.61
	Thenmozhi *et al.* (2006)	India	98/982	9.98
	Upadhyayula *et al.* (2012)	India	0/14	0.00
Anopheles tessellatus	Hsu, Huang, and Cross (1978)	Taiwan	0/30	0.00
	Olson *et al.* (1985)	Indonesia	0/33	0.00
	Weng *et al.* (1999)	Taiwan	0/1	0.00
	Weng, Lien, and Ji (2005)	Taiwan	0/7	0.00
	Su *et al.* (2014)	Taiwan	2/31	6.45
Anopheles vagus	Dandawate *et al.* (1969)	India	0/4	0.00
	Khan *et al.* (1981)	Bangladesh	0/5	0.00
	Olson *et al.* (1985)	Indonesia	1/42	2.38
	Dhanda *et al.* (1989)	India	0/7	0.00
	Mourya *et al.* (1989)	India	0/30	0.00
	Tan *et al.* (1993)	Indonesia	3/93	3.23
	Bryant *et al.* (2005)	Vietnam	0/802	0.00
Armigeres spp.	Vythilingam *et al.* (1997)	Malaysia	0/5	0.00
Armigeres obturbans	Dandawate *et al.* (1969)	India	0/4	0.00
	Khan *et al.* (1981)	Bangladesh	0/1	0.00
Armigeres subalbatus	Fukumi *et al.* (1975)	Japan	1/11,666	0.01
	Hsu, Huang, and Cross (1978)	Taiwan	0/111	0.00
	Mourya *et al.* (1989)	India	0/22	0.00
	Tan *et al.* (1993)	Indonesia	3/114	2.63
	Vythilingam *et al.* (1997)	Malaysia	0/43	0.00
	Weng *et al.* (1999)	Taiwan	8/20	40.00
	Chen *et al.* (2000)	Taiwan	1/123	0.81
	Bryant *et al.* (2005)	Vietnam	0/532	0.00
	Sun *et al.* (2009)	China	0/1,249	0.00
	Kim *et al.* (2011)	South Korea	0/6	0.00
	Feng *et al.* (2012)	China	2/394	0.51
	Tiawsirisup, Junpee, and Nuchprayoon (2012)	Thailand	0/29	0.00
	Seo *et al.* (2013)	South Korea	0/9	0.00
	Su *et al.* (2014)	Taiwan	3/30	10.00
	Kim *et al.* (2015)	South Korea	0/145	0.00
Coquillettidia crassipes	Dhanda *et al.* (1989)	India	0/1	0.00
	Mourya *et al.* (1989)	India	0/1	0.00
	Vythilingam *et al.* (1997)	Malaysia	0/1	0.00
	van den Hurk *et al.* (2001)	Australia	0/86	0.00
	van den Hurk *et al.* (2003)	India	2/3	66.67
	Su *et al.* (2014)	Taiwan	0/3	0.00
Coquillettidia ochracea	Kim *et al.* (2015)	South Korea	0/14	0.00
Coquillettidia xanthogaster	Johansen *et al.* (2003)	Australia	0/129	0.00
Culex spp.	Mitchell *et al.* (1993)	Saipan (Mariana islands)	0/39	0.00

	Vythilingam *et al.* (1997)	Malaysia	0/1	0.00
	Johansen *et al.* (2003)	Australia	0/184	0.00
	Bryant *et al.* (2005)	Vietnam	0/1,210	0.00
	Tewari *et al.* (2008)	India	59/2,816	2.10
Culex annulirostris	Hanna *et al.* (1996)	Australia	8/2,871	0.28
	Ritchie *et al.* (1997)	Australia	8/134	5.97
	van den Hurk *et al.* (2001)	Australia	0/25,352	0.00
	van den Hurk *et al.* (2003)	India	2,368/3,197	74.07
Culex annulirostris marianae	Mitchell *et al.* (1993)	Saipan (Mariana islands)	0/5	0.00
Culex annulus	Cates and Detels (1969)	Taiwan	3/174	1.72
	Okuno *et al.* (1973)	Taiwan	6/91	6.59
	Wang (1975)	Taiwan	220/223	98.65
	Hsu, Huang, and Cross (1978)	Taiwan	31/703	4.41
	Rosen, Lien, and Lu (1989)	Taiwan	0/84,305	0.00
	Weng *et al.* (1999)	Taiwan	1/3	33.33
	Weng, Lien, and Ji (2005)	Taiwan	0/1	0.00
	Su *et al.* (2014)	Taiwan	9/79	11.39
Culex bitaeniorhynchus	Dandawate *et al.* (1969)	India	0/537	0.00
	Khan *et al.* (1981)	Bangladesh	0/1	0.00
	Olson *et al.* (1985)	Indonesia	0/13	0.00
	Dhanda *et al.* (1989)	India	0/41	0.00
	Mourya *et al.* (1989)	India	0/21	0.00
	Tan *et al.* (1993)	Indonesia	1/85	1.18
	Ritchie *et al.* (1997)	Australia	0/3	0.00
	Vythilingam *et al.* (1997)	Malaysia	0/1	0.00
	van den Hurk *et al.* (2001)	Australia	0/200	0.00
	Johansen *et al.* (2003)	Australia	0/386	0.00
	van den Hurk *et al.* (2003)	India	7/10	70.00
	Bryant *et al.* (2005)	Vietnam	0/64	0.00
	Tewari *et al.* (2008)	India	/1	0.00
	Kim *et al.* (2011)	South Korea	1/45	2.22
	Seo *et al.* (2013)	South Korea	1/26	3.85
	Su *et al.* (2014)	Taiwan	0/7	0.00
	Kim *et al.* (2015)	South Korea	0/16	0.00
Culex brevipalpis	Su *et al.* (2014)	Taiwan	0/1	0.00
Culex edwardsii	Dandawate *et al.* (1969)	India	0/3	0.00
Culex fatigans	Dandawate *et al.* (1969)	India	0/388	0.00
	Hsu, Huang, and Cross (1978)	Taiwan	0/53	0.00
Culex fuscanus	Dandawate *et al.* (1969)	India	0/50	0.00
	Dhanda *et al.* (1989)	India	0/7	0.00
	Mourya *et al.* (1989)	India	0/5	0.00
	Mitchell *et al.* (1993)	Saipan (Mariana islands)	0/2	0.00
	Weng *et al.* (1999)	Taiwan	1/2	50.00
	Bryant *et al.* (2005)	Vietnam	0/1	0.00
	Weng, Lien, and Ji (2005)	Taiwan	0/8	0.00
	Su *et al.* (2014)	Taiwan	0/3	0.00

Culex fuscocephala	Gould *et al.* (1973)	Thailand	2/142,375	0.00
	Wang (1975)	Taiwan	353/359	98.33
	Olson *et al.* (1985)	Indonesia	0/49	0.00
	Dhanda *et al.* (1989)	India	1/85	1.18
	Mourya *et al.* (1989)	India	2/257	0.78
	Peiris *et al.* (1992)	Sri Lanka	0/4,465	0.00
	Gajanana *et al.* (1997)	India	6/305	1.97
	Vythilingam *et al.* (1997)	Malaysia	2/76	2.63
	Bryant *et al.* (2005)	Vietnam	0/47	0.00
	Su *et al.* (2014)	Taiwan	3/19	15.79
Culex fuscocephalus	Dandawate *et al.* (1969)	India	0/113	0.00
	van Peenen and Joseph (1975)	Indonesia	1/12	8.33
	Hsu, Huang, and Cross (1978)	Taiwan	19/282	6.74
	Khan *et al.* (1981)	Bangladesh	0/6	0.00
	Burke *et al.* (1985)	Thailand	0/2,792	0.00
	Tan *et al.* (1993)	Indonesia	3/185	1.62
Culex gelidus	Dandawate *et al.* (1969)	India	0/82	0.00
	Gould *et al.* (1973)	Thailand	3/11,495	0.03
	van Peenen and Joseph (1975)	Indonesia	2/12	16.67
	Khan *et al.* (1981)	Bangladesh	0/11	0.00
	Burke *et al.* (1985)	Thailand	0/2,792	0.00
	Dhanda *et al.* (1989)	India	0/5	0.00
	Mourya *et al.* (1989)	India	4/127	3.15
	Peiris *et al.* (1992)	Sri Lanka	4/13,043	0.03
	Gajanana *et al.* (1997)	India	5/194	2.58
	Vythilingam *et al.* (1997)	Malaysia	12/224	5.36
	Johansen *et al.* (2003)	Australia	0/2	0.00
	van den Hurk *et al.* (2003)	India	13/16	81.25
	Bryant *et al.* (2005)	Vietnam	0/2,953	0.00
	Nitatpattana *et al.* (2005)	Thailand	0/2	0.00
	Samuel *et al.* (2008)	India	56/64	87.50
	Tewari *et al.* (2008)	India	4/177	2.26
	Arunachalam *et al.* (2009)	India	11/594	1.85
	Tiawsirisup and Nuchprayoon (2010)	Thailand	0/60	0.00
	Tiawsirisup, Junpee, and Nuchprayoon (2012)	Thailand	0/3	0.00
	Upadhyayula *et al.* (2012)	India	12/590	2.03
Culex hayshii	Kim *et al.* (2015)	South Korea	0/2	0.00
Culex inatomii	Seo *et al.* (2013)	South Korea	0/1	0.00
	Kim *et al.* (2015)	South Korea	0/16	0.00
Culex infula	Dhanda *et al.* (1989)	India	0/6	0.00
	Mourya *et al.* (1989)	India	0/2	0.00
Culex mimeticus	Su *et al.* (2014)	Taiwan	0/1	0.00
	Kim *et al.* (2015)	South Korea	0/1	0.00
Culex mimulus	Dandawate *et al.* (1969)	India	0/2	0.00
Culex minutissimus	Dhanda *et al.* (1989)	India	0/3	0.00
Culex murrelli	Su *et al.* (2014)	Taiwan	0/3	0.00

Culex nigropunctatus	Vythilingam *et al.* (1997)	Malaysia	0/1	0.00
	Su *et al.* (2014)	Taiwan	0/1	0.00
Culex orientalis	Kim *et al.* (2011)	South Korea	0/6	0.00
	Seo *et al.* (2013)	South Korea	0/2	0.00
	Kim *et al.* (2015)	South Korea	5/83	6.02
Culex palpalis	van den Hurk *et al.* (2003)	India	57/69	82.61
Culex perplexus	Das *et al.* (2005)	India	0/2	0.00
Culex pipiens	Buescher *et al.* (1959)	Australia	2/1,490	0.13
	Takashima *et al.* (1989)	Japan	0/1,092	0.00
	Turell *et al.* (2003)	South Korea	0/150	0.00
	Seo *et al.* (2013)	South Korea	4/64	6.25
	Kim *et al.* (2015)	South Korea	1/264	0.38
Culex pipiens fatigans	Okuno *et al.* (1973)	Taiwan	0/288	0.00
	Wang (1975)	Taiwan	65/66	98.48
Culex pipiens pallens	Fukumi *et al.* (1975)	Japan	2/2,783	0.07
	Kim *et al.* (2011)	South Korea	0/42	0.00
Culex pipiens quinquefasciatus	Tan *et al.* (1993)	Indonesia	10/333	3.00
Culex pluvialus	Dandawate *et al.* (1969)	India	0/39	0.00
Culex pseudovishnui	Fukumi *et al.* (1975)	Japan	8/21,012	0.04
	Olson *et al.* (1985)	Indonesia	0/7	0.00
	Dhanda *et al.* (1989)	India	3/81	3.70
	Mourya *et al.* (1989)	India	1/112	0.89
	Peiris *et al.* (1992)	Sri Lanka	0/116	0.00
	Victor *et al.* (2000)	India	0/2	0.00
	Bryant *et al.* (2005)	Vietnam	0/1	0.00
	Tewari *et al.* (2008)	India	/3	0.00
	Upadhyayula *et al.* (2012)	India	0/2	0.00
	Borah *et al.* (2013)	India	3/107	2.80
Culex pullus	van den Hurk *et al.* (2001)	Australia	0/7	0.00
	Johansen *et al.* (2003)	Australia	0/22	0.00
Culex quinquefasciatus	Dhanda *et al.* (1989)	India	0/12	0.00
	Mourya *et al.* (1989)	India	1/18	5.56
	Ritchie *et al.* (1997)	Australia	0/1	0.00
	Vythilingam *et al.* (1997)	Malaysia	1/48	2.08
	Weng *et al.* (1999)	Taiwan	7/31	22.58
	van den Hurk *et al.* (2001)	Australia	0/6	0.00
	van den Hurk *et al.* (2003)	India	7/8	87.50
	Bryant *et al.* (2005)	Vietnam	0/44	0.00
	Nitatpattana *et al.* (2005)	Thailand	2/25	8.00
	Weng, Lien, and Ji (2005)	Taiwan	0/31	0.00
	Tiawsirisup, Junpee, and Nuchprayoon (2012)	Thailand	0/75	0.00
	Su *et al.* (2014)	Taiwan	2/74	2.70
Culex raptor	Dandawate *et al.* (1969)	India	0/29	0.00
Culex rubensis	Kim *et al.* (2015)	South Korea	0/1	0.00
Culex rubithoracis	Weng, Lien, and Ji (2005)	Taiwan	4/22	18.18
	Su *et al.* (2014)	Taiwan	0/8	0.00

Culex sinensis	van Peenen and Joseph (1975)	Indonesia	0/2	0.00
	Khan *et al.* (1981)	Bangladesh	0/1	0.00
Culex sitiens	Mitchell *et al.* (1993)	Saipan (Mariana islands)	0/11	0.00
	Vythilingam *et al.* (1997)	Malaysia	0/58	0.00
	Weng *et al.* (1999)	Taiwan	2/2	100.00
	Johansen *et al.* (2001)	Australia	42/25,292	0.17
	van den Hurk *et al.* (2001)	Australia	0/21	0.00
	Johansen *et al.* (2003)	Australia	0/160,939	0.00
	van den Hurk *et al.* (2003)	India	3/8	37.50
	Weng, Lien, and Ji (2005)	Taiwan	1/34	2.94
	van den Hurk *et al.* (2006)	Australia	1/22,833	0.00
	Hall-Mendelin *et al.* (2012)	Australia	17/39,698	0.04
	Su *et al.* (2014)	Taiwan	0/128	0.00
Culex squamosus	van den Hurk *et al.* (2001)	Australia	0/20	0.00
Culex starckeae	Ritchie *et al.* (1997)	Australia	0/10	0.00
Culex tritaeniorhynchus	Buescher *et al.* (1959)	Australia	307/2,400	12.79
	Konno *et al.* (1966)	Japan	16/153	10.46
	Cates and Detels (1969)	Taiwan	0/121	0.00
	Gould *et al.* (1973)	Thailand	8/182,940	0.00
	Okuno *et al.* (1973)	Taiwan	6/91	6.59
	Fukumi *et al.* (1975)	Japan	435/598,434	0.07
	Hayashi *et al.* (1975)	Japan	19/216	8.80
	Wang (1975)	Taiwan	110/110	100.00
	van Peenen and Joseph (1975)	Indonesia	3/93	3.23
	Hsu, Huang, and Cross (1978)	Taiwan	18/267	6.74
	Khan *et al.* (1981)	Bangladesh	0/3	0.00
	Burke *et al.* (1985)	Thailand	0/2,792	0.00
	Olson *et al.* (1985)	Indonesia	1/596	0.17
	Dhanda *et al.* (1989)	India	3/117	2.56
	Mourya *et al.* (1989)	India	3/272	1.10
	Rosen, Lien, and Lu (1989)	Taiwan	165/524,290	0.03
	Takashima *et al.* (1989)	Japan	0/27	0.00
	Peiris *et al.* (1992)	Sri Lanka	4/17,436	0.02
	Mitchell *et al.* (1993)	Saipan (Mariana islands)	0/36	0.00
	Tan *et al.* (1993)	Indonesia	3/165	1.82
	Pant *et al.* (1994)	India	1/753	0.13
	Dhanda *et al.* (1997)	India	7/163	4.29
	Gajanana *et al.* (1997)	India	58/4,128	1.41
	Vythilingam *et al.* (1997)	Malaysia	24/731	3.28
	Weng *et al.* (1999)	Taiwan	97/294	32.99
	Victor *et al.* (2000)	India	2/10	20.00

	Turell *et al.* (2003)	South Korea	14/4,281	0.33
	Bryant *et al.* (2005)	Vietnam	0/18,634	0.00
	Das *et al.* (2005)	India	1/15	6.67
	Nitatpattana *et al.* (2005)	Thailand	0/2	0.00
	Weng, Lien, and Ji (2005)	Taiwan	95/1,061	8.95
	Tewari *et al.* (2008)	India	13/429	3.03
	Arunachalam *et al.* (2009)	India	19/951	2.00
	Sun *et al.* (2009)	China	12/14,840	0.08
	Tiawsirisup and Nuchprayoon (2010)	Thailand	0/60	0.00
	Kim *et al.* (2011)	South Korea	50/207	24.15
	Li *et al.* (2011)	China	1/97	1.03
	Feng *et al.* (2012)	China	15/37,119	0.04
	Tiawsirisup, Junpee, and Nuchprayoon (2012)	Thailand	0/55	0.00
	Upadhyayula *et al.* (2012)	India	19/972	1.95
	Borah *et al.* (2013)	India	19/281	6.76
	Seo *et al.* (2013)	South Korea	29/121	23.97
	Su *et al.* (2014)	Taiwan	468/2,242	20.87
	Kim *et al.* (2015)	South Korea	0/7	0.00
Culex univittattus	Dhanda *et al.* (1989)	India	1/29	3.45
	Mourya *et al.* (1989)	India	0/5	0.00
Culex vagans	Kim *et al.* (2015)	South Korea	0/2	0.00
Culex vicinus	van den Hurk *et al.* (2001)	Australia	0/5	0.00
	Johansen *et al.* (2003)	Australia	0/2,233	0.00
Culex vishnui	Dandawate *et al.* (1969)	India	2/5,553	0.04
	Khan *et al.* (1981)	Bangladesh	0/10	0.00
	Burke *et al.* (1985)	Thailand	0/2,792	0.00
	Olson *et al.* (1985)	Indonesia	0/104	0.00
	Dhanda *et al.* (1989)	India	0/59	0.00
	Mourya *et al.* (1989)	India	2/290	0.69
	Tan *et al.* (1993)	Indonesia	0/153	0.00
	Gajanana *et al.* (1997)	India	22/1,080	2.04
	Vythilingam *et al.* (1997)	Malaysia	0/2	0.00
	Victor *et al.* (2000)	India	0/1	0.00
	Bryant *et al.* (2005)	Vietnam	0/3,645	0.00
	Nitatpattana *et al.* (2005)	Thailand	0/1	0.00
	Tewari *et al.* (2008)	India	42/2,203	1.91
	Borah *et al.* (2013)	India	7/198	3.54
Culex whitmorei	Dandawate *et al.* (1969)	India	0/28	0.00
	van Peenen and Joseph (1975)	Indonesia	0/6	0.00
	Khan *et al.* (1981)	Bangladesh	0/1	0.00
	Olson *et al.* (1985)	Indonesia	0/62	0.00
	Dhanda *et al.* (1989)	India	1/20	5.00
	Mourya *et al.* (1989)	India	1/132	0.76
	Peiris *et al.* (1992)	Sri Lanka	1/167	0.60
	van den Hurk *et al.* (2001)	Australia	0/32	0.00
	Johansen *et al.* (2003)	Australia	0/4,873	0.00

	van den Hurk *et al.* (2003)	India	2/2	100.00
	Nitatpattana *et al.* (2005)	Thailand	0/2	0.00
Culiciomyia sp.	Nitatpattana *et al.* (2005)	Thailand	0/2	0.00
Culiseta bergrothi	Kim *et al.* (2015)	South Korea	0/1	0.00
Mansonia annulifera	Dandawate *et al.* (1969)	India	0/6	0.00
	Bryant *et al.* (2005)	Vietnam	0/315	0.00
Mansonia indiana	Dhanda *et al.* (1997)	India	1/163	0.61
	Bryant *et al.* (2005)	Vietnam	0/160	0.00
Mansonia septempunctata	van den Hurk *et al.* (2001)	Australia	0/1	0.00
	Johansen *et al.* (2003)	Australia	0/101	0.00
	van den Hurk *et al.* (2003)	India	4/14	28.57
Mansonia uniformis	Dandawate *et al.* (1969)	India	0/2	0.00
	Hsu, Huang, and Cross (1978)	Taiwan	0/20	0.00
	Mourya *et al.* (1989)	India	2/281	0.71
	Peiris *et al.* (1992)	Sri Lanka	0/582	0.00
	Dhanda *et al.* (1997)	India	3/163	1.84
	Vythilingam *et al.* (1997)	Malaysia	0/12	0.00
	van den Hurk *et al.* (2001)	Australia	0/92	0.00
	Johansen *et al.* (2003)	Australia	0/4,645	0.00
	van den Hurk *et al.* (2003)	India	11/11	100.00
	Weng, Lien, and Ji (2005)	Taiwan	0/10	0.00
	Kim *et al.* (2011)	South Korea	0/13	0.00
	Seo *et al.* (2013)	South Korea	0/1	0.00
	Su *et al.* (2014)	Taiwan	1/19	5.26
	Kim *et al.* (2015)	South Korea	0/66	0.00
Mimomyia chamberlaini	Dhanda *et al.* (1989)	India	0/2	0.00
Mimomyia luzonensis	Weng, Lien, and Ji (2005)	Taiwan	0/13	0.00
Ochleratus dorsalis	Kim *et al.* (2015)	South Korea	0/4	0.00
Ochleratus korelcus	Seo *et al.* (2013)	South Korea	0/24	0.00
	Kim *et al.* (2015)	South Korea	0/70	0.00
Ochleratus nipponicus	Seo *et al.* (2013)	South Korea	0/1	0.00
Ochleratus normanensis	van den Hurk *et al.* (2003)	India	100/310	32.26
Ochleratus vigilax	van den Hurk *et al.* (2003)	India	3/3	100.00
Ochleratus vittiger	van den Hurk *et al.* (2003)	India	1/1	100.00
Ochlerotatus albolateralis	Su *et al.* (2014)	Taiwan	0/1	0.00
Ochlerotatus kochi	Johansen *et al.* (2003)	Australia	0/2,047	0.00
Ochlerotatus littlechildi	Johansen *et al.* (2003)	Australia	0/3	0.00
Ochlerotatus normanensis	Johansen *et al.* (2003)	Australia	0/8,908	0.00
Ochlerotatus notoscriptus	Johansen *et al.* (2003)	Australia	0/37	0.00
Ochlerotatus palmarum	Johansen *et al.* (2003)	Australia	0/6	0.00
Ochlerotatus purpureus	Johansen *et al.* (2003)	Australia	0/2	0.00
Ochlerotatus rupestris	Johansen *et al.* (2003)	Australia	0/102	0.00
Ochlerotatus stoneorum	Johansen *et al.* (2003)	Australia	0/1	0.00
Ochlerotatus togoi	Su *et al.* (2014)	Taiwan	0/1	0.00
Ochlerotatus vigilax	Johansen *et al.* (2001)	Australia	1/3,073	0.03
	Johansen *et al.* (2003)	Australia	0/2,645	0.00
Topomyia spp.	Bryant *et al.* (2005)	Vietnam	0/1	0.00
Tripteroides bambusa	Kim *et al.* (2015)	South Korea	0/9	0.00

	Takashima *et al.* (1989)	Japan	0/128	0.00
Tripteroides magnesianus	van den Hurk *et al.* (2001)	Australia	0/10	0.00
	Johansen *et al.* (2003)	Australia	0/4	0.00
Uranotaenia spp.	Dandawate *et al.* (1969)	India	0/2	0.00
	Vythilingam *et al.* (1997)	Malaysia	0/5	0.00
	Johansen *et al.* (2003)	Australia	0/3	0.00
	Bryant *et al.* (2005)	Vietnam	0/57	0.00
Uranoteania novobscura	Weng *et al.* (1999)	Taiwan	0/1	0.00
Uranotenia macfarlanei	Su *et al.* (2014)	Taiwan	0/1	0.00
Verrallina carmenti	Johansen *et al.* (2003)	Australia	0/49	0.00
Verrallina funerea	van den Hurk *et al.* (2003)	India	3/5	60.00

[1] Mosquito species refer to genus, (Subgenus), species, and subspecies, as reported by the authors of the articles.
[2] Mosquito pools = 1 to 800 mosquitoes

Appendix C - JEV infection, dissemination, and transmission rates, for days post infection (DPI) across all experimental studies (n=33), by author, year of publication, and by mosquito species (ordered alphabetically).

Author (year)	Mosquito Name	DPI	Proportion infected[1]	Infection rate (%)[2]	Proportion disseminated[3]	Dissemination rate (%)[4]	Proportion transmitted[5]	Transmission rate (%)[6]	Mosquitoes/pool
Reeves and Hammon (1946)	*Aedes dorsalis*	16	1/31	3.23	-	-	-	-	-
	Aedes nigromaculis	8-14	4/217	1.84	-	-	-	-	-
	Aedes varipalpus	6-14	0/153	0.00	-	-	-	-	-
	Aedes vexans	8-27	0/98	0.00	-	-	-	-	-
	Anopheles maculipennis freeborni	0-16	0/119	0.00	-	-	-	-	-
	Culex pipiens molestus	7-20	3/216	1.39	-	-	-	-	-
	Culex pipiens (pipiens)	20	2/15	13.33	-	-	-	-	-
	Culex quinquefasciatus	11-25	4/664	0.60	-	-	-	-	-
	Culex tarsalis	6-10	2/165	1.21	-	-	-	-	-
	Culiseta incidens	8-14	3/74	4.05	-	-	-	-	-
	Culiseta inornata	10-20	3/82	3.66	-	-	-	-	-
Hurlbut (1950)	*Culex quinquefasciatus*	6	5/5	100.00	-	-	-	-	-
		3	5/5	100.00	-	-	-	-	-
Gresser *et al.* (1958)	*Culex tritaeniorhynchus*	18	7/8	87.50	-	-	-	-	-
		18	1/1	100.00	-	-	-	-	-

		18	2/2	100.00	-	-	-	-	-
		18	7/8	87.50	-	-	-	-	-
		18	1/1	100.00	-	-	-	-	-
		18	1/1	100.00	-	-	-	-	-
		18	1/1	100.00	-	-	-	-	-
Gould, Barnett, and Suyemoto (1962)	*Culex gelidus*	6 - 21	-	-	-	-	1/13	8.00	-
		6 - 21	-	-	-	-	16/73	21.92	-
		6 - 21	-	-	-	-	19/38	50.00	-
		6 - 21	-	-	-	-	79/126	62.70	-
		21-22	-	-	-	-	0/275	0.00	-
Gould, Byrne, and Hayes (1964)	*Culex gelidus*	4	-	-	-	-	0/27	0.00	-
		5	-	-	-	-	0/6	0.00	-
		6	-	-	-	-	0/13	0.00	-
	Culex tritaeniorhynchus	4	-	-	-	-	0/25	0.00	-
		5	-	-	-	-	0/8	0.00	-
		6	-	-	-	-	1/29	3.45	-
Hurlbut (1964)	*Aedes albopictus*	14	0/10	0.00	-	-	-	-	-
		14	0/12	0.00	-	-	-	-	-
		14	0/13	0.00	-	-	-	-	-
		14	0/12	0.00	-	-	-	-	-
		14	0/10	0.00	-	-	-	-	-
		14	0/27	0.00	-	-	-	-	-
		14	0/14	0.00	-	-	-	-	-

		14	0/16	0.00	-	-	-	-	-
	Culex tritaeniorhynchus	14	4/26	15.38	-	-	-	-	-
		14	1/19	5.26	-	-	-	-	-
		14	12/25	48.00	-	-	-	-	-
		14	23/24	95.83	-	-	-	-	-
		14	1/22	4.55	-	-	-	-	-
		14	4/27	14.81	-	-	-	-	-
		14	11/30	36.67	-	-	-	-	-
		14	2/28	7.14	-	-	-	-	-
Doi, Shirasaka, and Sasa (1967)	*Culex tritaeniorhynchus*	6	4/5	80.00	-	-	-	-	-
		9	6/7	85.71	-	-	-	-	-
		12	7/7	100.00	-	-	-	-	-
		15	6/6	100.00	-	-	-	-	-
		5	1/4	25.00	-	-	-	-	-
		6	1/5	20.00	-	-	-	-	-
		7	3/5	60.00	-	-	-	-	-
		8	0/5	0.00	-	-	-	-	-
		9	4/5	80.00	-	-	-	-	-
		10	3/5	60.00	-	-	-	-	-
		28	2/4	50.00	-	-	-	-	-
		42	3/3	100.00	-	-	-	-	-
Doi *et al.* (1970)	*Culex pipiens*	31	7/7	100.00	-	-	-	-	-
		4	0/5	0.00	-	-	-	-	-
		6	2/5	40.00	-	-	-	-	-

	8	3/5	60.00	-	-	-	-	-
	10	2/5	40.00	-	-	-	-	-
	14	2/5	40.00	-	-	-	-	-
	20	1/5	20.00	-	-	-	-	-
	30	1/6	16.67	-	-	-	-	-
	15	7/11	63.64	-	-	-	-	-
	19	4/5	80.00	-	-	-	-	-
	24	5/5	100.00	-	-	-	-	-
	27	1/5	20.00	-	-	-	-	-
	14	4/7	57.14	-	-	-	-	-
	3	6/6	100.00	-	-	-	-	-
	4	8/8	100.00	-	-	-	-	-
	6	7/7	100.00	-	-	-	-	-
	8	5/5	100.00	-	-	-	-	-
	10	5/5	100.00	-	-	-	-	-
	11	5/6	83.33	-	-	-	-	-
	16	12/12	100.00	-	-	-	-	-
Culex tritaeniorhynchus	10	8/15	53.33	-	-	-	-	-
	17	2/2	100.00	-	-	-	-	-
	3	10/10	100.00	-	-	-	-	-
	7	10/10	100.00	-	-	-	-	-
	10	5/5	100.00	-	-	-	-	-
	15	9/9	100.00	-	-	-	-	-
	21	7/7	100.00	-	-	-	-	-

Muangman *et al.* (1972)	*Culex fuscocephala*	10	19/20	95.00	-	-	1/10	10.00	-
		11	20/20	100.00	-	-	2/10	20.00	-
		19	20/20	100.00	-	-	2/10	20.00	-
		27	14/15	93.33	-	-	1/8	12.50	-
	Culex tritaeniorhynchus	10	20/20	100.00	-	-	0/10	0.00	-
		11	18/20	90.00	-	-	1/10	10.00	-
		19	18/20	90.00	-	-	4/10	40.00	-
		27	18/20	90.00	-	-	2/10	20.00	-
Doi *et al.* (1977)	*Culex pipiens fatigans*	10-14	0/17	0.00	-	-	-	-	-
		10-14	1/24	4.20	-	-	-	-	-
		10-14	8/21	38.10	-	-	-	-	-
	Culex pipiens pallens	10-14	0/23	0.00	-	-	-	-	-
		10-14	0/17	0.00	-	-	-	-	-
		10-14	1/12	8.30	-	-	-	-	-
		10-14	5/34	14.70	-	-	-	-	-
		10-14	19/28	67.80	-	-	-	-	-
		10-14	18/18	100.00	-	-	-	-	-
	Culex pseudovishnui	10-14	0/19	0.00	-	-	-	-	-
		10-14	8/31	25.80	-	-	-	-	-
		10-14	2/27	7.40	-	-	-	-	-
		10-14	3/59	5.10	-	-	-	-	-
	Culex tritaeniorhynchus	10-14	8/9	88.90	-	-	-	-	-
		10-14	11/14	78.60	-	-	-	-	-
		10-14	17/17	100.00	-	-	-	-	-

Rosen *et al.* (1978)	Aedes albopictus	-	17/40	42.50	-	-	-	-	100
		-	7/41	17.07	-	-	-	-	1-120
		-	5/52	9.62	-	-	-	-	1-120
		-	3/4	75.00	-	-	-	-	1-120
		-	1/10	10.00	-	-	-	-	1-120
	Aedes togoi	-	4/12	33.33	-	-	-	-	1-120
		-	8/19	42.11	-	-	-	-	1-120
		-	5/6	83.33	-	-	-	-	1-120
		-	3/7	42.86	-	-	-	-	1-120
Rosen, Shroyer, and Lien (1980)	Culex tritaeniorhynchus	-	22/51	43.14	-	-	-	-	-
		-	7/14	50.00	-	-	-	-	-
		-	12/34	35.29	-	-	-	-	-
		-	0/22	0.00	-	-	-	-	-
Takahashi (1982)	*Culex tritaeniorhynchus*	10-14	19/20	95.00	-	-	19/19	100.00	-
		10-14	18/20	90.00	-	-	16/20	80.00	-
		10-14	16/20	80.00	-	-	16/16	100.00	-
		10-14	20/20	100.00	-	-	7/20	35.00	-
		10-14	16/20	80.00	-	-	9/20	45.00	-
		10-14	14/20	70.00	-	-	11/14	78.57	-
Rosen and Shroyer (1985)	*Toxorhynchites amboinensis*	14	5/5	100.00	-	-	-	-	-
	Toxorhynchites brevipalpis	14	5/5	100.00	-	-	-	-	-
	Toxorhynchites rutilus	14	5/5	100.00	-	-	-	-	-
	Toxorhynchites Splendens	14	5/5	100.00	-	-	-	-	-
	Toxorhynchites Theobaldi	14	5/5	100.00	-	-	-	-	-

Rosen (1988)	*Aedes albopictus*	2-3	0/32	0.00	-	-	-	-	≤100
		3-4	1/37	2.70	-	-	-	-	≤100
		4-5	10/44	22.73	-	-	-	-	≤100
		5-6	11/35	31.43	-	-	-	-	≤100
		6-7	8/34	23.53	-	-	-	-	≤100
		7-8	3/37	8.11	-	-	-	-	≤100
		8-9	4/24	16.67	-	-	-	-	≤100
		9-10	0/26	0.00	-	-	-	-	≤100
Rosen *et al.* (1989)	Aedes alcasidi	-	2/7	28.57	-	-	-	-	100
	Aedes vexans	-	1/24	4.17	-	-	-	-	100
	Armigeres flavus	-	1/15	6.67	-	-	-	-	100
	Armigeres subalbatus	-	14/36	38.89	-	-	-	-	100
	Culex annulus	-	0/24	0.00	-	-	-	-	100
		-	5/8	62.50	-	-	-	-	100
		-	8/9	88.89	-	-	-	-	100
		-	1/4	25.00	-	-	-	-	100
		-	0/25	0.00	-	-	-	-	100
		-	1/8	12.50	-	-	-	-	100
		-	1/5	20.00	-	-	-	-	100
		-	0/2	0.00	-	-	-	-	100
	Culex pipiens molestus	-	5/37	13.51	-	-	-	-	100
	Culex pipiens pallens	-	1/52	1.92	-	-	-	-	100
	Culex quinquefasciatus	-	3/78	3.85	-	-	-	-	100
		-	1/64	1.56	-	-	-	-	100
		-	1/24	4.17	-	-	-	-	100

		-	7/94	7.45	-	-	-	-	100
		-	1/21	4.76	-	-	-	-	100
	Culex tritaeniorhynchus	-	66/174	37.93	-	-	-	-	100
		-	27/173	15.61	-	-	-	-	100
		-	73/208	35.10	-	-	-	-	100
		-	40/208	19.23	-	-	-	-	100
Takashima and Rosen (1989)	*Aedes japonicus*	1-20	18/20	90.00	-	-	3/4	75.00	-
		1-20	9/20	45.00	-	-	2/6	33.30	-
	Aedes vexans nipponii	1-20	3/12	25.00	-	-	-	-	-
	Culex pipiens pallens	1-20	3/10	30.00	-	-	-	-	-
	Culex tritaeniorhynchus	1-20	15/15	100.00	-	-	6/6	100.00	-
Weng *et al.* (1997)	*Aedes albopictus*	14	-	-	-	-	5/13	38.46	-
		14	-	-	-	-	5/11	45.45	-
		14	-	-	-	-	3/11	27.27	-
Samuel *et al.* (1998)	*Culex tritaeniorhynchus*	12-14	17/26	65.40	-	-	3/19	15.79	-
		12-14	19/24	79.00	-	-	1/24	4.17	-
		12-14	21/33	63.60	-	-	0/14	0.00	-
		12-14	-	-	-	-	6/19	31.58	-
		12-14	23/24	95.80	-	-	17/23	73.91	-
		12-14	10/14	71.40	-	-	5/14	35.71	-
Weng *et al.* (2000)	*Culex pipiens molestus*	0	-	-	-	-	4/5	80.00	-
		3	-	-	-	-	3/3	100.00	-
		7	-	-	-	-	3/3	100.00	-
	Culex tritaeniorhynchus	0	-	-	-	-	6/6	100.00	-

		10	-	-	-	-	8/8	100.00	-
		13	-	-	-	-	6/6	100.00	-
Chen *et al.* (2000)	*Aedes aegypti*	14	0/6	0.00	-	-	-	-	-
	Aedes albopictus	14	7/15	46.67	-	-	-	-	-
	Armigeres subalbatus	14	7/8	87.50	-	-	-	-	-
		1	0/8	0.00	-	-	-	-	-
		5	1/9	11.11	-	-	-	-	-
		10	2/8	25.00	-	-	-	-	-
		15	4/10	40.00	-	-	-	-	-
		20	11/14	78.57	-	-	-	-	-
	Culex quinquefasciatus	14	2/5	40.00	-	-	-	-	-
Mourya and Mishra (2000)	*Culex pseudovishnui*	1	0/10	0.00	-	-	-	-	-
		2	3/10	30.00	-	-	-	-	-
		3	5/10	50.00	-	-	-	-	-
		4	6/10	60.00	-	-	-	-	-
		5	4/10	40.00	-	-	-	-	-
		6	7/10	70.00	-	-	-	-	-
		7	7/10	70.00	-	-	-	-	-
		8	7/10	70.00	-	-	-	-	-
		9	4/10	40.00	-	-	-	-	-
		10	6/10	60.00	-	-	-	-	-
		1	3/10	30.00	-	-	-	-	-
		2	5/10	50.00	-	-	-	-	-
		3	6/10	60.00	-	-	-	-	-

	4	4/10	40.00	-	-	-	-	-
	5	6/10	60.00	-	-	-	-	-
	6	6/10	60.00	-	-	-	-	-
	7	4/10	40.00	-	-	-	-	-
	8	6/10	60.00	-	-	-	-	-
	9	5/10	50.00	-	-	-	-	-
	10	6/10	60.00	-	-	-	-	-
	1	3/108	2.78	-	-	-	-	-
	2	9/114	7.89	-	-	-	-	-
	3	20/108	18.52	-	-	-	-	-
	4	19/114	16.67	-	-	-	-	-
	5	23/120	19.17	-	-	-	-	-
	6	23/114	20.18	-	-	-	-	-
	7	24/114	21.05	-	-	-	-	-
	8	40/114	35.09	-	-	-	-	-
	9	34/108	31.48	-	-	-	-	-
	10	31/102	30.39	-	-	-	-	-
Culex tritaeniorhynchus	1	0/10	0.00	-	-	-	-	-
	2	3/10	30.00	-	-	-	-	-
	3	4/10	40.00	-	-	-	-	-
	4	6/10	60.00	-	-	-	-	-
	5	6/10	60.00	-	-	-	-	-
	6	4/10	40.00	-	-	-	-	-
	7	6/10	60.00	-	-	-	-	-
	8	7/10	70.00	-	-	-	-	-

	9	7/10	70.00	-	-	-	-	-
	10	8/10	80.00	-	-	-	-	-
	1	3/10	30.00	-	-	-	-	-
	2	5/10	50.00	-	-	-	-	-
	3	7/10	70.00	-	-	-	-	-
	4	6/10	60.00	-	-	-	-	-
	5	6/10	60.00	-	-	-	-	-
	6	6/10	60.00	-	-	-	-	-
	7	5/10	50.00	-	-	-	-	-
	8	7/10	70.00	-	-	-	-	-
	9	6/10	60.00	-	-	-	-	-
	10	6/10	60.00	-	-	-	-	-
	1	3/108	2.78	-	-	-	-	-
	2	8/108	7.41	-	-	-	-	-
	3	27/102	26.47	-	-	-	-	-
	4	42/108	38.89	-	-	-	-	-
	5	44/102	43.14	-	-	-	-	-
	6	41/108	37.96	-	-	-	-	-
	7	38/96	39.58	-	-	-	-	-
	8	58/108	53.70	-	-	-	-	-
	9	44/108	40.74	-	-	-	-	-
	10	53/108	49.07	-	-	-	-	-
Culex vishnui	1	0/10	0.00	-	-	-	-	-
	2	2/10	20.00	-	-	-	-	-
	3	4/10	40.00	-	-	-	-	-

4	3/10	30.00	-	-	-	-	-
5	5/10	50.00	-	-	-	-	-
6	4/10	40.00	-	-	-	-	-
7	5/10	50.00	-	-	-	-	-
8	4/10	40.00	-	-	-	-	-
9	3/10	30.00	-	-	-	-	-
10	4/10	40.00	-	-	-	-	-
1	2/10	20.00	-	-	-	-	-
2	4/10	40.00	-	-	-	-	-
3	6/10	60.00	-	-	-	-	-
4	6/10	60.00	-	-	-	-	-
5	5/10	50.00	-	-	-	-	-
6	6/10	60.00	-	-	-	-	-
7	4/10	40.00	-	-	-	-	-
8	5/10	50.00	-	-	-	-	-
9	4/10	40.00	-	-	-	-	-
10	6/10	60.00	-	-	-	-	-
1	2/108	1.85	-	-	-	-	-
2	7/114	6.14	-	-	-	-	-
3	14/102	13.73	-	-	-	-	-
4	19/114	16.67	-	-	-	-	-
5	24/120	20.00	-	-	-	-	-
6	20/114	17.54	-	-	-	-	-
7	26/102	25.49	-	-	-	-	-
8	24/114	21.05	-	-	-	-	-

		9	26/114	22.81	-	-	-	-	-
		10	26/114	22.81	-	-	-	-	-
van den Hurk *et al.* (2003)	*Aedes aegypti*	14-15	16/60	26.67	0/60		15/60	25.00	-
	Coquillettidia xanthogaster	14-15	4/36	11.11	-	-	1/15	6.67	-
		14-15	0/1	0.00	-	-	0/1	0.00	-
	Culex annulirostris	5	14/18	78.00	1/18	5.56	-	-	-
		7	16/18	89.00	6/18	33.33	4/17	82.35	-
		10	17/18	94.00	14/18	77.78	8/14	57.14	-
		14	36/36	100.00	23/36	63.89	13/16	81.25	-
		14-15	2/2	100.00	-	-	0/2	0.00	-
	Culex gelidus	14-15	4/4	100.00	-	-	1/1	100.00	-
	Culex quinquefasciatus	17-19	50/51	98.00	14/51	27.45	4/8	50.00	-
		14-15	51/55	92.73	-	-	14/23	60.87	-
		14-15	15/27	55.56	-	-	0/16	0.00	-
	Culex sitiens	5	15/18	83.00	1/18	5.56	-	-	-
		7	15/18	83.00	5/18	27.78	2/15	13.33	-
		10	16/18	89.00	6/18	33.33	1/15	6.67	-
		14	33/36	92.00	4/36	11.11	10/15	66.67	-
		14-15	1/1	100.00	0/1	0.00	1/1	100.00	-
	Mansonia septempunctata	9	16/24	66.67	0/24	0.00	13/24	54.17	-
	Mansonia uniformis	14-15	1/1	100.00	0/1	0.00	1/1	100.00	-
	Ochlerotatus kochi	14-15	6/28	21.43	-	-	0/8	0.00	-
	Ochlerotatus normanensis	14-15	0/1	0.00	0/1	0.00	0/1	0.00	-
	Ochlerotatus notoscriptus	13-14	13/48	27.00	4/48	8.33	3/11	27.27	-

		14-15	1/5	20.00	0/5	0.00	1/5	20.00	-
	Ochlerotatus purpureus	14-15	2/2	100.00	0/2	0.00	2/2	100.00	-
	Ochlerotatus vigilax	9	12/62	19.00	11/62	17.74	-	-	-
		13	5/13	39.00	5/13	38.46	0/4	0.00	-
		14-15	1/9	11.11	-	-	1/8	12.50	-
	Verrallina carmenti	14-15	0/2	0.00	0/2	0.00	0/2	0.00	-
	Verrallina funerea	14-15	43/75	57.33	-	-	3/18	16.67	-
Turell *et al.* (2006a)	*Culex pipiens pallens*	12	0/40	0.00	-	-	-	-	-
		12	2/32	6.25	-	-	-	-	-
	Culex tritaeniorhynchus	12	10/10	100.00	-	-	-	-	-
		12	14/14	100.00	-	-	-	-	-
Turell *et al.* (2006b)	*Culex pipiens*	16-17	28/50	56.00	-	26.00	-	-	-
		16-17	25/53	47.17	-	25.00	2/24	8.33	-
		25-27	20/39	51.28	-	26.00	1/13	7.69	-
van den Hurk *et al.* (2007)	*Culex annulirostris Skuse*	13	22/23	95.65	-	-	-	96.00	-
	Culex gelidus	13	20/25	80.00	-	-	-	12.00	-
		15	6/7	85.71	-	-	-	86.00	-
		15	5/20	25.00	-	-	-	25.00	-
Johnson *et al.* (2009)	*Culex annulirostris*	12	20/25	80.00	14/56	25.00	3/12	25.00	-
	Culex gelidus	12	22/23	96.00	22/96	22.92	22/96	23.00	-
van den Hurk *et al.* (2009)	*Culex annulirostris*	3	2/3	66.67	-	-	-	-	-
		4	1/3	33.33	-	-	-	-	-
		4	2/2	100.00	-	-	-	-	-

		4	1/4	25.00	-	-	-	-	-
		5	1/4	25.00	-	-	-	-	-
Kramer *et al.* (2011)	*Aedes notoscriptus*	14	0/39	0.00	-	-	-	-	-
	Culex pipiens	14	5/50	10.00	2/5	40.00	0/5	0.00	-
	Culex quinquefasciatus	14	6/36	16.67	0/6	0.00	-	-	-
		14	43/50	86.00	0/43	0.00	0/43	0.00	-
		120	1/16	6.25	1/1	100.00	-	-	-
	Opifex fuscus	14	37/50	74.00	26/37	70.27	0/37	0.00	-
Huber *et al.* (2014)	*Aedes japonicus japonicus*	0-14	3/3	100.00	-	-	-	-	-
		0-15	4/4	100.00	-	-	-	-	-
Nicholson, Ritchie, and van Den Hurk (2014)	*Aedes albopictus*	14	-	-	-	16.00	-	16.00	-
		14	5/25	20.00	-	80.00	-	100.00	-
		14	1/25	4.00	-	-	-	-	-
		14	0/25	0.00	-	-	-	-	-
Huang *et al.* (2015)	*Culex quinquefasciatus*	7	12/12	100.00	0/8	0.00	-	-	-
		14	22/26	84.60	7/14	50.00	-	-	-
Mackenzie-Impoinvil *et al.* (2015)	*Culex quinquefasciatus*	7	6/24	25.00	20/24	83.33	16/24	66.67	-
		14	20/32	62.00	18/32	56.25	2/32	6.25	-
		21	7/10	70.00	10/10	100.00	7/10	70.00	-
		7	4/9	44.00	0/9	0.00	0/9	0.00	-
		14	8/12	66.00	10/12	83.33	3/12	25.00	-
		21	7/10	70.00	10/10	100.00	10/10	100.00	-
	Ochlerotatus detritus	7	8/25	32.00	16/25	64.00	9/25	36.00	-

14	25/32	78.00	29/32	90.63	1/32	3.13	-
21	6/6	100.00	6/6	100.00	4/6	66.67	-
7	9/15	60.00	15/15	100.00	7/15	46.67	-
14	3/6	50.00	4/6	66.67	2/6	33.33	-
21	3/3	100.00	3/3	100.00	1/3	33.33	-

[1] Proportion infected is the number of positive infected mosquitoes divided by the total number of mosquitoes tested.
[2] Infection rate is an estimate of the prevalence of infection in a mosquito population (Bustamante and Lord, 2010).
[3] Proportion disseminated is the number of positive mosquitoes with disseminated infection divided by the total number of mosquitoes tested.
[4] Dissemination rate refers to the proportion of mosquitoes containing virus in their legs, regardless of their infection status (Golnar *et al.*, 2015).

[5] Proportion transmitted is the number of positive mosquitoes that transmit the virus divided by the total number of mosquitoes tested.
[6] Transmission rate refers to the proportion of mosquitoes with a disseminated infection that transmit the virus after refeeding (Golnar *et al.*, 2015).