Am. J. Trop. Med. Hyg., 98(3), 2018, pp. 883–890
doi:10.4269/ajtmh.17-0622

Meta-Analyses of Japanese Encephalitis Virus Infection, Dissemination, and Transmission Rates in Vectors

Ana R. S. Oliveira,[1] Lee W. Cohnstaedt,[2] Erin Strathe,[3] Luciana Etcheverry,[1] D. Scott McVey,[2] José Piaggio,[4] and Natalia Cernicchiaro[1]*

[1]*Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas;* [2]*USDA-ARS Arthropod-Borne Animal Diseases Research, Manhattan, Kansas;* [3]*Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas;* [4]*School of Veterinary Medicine, University of the Republic, Montevideo, Uruguay*

Abstract. The objective of this work was to summarize and quantify Japanese encephalitis virus (JEV) infection, dissemination, and transmission rates in mosquitoes, using a meta-analysis approach. Data were obtained from experimental studies, gathered by means of a systematic review of the literature. Random-effects subgroup meta-analysis models by mosquito species were fitted to estimate pooled estimates and to calculate the variance between studies for 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 outcome JEV infection rate in vectors, we fitted univariable meta-regression models. Mosquito species and administration route represented the main sources of heterogeneity associated with JEV infection rate in vectors. This study provided summary effect size estimates to be used as reference for other investigators when assessing transmission efficiency of vectors and explored sources of variability for JEV infection rates 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 toward more informed and targeted actions to prevent and control JEV in both affected and susceptible regions worldwide.

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.[1,2] Most of China, Southeast Asia, and the Indian subcontinent experience JE outbreaks, with 75% of JE cases occurring in children up to 14 years old. Clinical disease varies from flu-like to severe neuropsychiatric symptoms.[3,4]

Across all endemic regions, JEV is transmitted by mosquitoes, mainly from the *Culex* genus, and its epidemiology is complex and dynamic. The most known competent vectors within the *Culex* genus are: *Culex tritaeniorhynchus*, *Culex annulirostris*, *Culex annulus*, *Culex fuscocephala*, *Culex gelidus*, *Culex sitiens*, and the *Culex vishnui* species complex.[5] 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.[6] 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.[1,2,5] 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.[7] 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.[6,8–10]

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.[10] 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.[11]

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.[12,13] 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.[12–16] To thoroughly assess infection, dissemination, and transmission rates of mosquitoes, we carried out a meta-analysis with the objective of quantitatively assessing vector competence from experimental studies, using data obtained from a systematic review of the literature.

METHODS

Systematic review of the literature. The first step of the systematic review was to determine the research question and search the literature in eight electronic databases and journal

---

* Address correspondence to Natalia Cernicchiaro, Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Coles Hall 332, 1800 Denison Avenue, Manhattan, KS 66506. E-mail: ncernic@vet.k-state.edu

websites. These consisted of 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. We performed an additional hand search of references of nine key publications on JE and JEV (available in Supplemental Table 1 of the Supplemental Materials) and all literature searches were conducted between March and April 2016.

After identifying the articles, we screened the abstracts for relevance, according to a set of inclusion and exclusion criteria (available in the supplementary materials). The relevance screening step was performed by two reviewers (A.R.S.O. and L.E.), who worked independently and resolved all conflicts by consensus or by consulting a third reviewer (N.C.). Data from the relevant articles were subsequently retrieved and recorded in an Excel (Microsoft Corp., Redmond WA) spreadsheet template.

The assessment of the risk of bias, which aimed at evaluating the internal and external validity of the relevant articles, was performed based on a set of specific criteria that considered 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).

A complete list of search terms, summary of search results, inclusion and exclusion criteria, outcomes, and identification of key domains for the risk of bias assessment in observational and experimental studies are available elsewhere.[17] The references of the articles included in the systematic review are included in the supplementary materials (Supplemental Table 2). We followed the guidelines described by Sargeant and O'Connor[13,18] and O'Connor et al.[12,19] to perform the systematic review, and the Cochrane Review Handbook guidelines[20] to conduct the risk of bias assessment.

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 rate in vectors, JEV dissemination rate in vectors, and JEV transmission rate 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.,[21] 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 that were orally exposed to JEV and transmitted the virus on refeeding or contained the virus in their saliva or salivary glands. All observations pertaining to all mosquito species were included in the assessment of our outcomes of interest with each entry corresponding to an observation within each outcome. Several entries may pertain to the same article, given that different records of the same mosquito species (under different temperatures or incubation periods) may be reported within the same article. Although the outcomes are referred to as rates, infection, dissemination, and transmission events are actually proportions, as the denominator does not include a time component.[22] Nonetheless, because these are the terms most 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 (SE) of the logit of the rates were computed, following the formulae provided by Sanchez et al.[23]:

$$\text{Logit proportion} = \ln\left(\frac{p}{1-p}\right) \quad \text{SE} = \sqrt{\frac{1}{n \times p \times (1-p)}},$$

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 (CI) were back-transformed for interpretation purposes,[24] using the following formula:

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

Because we assumed a priori that there was substantial heterogeneity among the studies, we fitted a random-effects meta-analysis model. We used the method of DerSimonian and Laird[25] to estimate the variance between studies, using a restricted maximum likelihood (REML) algorithm. Weights are reported as the percentage of the overall total and were computed by mosquito species using a variation of the inverse-variance approach.[25] Using the *metan* command in Stata-SE 12.0 (Stata Corp., College Station, TX), 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 fitted with a REML method using the *metareg* command in Stata-SE 12.0. Outcomes and within-study SE were logit transformed. Random effects meta-regression assumes that the true effects follow a normal distribution around the linear predictor accounting for residual heterogeneity and including study-level predictors[22] as follows:

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

where β_0 is the intercept, βX_j is the coefficient for the jth predictor, μ_j is the effect of study j, ε_j is the error term (differences between studies due to sampling variation), $\mu_j \sim N(0, \tau^2)$, and $\varepsilon \sim N(0, \sigma_j^2)$.

For the quantification of heterogeneity between studies, we used I-squared (I^2), which depicts the proportion of total variability in point estimates that can be attributed to heterogeneity.[19] I-squared values were interpreted following the recommendations by O'Connor et al.[19] 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, when possible, 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 1 provides a detailed description of these predictors.

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, which would prevent us from building multivariable models, 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 [22]).

Predictors and outcomes. Predictors of interest were selected for model inclusion based on biological importance and completeness of observations, and their definition is given in Table 1.

Our main predictor of interest was mosquito species, which included 50 genera or species (as reported in the articles).

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

RESULTS

Systematic review of the literature. Thirty-three experimental studies (out of the total 171 relevant articles) extracted in the systematic review reported JEV infection, dissemination, or transmission rates in vectors and thus were considered in this meta-analysis.

Meta-analyses. A subgroup analysis by mosquito species was performed for the three outcomes of interest. Tables 2–4 display the pooled estimates for each outcome and their 95% CI (logit and back-transformed estimates).

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

Heterogeneity was considered unimportant for articles reporting infection rates in *Aedes togoi* (I^2 = 11.9%) and *Ochlerotatus vigilax* (I^2 = 28.6%), moderate (I^2 values between 50% and 60%) for *Culex pipiens*, *C. annulirostris*, and *C. annulus*, substantial (I^2 greater than 60%) for studies reporting *Aedes albopictus*, *Culex pseudovishnui*, and *Ochlerotatus detritus* and considerable heterogeneity (I^2 greater than 80%) for *C. gelidus*, *Culex pipiens molestus*, *C. 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 *O. detritus*. The overall pooled estimate of JEV dissemination rate across all mosquito species was 42% (Table 3).

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

Pooled estimates from subgroup meta-analysis of studies reporting JEV transmission rates varied between 0% in *A. albopictus* and 80% in *C. pipiens molestus* (overall estimate = 33%) (Table 4). Pooled estimates of JEV transmission rates showed considerable heterogeneity across studies in *C. gelidus* (I^2 = 92.4%) and *C. sitiens* (I^2 = 83.9%); substantial heterogeneity (I^2 greater than 70%) in *C. annulirostris*, *C. quinquefasciatus*, and *C. tritaeniorhynchus*; and moderate heterogeneity in *O. detritus* (I^2 = 54.6%).

Because of the high heterogeneity found in the meta-analysis models for the three outcomes reported, pooled estimates were provided for reference only, and a meta-regression model was fitted to explore sources of heterogeneity for the infection rate outcome.

Meta-regression. Despite our a priori plan to conduct univariable and multivariable meta-regression models for the three outcomes of interest, we could only perform a univariable

TABLE 1

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 (reported as recorded)	Several species (*N* = 50)
Administration route	Administration route used to experimentally infect mosquitoes	Oral feeding, intrathoracic inoculation, and vertical transmission*
Diagnostic method	Diagnostic method used for detecting JEV	PCR, virus isolation (cell culture techniques or insect bioassays), and virus isolation (with immunofluorescence, hemagglutination inhibition tests, or neutralization tests)†
Incubation period	Period (in days) between experimental infection and testing‡	–

JEV = Japanese encephalitis virus; PCR = polymerase chain reaction; RT-PCR = reverse transcription-polymerase chain reaction.

* 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.

† PCR includes real-time RT-PCR, RT-PCR alone, or in combination with antigen-capture enzyme assays (e.g., enzyme-linked immunosorbent assay) 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.

‡ 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 2

Subgroup meta-analysis* of studies reporting JEV infection rates in vectors by mosquito species

Mosquito species†	N‡	Effect size (logit)	95% CI (logit)	JEV infection rates§	95% CI (rates)	% Weight	I-squared (%)‖
Aedes aegypti	1	−1.01	−1.58, −0.44	0.27	0.17, 0.39	0.51	–
Aedes albopictus	14	−1.37	−1.84, −0.90	0.20	0.14, 0.29	5.74	66.2
Aedes alcasidi	1	−0.92	−2.57, 0.73	0.28	0.07, 0.67	0.34	–
Aedes dorsalis	1	−3.40	−5.40, −1.40	0.03	0.00, 0.20	0.29	–
Aedes japonicus	1	2.20	0.73, 3.67	0.90	0.67, 0.98	0.37	–
Aedes nigromaculis	1	−3.97	−4.95, −2.99	0.02	0.01, 0.05	0.45	–
Aedes togoi	4	−0.24	−0.91, 0.45	0.44	0.29, 0.61	1.50	11.9
Aedes vexans	1	−3.14	−5.14, −1.14	0.04	0.01, 0.24	0.29	–
Aedes vexans nipponi	1	−0.20	−1.08, 0.68	0.45	0.25, 0.66	0.46	–
Armigeres flavus	1	−2.64	−4.68, −0.60	0.07	0.01, 0.35	0.28	–
Armigeres subalbatus	6	−0.13	−1.08, 0.82	0.47	0.25, 0.69	2.18	65.0
Coquillettidia xanth	1	−2.08	−3.12, −1.04	0.11	0.04, 0.26	0.44	–
Culex annulirostris	9	1.11	0.24, 1.98	0.75	0.56, 0.88	2.79	55.7
Culex annulus	5	−0.30	−1.70, 1.10	0.43	0.15, 0.75	1.44	59.9
Culex fuscocephala	2	2.79	1.36, 4.23	0.94	0.80, 0.99	0.57	0.0
Culex gelidus	4	1.17	−0.65, 2.98	0.76	0.34, 0.95	1.45	85.3
Culex pipiens	12	−0.35	−1.11, 0.42	0.41	0.25, 0.60	3.82	58.9
C. pipiens (pipiens)	1	−1.87	−3.36, −0.38	0.13	0.03, 0.41	0.36	–
Culex pipiens fatiga	2	−1.66	−4.24, 0.92	0.16	0.01, 0.72	0.75	82.3
Culex pipiens molestus	2	−3.04	−5.39, −0.69	0.05	0.00, 0.33	0.88	90.2
Culex pipiens pallen	6	−1.73	−3.15, −0.32	0.15	0.04, 0.42	2.27	86.6
Culex pseudovishnui	32	−0.74	−1.05, −0.42	0.32	0.26, 0.40	13.90	77.9
Culex quinquefasciatus	20	−0.67	−1.66, 0.31	0.34	0.16, 0.58	7.93	93.6
Culex sitiens	4	1.93	1.29, 2.56	0.87	0.78, 0.93	1.60	0.0
Culex tarsalis	1	−4.40	−5.79, −3.01	0.01	0.00, 0.05	0.38	–
Culex tritaeniorhynchus	66	0.09	−0.17, 0.35	0.52	0.46, 0.59	27.22	86.9
Culex vishnui	29	−0.94	−1.23, −0.65	0.28	0.23, 0.34	12.52	69.4
Culiseta incidens	1	−3.16	−4.32, −2.00	0.04	0.01, 0.12	0.42	–
Culiseta inornata	1	−3.27	−4.43, −2.11	0.04	0.01, 0.11	0.42	–
Mansonia septempunctata	1	0.69	−0.15, 1.53	0.67	0.46, 0.82	0.47	–
Ochlerotatus detritus	4	0.25	−0.74, 1.25	0.56	0.32, 0.78	1.72	73.2
Ochlerotatus kochi	1	−1.30	−2.20, −0.40	0.21	0.10, 0.40	0.46	–
Ochlerotatus notoscr	2	−1.02	−1.62, −0.42	0.27	0.16, 0.40	0.76	0.0
Ochlerotatus vigilax	3	−1.21	−1.94, −0.48	0.23	0.13, 0.38	1.20	28.6
Opifex fuscus	1	1.05	0.42, 1.68	0.74	0.60, 0.84	0.50	–
Toxorhynchites amboinensis	5	−0.25	−1.04, 0.55	0.44	0.26, 0.63	1.59	0.0
Toxorhynchites brevipalpis	2	−0.80	−2.18, 0.59	0.31	0.10, 0.64	0.58	0.0
Toxorhynchites rutilus	1	−0.41	−2.19, 1.37	0.40	0.10, 0.80	0.32	–
Toxorhynchites theobaldi	1	−0.41	−2.19, 1.37	0.40	0.10, 0.80	0.32	–
Verrallina funerea	1	0.30	−0.15, 0.75	0.57	0.46, 0.68	0.52	–
Overall	–	−0.43	−0.59, −0.28	0.39	0.36, 0.43	100.00	–

CI = confidence interval; JEV = Japanese encephalitis virus. Each effect size¶ represents pooled estimates of the outcome for each mosquito species, and the overall represents the overall pooled estimate across all mosquito species. N = 251 entries.

* Random-effects meta-analysis using the method of DerSimonian and Laird[25] to estimate the variance between studies, using a restricted maximum likelihood algorithm.

† Species are reported as recorded in the original articles.

‡ Entries correspond to the times a certain mosquito species was recorded within a specific outcome (≠ number of articles, i.e., several entries may pertain to the same article).

§ $p = (e^{logit}/[e^{logit} + 1])$.

‖ I-squared = proportion of total variability in point estimates that can be attributed to heterogeneity. I^2 range: 11.9% (*Aedes togoi*, P value = 0.50) to 93.6% (*Culex quinquefasciatus*, P value = 0.18).

¶ Computed for the group of studies reporting JEV infection rates in each mosquito species.

meta-regression model for JEV infection rate. No multivariable meta-regression models could be fitted because of the insufficient number of observations or lack of evidence of statistically significant conditional associations between predictors and outcomes.

Mosquito species and administration route were significantly associated (P value < 0.1) with JEV infection in vectors in the univariable screen (Table 5).

Aedes japonicus, *C. annulirostris*, *C. fuscocephala*, *C. gelidus*, *C. sitiens*, *Mansonia septempunctata*, *O. detritus*, *Opifex fuscus*, and *Verrallina funerea* showed higher proportion of JEV infection rates compared with *C. tritaeniorhynchus*, which is considered the most relevant vector species for JEV.

Furthermore, higher JEV infection rates were reported across studies in which intrathoracic inoculation was used, compared with oral feeding. Conversely, compared with oral feeding, vertical transmission was associated with lower infection rates (Table 5).

Associations between incubation period and diagnostic method with dissemination rate were not statistically significant (P value = 0.51; and P value = 0.37, respectively). We could not fit univariable meta-regression models to evaluate associations between the administration route or mosquito species with dissemination rate because of 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 the administration route with the JEV transmission rate. Associations between mosquito species, incubation period, and diagnostic method with JEV transmission rate were not statistically significant (P value = 0.46; P value = 0.51; and P value = 0.37, respectively).

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

TABLE 3
Subgroup meta-analysis* of studies reporting JEV dissemination rates in vectors grouped by mosquito species

Mosquito species†	N‡	Effect size (logit)	95% CI (logit)	JEV dissemination rates§	95% CI (rates)	% Weight	I-squared (%)‖
Culex annulirostris	5	−0.40	−1.48, 0.68	0.40	0.19, 0.66	21.90	85.4
Culex gelidus	1	−1.21	−1.68, −0.74	0.23	0.16, 0.32	5.26	–
Culex pipiens	1	−0.41	−2.19, 1.37	0.40	0.10, 0.80	3.20	–
Culex quinquefasciatus	5	0.42	−0.57, 1.41	0.60	0.36, 0.80	22.26	82.6
Culex sitiens	4	−1.44	−2.28, −0.60	0.19	0.09, 0.35	16.41	51.7
Ochlerotatus detritus	3	1.18	−0.11, 2.46	0.76	0.47, 0.92	12.21	69.7
Ochlerotatus notoscriptus	1	−2.44	−3.48, −1.40	0.08	0.03, 0.20	4.44	–
Ochlerotatus vigilax	2	−1.09	−2.12, −0.06	0.25	0.11, 0.49	9.35	62.1
Opifex fuscus	1	0.85	0.14, 1.56	0.70	0.54, 0.83	4.96	–
Overall	–	−0.33	−0.83, 0.16	0.42	0.30, 0.54	100.00	–

CI = confidence interval; JEV = Japanese encephalitis virus. Each effect size¶ represents pooled estimates of the outcome for each mosquito species and the overall represents the overall pooled estimate across all mosquito species. $N = 23$ entries.

* Random-effects meta-analysis using the method of DerSimonian and Laird[25] to estimate the variance between studies, using a restricted maximum likelihood algorithm.

† Species are reported as recorded in the original articles.

‡ Entries correspond to the times a certain mosquito species was recorded within a specific outcome (≠ number of articles, i.e., several entries may pertain to the same article).

§ $p = (e^{logit}/[e^{logit} + 1])$.

‖ I-squared = proportion of total variability in point estimates that can be attributed to heterogeneity. I^2 range: 62.1% (*Ochlerotatus vigilax*, P-value = 0.04) to 85.4% (*Culex annulirostris*, P value = 0.47).

¶ Computed for the group of studies reporting JEV dissemination rates in each mosquito species.

research articles gathered using a systematic review of the literature. Moreover, by performing meta-regression models, we explored some sources of heterogeneity that could explain the variation reported in pooled estimates from the meta-analysis models. Differences across studies, including those related to study design (e.g., observation period and duration, blinding, type of test used to measure outcomes, misclassification of the outcomes, etc.), can explain artifactual (methodological) heterogeneity. Real sources of variability, arising when there are true differences in effects across studies, along with artifactual variability are reflected in the evaluation of heterogeneity.[22] Because most studies included in this work were animal and entomological studies, real sources of variability are expected, as these correspond to the biological diversity inherently associated with this type of studies. Diverse ecological and geographical characteristics can also contribute toward biological variation. The statistical quantification of heterogeneity helped us assess the consistency of effects across studies to then determine the value and generalizability of the findings.

Highest JEV infection rates were reported in *C. annulirostris*, *C. sitiens*, and *C. fuscocephala*, which supports previous research claiming that mosquito species with importance as JEV vectors belong to the *Culex* genus.[26] *A. 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.[6,10]

Nonetheless, and although 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.[27] 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,

TABLE 4
Subgroup meta-analysis* of studies reporting JEV transmission rates in vectors grouped by mosquito species

Mosquito species†	N‡	Effect size (logit)	95% CI (logit)	JEV transmission rates§	95% CI (rates)	% Weight	I-squared (%)‖
Aedes aegypti	1	−1.10	−1.69, −0.51	0.25	0.16, 0.37	2.66	–
Aedes albopictus	3	−5.57	−11.02, −0.13	0.00	0.00, 0.47	0.32	0.0
Aedes japonicus	1	1.10	−1.15, 3.35	0.75	0.24, 0.97	1.16	–
Aedes vexans nipponi	1	−0.69	−2.40, 1.02	0.33	0.08, 0.73	1.57	–
Coquillettidia xanthogaster	1	−2.64	−4.68, −0.60	0.07	0.01, 0.35	1.31	–
Culex annulirostris	4	−0.13	−1.31, 1.05	0.47	0.21, 0.74	8.27	74.9
Culex fuscocephala	4	−1.64	−2.52, −0.76	0.16	0.07, 0.32	5.96	0.0
Culex gelidus	5	−0.71	−1.64, 0.23	0.33	0.16, 0.56	12.25	92.4
Culex pipiens	2	−2.44	−3.62, −1.26	0.08	0.03, 0.22	3.12	0.0
Culex pipiens molestus	1	1.39	−0.81, 3.59	0.80	0.31, 0.97	1.20	–
Culex quinquefasciatus	6	−0.23	−1.21, 0.75	0.44	0.23, 0.68	12.20	76.4
Culex sitiens	3	−1.15	−3.30, 0.99	0.24	0.04, 0.73	5.28	83.9
Culex tritaeniorhynchus	13	−0.56	−1.25, 0.14	0.36	0.22, 0.53	25.40	76.4
Mansonia septempunctata	1	0.17	−0.63, 0.97	0.54	0.35, 0.73	2.46	–
Ochlerotatus detritus	6	−0.70	−1.56, 0.17	0.33	0.17, 0.54	10.44	54.6
Ochlerotatus notoscriptus	2	−1.10	−2.24, 0.04	0.25	0.10, 0.51	3.12	0.0
Ochlerotatus vigilax	1	−1.95	−4.05, 0.15	0.12	0.02, 0.54	1.27	–
Verrallina funerea	1	−1.61	−2.85, −0.38	0.17	0.05, 0.41	2.02	–
Overall	–	−0.71	−1.02, −0.40	0.33	0.26, 0.40	100.00	–

CI = confidence interval; JEV = Japanese encephalitis virus. Each effect size¶ represents pooled estimates of the outcome for each mosquito species and the overall represents the overall pooled estimate across all mosquito species. $N = 56$ entries.

* Random-effects meta-analysis using the method of DerSimonian and Laird[25] to estimate the variance between studies, using a restricted maximum likelihood algorithm.

† Species are reported as recorded in the original articles.

‡ Entries correspond to the times a certain mosquito species was recorded within a specific outcome (≠ number of articles, i.e., several entries may pertain to the same article).

§ $p = (e^{logit}/[e^{logit} + 1])$.

‖ I-squared = proportion of total variability in point estimates that can be attributed to heterogeneity. I^2 range: 54.6% (*Ochlerotatus detritus*, P-value = 0.11) to 92.4% (*Culex gelidus*, P-value = 0.14).

¶ Computed for the group of studies reporting JEV transmission rates in each mosquito species.

Table 5

Coefficients, *P* values, and 95% CI 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	< 0.01	–
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	< 0.01	–
Culex pipiens pallens	4	−1.79	0.58	−2.94, −0.65	< 0.01	–
Culex pseudovishnui	2	−0.82	0.28	−1.37, −0.27	< 0.01	–
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	< 0.01	–
Culex vishnui	1	−1.00	0.29	−1.57, −0.43	< 0.01	–
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	–
Toxorhynchites theobaldi	1	−0.55	1.49	−3.49, 2.38	0.71	–
Toxorhynchites amboinensis	1	−0.39	0.68	−1.73, 0.96	0.57	–
Toxorhynchites brevipalpis	1	−1.00	1.11	−3.18, 1.18	0.37	–
Toxorhynchites 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	< 0.01	–
Intercept	–	−0.27	0.11	−0.48, −0.06	0.01	–

CI = confidence intervals; JEV = Japanese encephalitis virus.

* Random effects meta-regression models using the restricted maximum likelihood method.

† Species are reported as recorded in the original articles.

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 the 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.[27]

Dissemination and transmission rates provide us with more information regarding mosquito infectiousness, as opposed to mosquito infection.[21] Mosquito species with the highest dissemination rates included *O. detritus* (76%) and *O. fuscus* (70%), none of which were among the species with the highest infection rates, actually supporting the hypothesis provided by Bustamante and Lord.[27] The highest JEV transmission rates were reported in *C. pipiens molestus* (80%) and *A. japonicus* (75%). Whereas the former reported a low infection rate (5%), the latter had one of the highest infection rates reported in the meta-analysis model for that outcome. According to the European Center for Disease Prevention and Control,[28] *A. japonicus* has become the third most abundant invasive mosquito species reported in Europe, mainly because of international trade in used tires. Similarly, this mosquito species has also been reported in the United States,[29] thus making *A. 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 are reported in very few studies (with the exception of *C. annulirostris*, which is represented in three articles, all other mosquito species mentioned as having high infection, dissemination, and transmission rates are reported in one article). The limited number of studies used in the meta-analysis 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 *C. tritaeniorhynchus*, which is considered the most significant JEV vector in Asia[3,5,6,30,31] and whose competence for JEV has been demonstrated in laboratory experiments,[32] resulted in a pooled estimate of the JEV transmission rate of 36%, which is lower than many other mosquito species not commonly associated with JEV infection and transmission, such as *A. japonicus* (although transmission results for *C. tritaeniorhynchus* pertained to six experimental studies, as opposed to only one for *A. 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 (*A. japonicus*, *C. annulirostris*, *C. fuscocephala*, *C. gelidus*, *C. sitiens*, *M. septempunctata*, *O. detritus*, *O. fuscus*, and *V. funerea*) reported higher proportion of JEV infection rates compared with *C. tritaeniorhynchus*. Pooled estimates for JEV infection showed unimportant heterogeneity for *A. togoi* and *O. vigilax*, and moderate for *C. pipiens fatigans*, *C. pipiens*, and *C. annulirostris*. Similarly, *O. 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 *C. pipiens* and *C. 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 another important source of heterogeneity, with higher JEV infection rates being reported across studies in which intrathoracic inoculation was the administration route used, compared with 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,[27] 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,[33] 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.

Incubation period and the diagnostic method, considered sources of methodological heterogeneity for these outcomes, did not explain the variability found across studies. In addition to the low number of studies eligible for data extraction, most studies reported general, as opposed to specific, methodological details (e.g., range of days versus specific number of days of incubation period and type of diagnostic test versus specific test used), which limited our ability to determine whether the variation in findings was compatible with chance alone. In addition, 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 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 association between incubation period with the vector competence outcomes.[22] 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 present 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 provided 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.,[11] 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.[11] 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.[34]

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 toward the populations and regions at higher risk, may be achieved and more informed efforts targeted.[11]

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, to use them as input parameters in risk assessment models aiming at studying risk profiles of JEV introduction in presently JEV-free regions.

Received August 4, 2017. Accepted for publication December 9, 2017.

Note: Supplemental tables appear at www.ajtmh.org.

Financial support: This research project was funded by the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Project No: 5430-32000-008-05S.

Authors' addresses: Ana R. S. Oliveira, Luciana Etcheverry, and Natalia Cernicchiaro, Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, E-mail: anarutesoliveira@gmail.com, l.etcheverry.h@gmail.com, and ncernic@vet.k-state.edu. Lee W. Cohnstaedt and D. Scott McVey, USDA-ARS Arthropod-Borne Animal Diseases Research, Manhattan, KS, E-mail: lee.cohnstaedt@ars.usda.gov and scott.mcvey@ars.usda.gov. Erin Strathe, Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS, E-mail: estrathe@vet.k-state.edu. José Piaggio, School of Veterinary Medicine, University of the Republic, Montevideo, Uruguay, E-mail: jpiaggio@fvet.edu.uy.

REFERENCES

1. Erlanger TE, Weiss S, Keiser J, Utzinger J, Wiedenmayer K, 2009. Past, present and future of Japanese encephalitis. *Emerg Infect Dis 15:* 1–7.
2. Solomon T, 2006. Control of Japanese encephalitis—within our grasp? *N Engl J Med 355:* 869–871.
3. Solomon T, Dung NM, Kneen R, Gainsborough M, Vaughn D, Khanh VT, 2000. Japanese encephalitis. *J Neurol Neurosurg Psychiatry 68:* 405–415.
4. Campbell GL et al., 2011. Estimated global incidence of Japanese encephalitis: a systematic review. *Bull World Health Organ 89:* 766–774.
5. Le Flohic G, Porphyre V, Barbazan P, Gonzalez JP, 2013. Review of climate, landscape, and viral genetics as drivers of the Japanese encephalitis virus ecology. *PLoS Negl Trop Dis 7:* 1–7.
6. van den Hurk AF, Ritchie SA, Mackenzie JS, 2009. Ecology and geographical expansion of Japanese encephalitis virus. *Annu Rev Entomol 54:* 17–35.
7. Gubler DJ, 2002. The global emergence/resurgence of arboviral diseases as public health problems. *Arch Med Res 33:* 330–342.
8. Nett RJ, Campbell GL, Reisen WK, 2009. Potential for the emergence of Japanese encephalitis virus in California. *Vector Borne Zoonotic Dis 9:* 511–517.
9. Nemeth N, Bosco-Lauth A, Oesterle P, Kohler D, Bowen R, 2012. North American birds as potential amplifying hosts of Japanese encephalitis virus. *Am J Trop Med Hyg 87:* 760–767.
10. Huang YJS, Higgs S, Horne KM, Vanlandingham DL, 2014. Flavivirus-mosquito interactions. *Viruses 6:* 4704–4730.
11. Lord JS, Gurley ES, Pulliam JRC, 2015. Rethinking Japanese encephalitis virus transmission: a framework for implicating host and vector species. *PLoS Negl Trop Dis 9:* 1–7.
12. Sargeant JM, Rajic A, Read S, Ohlsson A, 2006. The process of systematic review and its application in agri-food public-health. *Prev Vet Med 75:* 141–151.
13. Sargeant JM, O'Connor AM, 2014. Introduction to systematic reviews in animal agriculture and veterinary medicine. *Zoonoses Public Health 61 (Suppl 1):* 3–9.
14. Egger M, Smith GD, O'Rourke K, 2001. Rationale, potentials, and promise of systematic reviews. Egger M, Smith GD, Altman DG, eds. *Systematic Reviews in Health Care: Meta-Analysis in Context.* 2nd edition. London, United Kingdom: BMJ, 3–19.
15. Sutton AJ, Abrams KR, Jones DR, 2001. An illustrated guide to the methods of meta-analysis. *J Eval Clin Pract 7:* 135–148.
16. O'Connor AM, Anderson KM, Goodell CK, Sargeant JM, 2014. Conducting systematic reviews of intervention questions I: writing the review protocol, formulating the question and searching the literature. *Zoonoses Public Health 61 (Suppl 1):* 28–38.
17. Oliveira ARS, Cohnstaedt LW, Strathe E, Hernandez LE, McVey DS, Piaggio J, Cernicchiaro N, 2017. Meta-analyses of the proportion of Japanese encephalitis virus infection in vectors and vertebrate hosts. *Parasit Vectors 10:* 418.
18. Sargeant JM, O'Connor AM, 2014. Conducting systematic reviews of intervention questions II: relevance screening, data extraction, assessing risk of bias, presenting the results and interpreting the findings. *Zoonoses Public Health 61 (Suppl 1):* 39–51.
19. O'Connor AM, Sargeant JM, Wang C, 2014. Conducting systematic reviews of intervention questions III: synthesizing data from intervention studies using meta-analysis. *Zoonoses Public Health 61 (Suppl 1):* 52–63.
20. Higgins JPT, Green S, 2011. *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0.* The Cochrane Collaboration. [Updated March 2011]. Available at: http://handbook-5-1.cochrane.org/. Accessed March 15, 2017.
21. Golnar AJ, Turell MJ, LaBeaud AD, Kading RC, Hamer GL, 2015. Predicting the mosquito species and vertebrate species involved in the theoretical transmission of Rift Valley fever virus in the United States. *PLoS Negl Trop Dis 8:* e3163.
22. Dohoo I, Martin W, Stryhn H, 2009. *Veterinary Epidemiologic Research.* 2nd edition. Charlottetown, Prince Edward Island, Canada: Ver.
23. Sanchez J, Dohoo IR, Christensen J, Rajic A, 2007. Factors influencing the prevalence of *Salmonella* spp. in swine farms: a meta-analysis approach. *Prev Vet Med 81:* 148–177.
24. Lambert K, Coe J, Niel L, Dewey C, Sargeant JM, 2015. A systematic review and meta-analysis of the proportion of dogs surrendered for dog-related and owner-related reasons. *Prev Vet Med 118:* 148–160.
25. DerSimonian R, Laird N, 1986. Meta-analysis in clinical trials. *Control Clin Trials 7:* 177–188.
26. Misra UK, Kalita J, 2010. Overview: Japanese encephalitis. *Prog Neurobiol 91:* 108–120.
27. Bustamante DM, Lord CC, 2010. Sources of error in the estimation of mosquito infection rates used to assess risk of arbovirus transmission. *Am J Trop Med Hyg 82:* 1172–1184.
28. ECDC, 2017. *Health Topics:* Aedes japonicus. Available at: http://ecdc.europa.eu/en/healthtopics/vectors/mosquitoes/Pages/aedes-japonicus.aspx. Accessed May 15, 2017.
29. Darsie RF Jr, Ward RA, 2005. *Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico.* Gainesville, FL: University Press of Florida.
30. Mackenzie JS, Gubler DJ, Petersen LR, 2004. Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nat Med 10:* 98–109.
31. Weaver S, Barrett ADT, 2004. Transmission cycles, host range, evolution and emergence of arboviral disease. *Nat Rev Microbiol 2:* 789–801.
32. Gresser I, Hardy JL, Hu SMK, Scherer WF, 1958. Factors influencing transmission of Japanese B encephalitis virus by a colonized strain of *Culex tritaeniorhynchus* Giles, from infected pigs and chicks to susceptible pigs and birds. *Am J Trop Med Hyg 7:* 365–373.
33. Lequime S, Lambrechts L, 2014. Vertical transmission of arboviruses in mosquitoes: a historical perspective. *Infect Genet Evol 28:* 681–690.
34. Dye C, 1992. The analysis of parasite transmission by bloodsucking insects. *Annu Rev Entomol 37:* 1–19.