Vector competence of Culex mosquitoes (Diptera: Culicidae) in Zika virus transmission: an integrative review

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ABSTRACT

Objective. To identify studies on the competence of Culex mosquitoes as vectors for the transmission of Zika virus (ZIKV) around the globe.

Methods. We performed an integrative review to identify relevant articles on specific experiments to determine whether Culex mosquitoes are vectors for ZIKV. The sources we used for our research were the Brazilian Periódicos CAPES electronic portal (MEDLINE/PubMed, ScienceDirect Journals, Nature Publishing Group, SciELO, Springer Link, and 250 other databases) and gray literature.

Results. We identified 344 studies, of which 36 were considered for this review. In 8 studies, infection in salivary glands of Culex quinquefasciatus, Culex restuans, Culex tarsalis, and Culex coronator was detected. Cx. quinquefasciatus was the most studied among those confirmed as potential ZIKV vectors, and only strains of Asian lineages (THA/2014/SV0127-14; S201 (2016)) and American lineages (BRPE243 (2015); PRVABC59 (2015)) can infect the salivary glands of Culex mosquitoes. The tested African strains (MR766 and DAK AR 41525) were unable to infect salivary glands.

Conclusions. There is still a lack of compelling evidence that indicates Culex spp. are a competent ZIKV vector, but they should remain a target for further monitoring studies, especially regarding ZIKV transmission to other species. Furthermore, studies should not be limited to studying whether their salivary glands are infected.

Keywords Public health; Zika virus; mosquito vectors; Culex.

Zika virus (ZIKV) is known to be transmitted among humans mainly through bites of Aedes aegypti (Linnaeus) and Aedes albopictus (Skuse) mosquitoes (1). The virus was initially isolated in a rhesus monkey in 1947. There was a second isolation from Aedes africanus (Theobald) in 1948 in an attempt to isolate yellow fever virus from mosquitoes in the Zika Forest of Uganda (2). Aedes mosquitoes are considered the only competent vectors for ZIKV transmission (3, 4). Transmission can occur sexually (5), through blood transfusion and saliva, and from mother to child during pregnancy, birth, and breast-feeding (6).

ZIKV is a positively enveloped RNA virus member of the Flaviviridae family, genus Flavivirus (1, 7). It was discovered in 1947 in the Zika Forest, in Uganda, and remained confined to some areas of Africa and Asia. In 2007, ZIKV emerged in the Yap Islands in the Federated States of Micronesia and also in the African country of Gabon. In addition, in 2013, the virus appeared in French Polynesia. By 2014, ZIKV had spread to other Pacific islands: New Caledonia, the Cook Islands, and Easter Island. In early 2015, the virus was identified in Brazil and then, later, throughout continental South America and Latin America (1, 7). This fast and massive spread is worrisome because there are no available drugs or vaccines for the treatment of ZIKV infection, and a possible marked, severe outcome of ZIKV infection in pregnant women is microcephaly in newborns (6).

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Within the genus *Aedes*, other anthropophilic species have been considered to be vectors, including *Aedes hensilli* (Farner) and *Aedes texans* (Meigen). In addition, other mosquito genera have been investigated as vectors, especially *Culex*. (3, 4, 8, 9). *Culex* spp. are already considered competent vectors to transmit such flaviviruses as human-like Japanese encephalitis virus (JEV), West Nile virus (WNV), and Saint Louis encephalitis (10). This fact makes them candidates for further investigations as a vector for other flaviviruses, such as ZIKV.

In a study performed in China, *Culex quinquefasciatus* (Say) was identified as a potential laboratory vector for ZIKV. In that research, mosquitoes were infected through a blood meal with a ZIKV strain (SZ01) isolated from a patient, and viral RNA was found in the salivary glands, midgut, and ovary. Importantly, *Cx. quinquefasciatus* mosquitoes were able to infect infant mice (11). In a session of the First International Workshop on Zika Virus, organized by the Oswaldo Cruz Foundation (FIOCRUZ) and held in Brazil in 2016, researchers from the Ageu Magalhães Institute (Recife, Pernambuco, Brazil) presented results of experiments with mosquitoes artificially fed with blood infected with the ZIKV. The virus was detected in the body and in salivary glands of *Cx. quinquefasciatus* 7 and 15 days after feeding, with a confirmed infection rate of 100% and 67%, respectively (12).

Currently, there are no vaccines or drugs against ZIKV (1, 4, 5), and the only precaution is to prevent mosquito bites by integrated vector control, including surveillance, biological and chemical control, source reduction, and environmental management (4, 5). For example, the development of sanitation and management of urban water collection and vector control with DDT was responsible for vector *Aedes* mosquitoes disappearing after 1950, although the species has recently reinvaded European territory (4). Furthermore, the World Health Organization (WHO) also recommends the practice of safe sex for women living in areas of high virus transmission (13).

Therefore, considering that the genus *Aedes* is the principal target among the strategies for the prevention and control of ZIKV, we reasoned that it is essential to understand whether there are other vectors capable of or even competent in transmitting ZIKV. Thus, we aimed to identify studies on the competence of the *Culex* mosquitoes as vectors for ZIKV transmission through a review of the literature on this subject.

**METHODS**

We performed a literature review in July 2019 to identify relevant articles on the vector competence of *Culex* spp. mosquitoes for ZIKV. The review was based on an advanced search of the Brazilian Periódicos CAPES electronic portal (www.periodicos.capes.gov.br), which includes MEDLINE/PubMed, ScienceDirect Journals, Nature Publishing Group, SciELO, Springer Link, and 250 other databases. Additional studies were identified by searching for gray literature, using the Google Scholar search engine, and with a manual search of the bibliographical references of the relevant identified publications.

The search strategy was drawn from the descriptors (“Culex” and “vector”) AND (zik*), marking “any field” for the search field of the thesauri, in the advanced-search area of the Periódicos CAPES electronic portal. All studies from the literature search were analyzed to eliminate duplicates from the manual search strategies, specifically by comparing authors, titles, and name of the journal, along with their volume, number, and year of publication. After the duplicates were removed, the remaining studies were examined based on their titles and abstracts. At this stage, the eligibility criteria for the articles were: original studies; mentions of ZIKV and a mosquito from the genus *Culex* establishes *Culex* spp. as a vector of ZIKV through specific results in experiments identifying the virus at their organisms; and in Portuguese, Spanish, or English. Reviews and opinion letters were discarded. The documents selected as potentially relevant went to the next step, where they were judged carefully from the reading of the full text. Only those that met all the eligibility criteria mentioned above were included in this review. The studies from each database were placed in spreadsheets in the Microsoft Excel program (version 2010), to eliminate duplicates and to create a database for references.

**RESULTS**

The study designs were classified as cohort studies (10 articles), case-control studies (26 articles), and a conference abstract (1 article). One of the documents presented two different study classifications, case-control and cohort.

**Study selection**

We identified 333 studies in the bibliographic search in the Periódicos CAPES website, 7 studies through gray literature, and 7 additional studies from the manual search of the bibliographical references of the relevant publications, for a total of 347 studies. Three duplicate papers were discarded, and then 278 articles were excluded after reading the title. We incorporated 66 studies whose titles were not clear concerning inclusion criteria into the next stage, which included reading the abstracts to avoid missing any article that matched our criteria (all the articles were written in English, so no study was excluded based on language). In the end, after discarding 26 articles by reading the abstract and 4 articles by reading the full text, we included 36 studies in this review. Figure 1 is a flow diagram of the methodology steps. Table 1 summarizes the articles used in this review.

**FIGURE 1. Flow diagram of the methodology used to identify studies.**

| Articles found in Periódicos Capes (n=333) | Articles found in citations (n=7) | Articles found in gray literature (n=7) |
|------------------------------------------|-----------------------------------|-----------------------------------------|
| Articles included for title reading (n=344) | Duplicates discarded (n=3) |
| Articles included for abstract reading (n=66) | Discarded (n=278) |
| Articles included for full-text reading (n=40) | Discarded (n=26) |
| Articles selected for the review (n=36) | Discarded (n=4) |

Source: prepared by the authors
### TABLE 1. Studies included in the review.

| Author (Reference) | Study Design | Country/Year | Blood Meals Titers | Infection | S.G Infection | Observations |
|--------------------|-------------|--------------|-------------------|-----------|---------------|--------------|
| Guo et al. (11)    | CC          | China 2016   | 3×10^8 PFU/ml     | +         | +             | 89% of mice infected had viral RNA of strain SZ01 in their brain at 10 DPI with 7.85 log RNA copies/ml. Cx. quinquefasciatus tested. |
| Liu et al. (37)    | CC          | China 2017   | 5.45 ± 0.38 log_{10} Copies/ml | +         | -             | Strain ZJ03 used. 15.94 % of Cx. quinquefasciatus infected. |
| Fu et al. (33)     | CH          | China 2017   | MC                | +         | NT            | Cx. quinquefasciatus collected from the field with strain GZD1685-2 detected. |
| Phumee et al. (47) | CC          | Thailand 2019| 1.7×10^6 FFU/ml   | +         | +             | Vertical transmission of ZIKV in Ae. aegypti, Aedes albopictus and Cx. quinquefasciatus confirmed. Strain used: Zika virus/H. sapiens-tc/THA/2014/SV0127-14. |
| Pompon et al. (34) | CC          | Singapore 2017| 10^4 to 10^5 PFU/ml | +         | -             | Strain H/PF used. Cx. quinquefasciatus tested. |
| Ho et al. (25)     | CH          | Singapore 2017| MC                | -         | -             | Aedes aegypti infected in many pools. No Culex spp. infected. |
| Tien et al. (26)   | CH          | Singapore 2017| MC                | -         | -             | Aedes aegypti infected in many pools. No Culex spp. infected. |
| Franca et al. (12) | CA          | Brazil 2016  | Not revealed      | +         | +             | Transmission to other species not tested. |
| Guedes et al. (43) | CC; CH      | Brazil 2017  | 10^8 PFU/ml and MC| +         | +             | A study made with mosquito Cx. quinquefasciatus and strain BRPE243/2015. Collected mosquitoes with salivary glands infected. No traces of recent feeding. |
| Fernandes et al. (31) | CC         | Brazil 2016  | 10^5 PFU/ml      | +         | -             | Strains Rio-U1 and Rio S-1 tested. 3.3% of Cx. quinquefasciatus infected. |
| Fernandes et al. (35) | CC         | Brazil 2017  | 2.3 × 10^5, 3.55 × 10^5 and 1.68 × 10^5 PFU/ml | +         | -             | 5% of Cx. quinquefasciatus infected. Strains: BRPE243/2015, U1, and SPH2015. |
| Ayres et al. (42)  | CH          | Brazil 2019  | MC                | +         | NT            | A study made with Cx. quinquefasciatus collected from the field at Espírito Santo. Ae. aegypti also infected. Strains detected: PE243 2015 (from Pernambuco), Haiti 1225 2014, and SPH2015 (from São Paulo). |
| Lourenço-de-Oliveira et al. (23) | CC       | Brazil 2018  | 10^7 TCID_{50}/ml | -         | -             | Cx. quinquefasciatus tested with strains NC-2014-5132. |
| Dibernardo et al. (44) | CC         | Canada 2017  | 10^4 PFU/ml      | +         | +             | Intrathoracic inoculation of strain PRVABC59 of ZIKV at Cx. restuans and Cx. tarsalis. In 3/58 of Cx. restuans was detected ZIKV in the salivary glands. |
| Smartt et al. (46) | CC          | United States 2018 | 3.5 log_{10} ZIKV PFU/ml | +         | +             | A study performed with Cx. quinquefasciatus and strain PRVABC59. RNA analysis at saliva eluted from the filter paper at 14 (Exp.1) and 16 DPI (Exp2) to detect ZIKV titers. |
| Weger-Lucarelli et al. (32) | CC         | United States 2016 | 1.6×10^6 and 5.0×10^6 PFU/ml | +         | -             | 2% of Cx. quinquefasciatus infected. Cx. pipiens nor Cx. tarsalis not infected. Strain: PRVABC59. |
| Hart et al. (20)   | CC          | United States 2017 | 10^4, 10^5, 10^6 and 10^7 PFU/ml | -         | -             | Cx. quinquefasciatus tested with strains FSS13025, PRVABC59, MEX1–7, and DAK AR 41525. |
| Kenney et al. (38) | CC          | United States 2017 | 4, 5, 9, 6, 7 and 7.6 log_{10} PFU/ml | +         | -             | 1-10% of mosquitoes infected. 15-70% of mosquitoes intrathoracically inoculated demonstrated virus-positive bodies. Mosquitoes of Cx. quinquefasciatus and Cx. pipiens. Strains: MR766, R103451 and PRVABC59. |
| Main et al. (40)   | CC          | United States 2018 | 4.6 log_{10} PFU/m | +         | -             | 30% of infection at 21 DPI for Cx. tarsalis. Dissemination of strain PRVABC59 at 4% at 14 DPI and 5% at 21 DPI. Cx. quinquefasciatus not infected. |
| Dodson et al. (18) | CC          | United States 2017 | 7.3 and 7.5 log_{10} PFU/ml | -         | -             | Cx. quinquefasciatus tested with strains PRVABC59 and MR766. |
| Dodson et al. (22) | CC          | United States 2018 | 8.4 log_{10} PFU/ml | -         | -             | Cx. tarsalis tested with strain NR-43026. |
| Aliota et al. (14) | CC          | United States 2016 | 4.74, 6.02 and 6.83 log_{10} PFU/ml | -         | -             | Cx. pipiens tested with strain PRVABC59. |
| Huang et al. (17)  | CC          | United States 2016 | 10^{25}, 10^{26}, 10^{29} logTCID_{50}/ml | -         | -             | Cx. quinquefasciatus tested with strain PRVABC59. |
| Yee et al. (21)    | CH          | Puerto Rico 2017 | MC                | -         | -             | Cx. quinquefasciatus not infected with no ZIKV. Aedes aegypti infected. |
| Elizondo-Quiroga et al. (45) | CH  | Mexico 2018     | MC                | +         | +             | Mosquitoes Cx. quinquefasciatus, Cx. coronator and Cx. tarsalis. Strains not identified. 2/5 of SG pools for Cx. quinquefasciatus showed CPE at 1 DPI. Pools of SG of Cx. coronator and Cx. tarsalis showed CPE at 3 and 4, respectively. Aedes aegypti showed the lowest MIR/1000 mosquitoes. |

(continued)
TABLE 1. Studies included in the review. (continued)

| Author (Reference) | Study Design | Country/Year | Blood Meals Titers | Infection | S.G Infection | Observations |
|--------------------|--------------|--------------|--------------------|-----------|---------------|--------------|
| Guerbois et al. (16) | CH | Mexico 2016 | MC | - | - | A study performed with Cx. quinquefasciatus collected. Ae. aegypti infected. |
| Diallo et al. (30) | CH | Senegal 2014 | MC | + | NT | A study made with Cx. perfuscus collected from the field. 1/7 pools infected. Strains not identified. |
| Amraoui et al. (28) | CC | Tunisia 2016 | 10^3.2 PFU/ml | + | - | Cx. pipiens and Cx. quinquefasciatus inoculated intrathoracically with ca. 2,530 PFU. Strain NC2014-5132 used. |
| Boccoli et al. (29) | CC | Italy 2016 | 10^4.5 PFU/ml | - | - | Strain H/PF for Cx. pipiens. |
| Foixi et al. (15) | CH | Italy 2016 | MC | - | - | A study performed with Culex spp. |
| Heitmann et al. (36) | CC | Germany 2017 | 10^6 PFU/ml | - | - | A study performed with strain MR766. Cx. molestus and Cx. torrentium infected but with no transmission detectable. |
| Hall-Mendelin et al. (27) | CC | Australia 2016 | 10^3.5±2 TCID_{50}/ml | + | - | A study performed with strain MR766. Cx. sitiens and Cx. annulirostris not infected. 7% of Cx. quinquefasciatus infected. |
| Duchemin et al. (19) | CC | Australia 2017 | TCID_{50}, 10^7/ml | - | - | A study performed with Cx. quinquefasciatus and Cx. annulirostris strain FSS13025. |
| Richard et al. (39) | CC | French Polynesia | 7 log_{10} TCID_{50}/ml | - | - | No dissemination nor transmission for Cx. quinquefasciatus. A study performed with strain PF13/251013-18 |

| Ae., Aedes CA, conference abstract; CC, case-control; CH, cohort; Cx., Culex DPI, days post-infection; ca., circa; Exp., experiment; FFU (fluorescence focus units); MC, mosquitoes collected from the field; blood meal titers expressed as PFU (plaque-forming unit) and PFUe (plaque-forming unit equivalents); NT, not tested; SG, Salivary glands; TCID_{50} (50% tissue culture infectious dose). Table prepared by the authors. |

Vector competence

Thirteen studies concluded that Culex spp. are not ZIKV competent vectors because they cannot be orally infected (14-26). These studies used a variety of ZIKV strains, including PRVABC59 from Puerto Rico (2015), FSS13025 from Cambodia (2010), MEX1-7 from Mexico City, Mexico (2015), DAKAR 41525 from Senegal (1985), PF13/251013-18 from French Polynesia (2013), BRPE243 from Pernambuco, Brazil (2015), SPPH2016 from São Paulo, Brazil (2015), NC-2014-5132 from New Caledonia (2015), SPH2015 from São Paulo, Brazil (2015), NC-2014-5132 from California (2016), GDZJ1685-2 from China (2016), PRVABC59 from Puerto Rico (2015), KFF993678 from Thailand (2013), RIO-U1 from Rio de Janeiro (2016), RIO-S1 from Rio de Janeiro (2016), PE243/2015 from the state of Pernambuco in Brazil (2015), Haiti 1225 from Haiti (2014), SPH2015 from São Paulo (2015), and strain MR766 from Uganda (1947). The infected mosquitoes were Cx. quinquefasciatus, Cx. pipiens, and Cx. tarsalis, with minimal blood meal titers of 5.7 log_{10} PFU/ml, 5.45 ± 0.38 log_{10} copies/ml and 7.0 log_{10} TCID_{50}/ml.

Finally, seven studies identified ZIKV in the salivary glands of Cx. restuans, Cx. quinquefasciatus, Cx. tarsalis, and Cx. coronator (Dyar and Knab) (43-49). Detection of the virus genetic material occurred via real-time polymerase chain reaction (RT-PCR or RT-qPCR), with minimal blood meal titers of 4.0 log_{10} PFU/ml and 5.7 plaque-forming unit equivalents per milliliter (3.5 ± 0.1 log_{10} ZIKV PFUe/ml titer for freshly fed). These seven studies were performed in Hainan (province), China (11); Recife (city in the state of Pernambuco), Brazil (12, 43); Winnipeg/Beausejour (city and nearby town in the province of Manitoba), Canada (44); Guadalajara (city in the state of Jalisco), Mexico (45); Florida (state), United States of America (46); and Thailand (47). The study in Mexico (45) analyzed mosquitoes collected from the metropolitan area of Guadalajara but did not disclose the strains. This design was different from the studies in Brazil, Canada, China, Thailand, and the United States, which used mosquitoes from existing laboratory colonies. The Brazilian paper (43) also analyzed field-caught mosquitoes from Recife, Pernambuco, Brazil.
DISCUSSION

In our work, we found 36 relevant studies. This was 18 more works than the already identified articles in five reviews in the literature that examined the capacity of Culex spp. to transmit ZIKV (3, 4, 8, 9, 48). Cx. quinquefasciatus was identified in 6 articles, while Cx. restuans, Cx. tarsalis, and Cx. coronator were identified as possibly competent in 1 study each.

As stated before, researchers from the Ageu Magalhães Institute have detected ZIKV in salivary glands of Cx. quinquefasciatus at 7 and 15 days after feeding, and confirmed an infection rate of 100% and 67%, respectively (12). Additionally, one Chinese paper (11) was the first to demonstrate the presence of ZIKV (Strain SZ01) in the salivary glands of Cx. Quinquefasciatus; it was also the only study to show the transmission capacity to another species. This work reported a transmission rate of 89% to mice; these animals had viral RNA in their brain at 10 days post-engorgement, with a titer of 7.85 RNA log_{10} copies/ml. On the other hand, this study did not provide experiments with the same methodology with Ae. aegypti as a positive control.

One study demonstrated the presence of ZIKV in the salivary glands of Cx. quinquefasciatus mosquitoes in concentrations similar (P > 0.05) to those found in Ae. aegypti, both fed with 1 × 10^6 PFU/ml (log_{10}) blood meal titers (43). Even with a minimum blood meal titer of 1 × 10^6 PFU/ml (log_{10}), Cx. quinquefasciatus salivary glands were infected. These findings indicate that Cx. quinquefasciatus can produce virus in the salivary gland even when fed with low titers of viral particles. This ability better mimics what occurs in nature, where mean human viremia is lower than 2.5 log_{10} PFU/ml (49). Likewise, the study in the Brazilian state of Pernambuco (43) investigated wild-caught mosquitoes from the city of Recife. They observed no traces of recent feeding and used electron microscopy to detect ZIKV in the salivary glands. That paper did not perform experiments to analyze ZIKV transmission from Cx. quinquefasciatus to another species.

Dibernardo et al. (44) detected ZIKV in 3 of 58 (5%) of salivary glands of Cx. restuans just after intrathoracic inoculation. Cx. tarsalis was refractory using the same methodology. The authors believe that Cx. restuans could transmit by bite but also suggested the presence of salivary and midgut barriers for Cx. restuans and Cx. tarsalis. They concluded that Cx. restuans is not a competent ZIKV vector due to its feeding behavior.

Elizondo-Quiroga et al. (45) detected ZIKV in wild-caught Cx. tarsalis, Cx. coronator, and Cx. quinquefasciatus. However, this study was not conclusive regarding the ZIKV infection titers in the saliva of the collected mosquitoes because the mosquitoes that presented the lowest maximum titer of infection were Ae. aegypti, the primary ZIKV vector. Unfortunately, for none of those mosquitoes did the authors indicate whether the mosquitoes had any trace of recent feeding or which ZIKV strains were involved. Such deficiencies impair a more detailed assessment of the competence of these Culex spp.

RNA analysis was performed in saliva from Cx. quinquefasciatus eluted from filter paper at 14 days postinfection (DPI) in a first experiment and then at 16 DPI in a second experiment (46). The ZIKV titers were 5.6 ± 4.5 log_{10} ZIKV PFUe/ml and 5.02 log_{10} ZIKV PFUe/ml, respectively. This same research group (46) neither investigated transmission to another species nor applied the same methodology for Ae. aegypti (as a positive control). The lack of positive controls diminishes the reliability of the results. On the other hand, this paper intriguingly showed that viruses isolated from Culex saliva can form plaques in Vero cells. These data prove that biologically active virus can be obtained from the saliva of those mosquitoes.

A Thai study investigated vertical transmission of ZIKV to larvae in Ae. aegypti, Ae. albopictus, and Cx. quinquefasciatus, with positive results for all the species (47). The ZIKV strain virus/H. sapiens-tc/THA/2014/SV0127-14 infected the salivary glands of these mosquitoes fed with 1.7×10^6 FFU/ml blood meal titers. These Thai investigators (47) did not demonstrate any kind of transmission to other species but applied the same methodology for Ae. aegypti as a positive control.

Six studies that examined mosquito colonies reported that only when infection rates (IRs) from bodies were over 50% was salivary gland infection noted (11, 12, 44, 46, 47). In addition, we found 13 other studies with IRs under 50% without any detected transmission capacity (28-32, 34-41). One article, with a range of 15% to 70% of mosquitoes with intrathoracic inoculation, demonstrated virus-positive bodies but no detectable viral RNA or infectious virus in saliva (38).

In two studies (43, 46), the infection of salivary glands declined over time, findings that are different from the Chinese study mentioned earlier (11). Thus, there are inconsistent results with Cx. quinquefasciatus, with a marked decrease from day 8 to day 12 postexposure, but an apparent increase again at days 16 and 18 postexposure. Another aspect in these three articles is that they all used different strains: the Chinese study (11) used SZ01, from an infected patient who had returned from Samoa to China; the Brazilian paper (43) used BRPE243/2015, from Pernambuco; and the United States paper (46) used PRCABC59, from Puerto Rico (2015). Another study about vector competence of Aedes mosquitoes argued that in Ae. albopictus and Ae. aegypti, ZIKV transmission can be relatively dependent on the virus strain (49). Thus, it is important to note that diverse strains, especially strains isolated from patients, can present distinct behaviors. This phenomenon may represent one factor responsible for the varied results found in the literature.

With regards to blood meal titers, only one work (43), which used 4.0 log_{10} ZIKV PFU/ml, reached an IR of 36% at 7 DPI and 10.53% at 15 DPI. The majority of studies that reached some IR used blood meal titers greater than 5.0 log_{10} ZIKV PFU/ml, 5.0 log_{10} ZIKV RNA copies/ml, or 1 × 10^7 TCID_{50}/ml. The results were refractory at lower blood meal concentrations. As described above, 13 studies did not detect the infection capacity, dissemination, or transmission of any Culex spp., even with higher blood meal titers. Some authors (28, 38, 44) suggested that the random inability to transmit in Culex mosquitoes may be linked to a gut barrier of some Culex spp., a place where viral particles attack and initiate penetration and replication. However, Amraoui et al. (28) did not demonstrate that inoculation of viral particles into the hemocoel tissue of Cx. quinquefasciatus favored viral ZIKV dissemination or transmission. A study from the United States (46) warned about the existence of specific populations with regard to variability in transmission competence. The authors concluded that some Cx. quinquefasciatus populations may be capable of salivating ZIKV under environmental and other unknown conditions. This statement is noteworthy. It cannot be ignored that some mosquito populations may be more prone to ZIKV infection and dissemination. All these aspects definitely merit further studies that could reveal new intervention approaches.
Another particular condition revealed by Cioti et al. (49) in a study on the vector competence of Aedes mosquitoes was that there were significant differences in the proportion of infected mosquitoes with equivalent ZIKV titers but two different types of meals. Fresh blood meals resulted in a significantly higher IR than did stocked meals frozen and stored at -80°C and then thawed before preparation (P < 0.0001). Thus, considering the documents of Table 1 that indicated the transmission capacity, the Brazilian work in Pernambuco (43) stored the viral stocks at -80°C and subsequently thawed them to prepare the blood meal. However, another paper (11) was unclear about storage conditions and used a stock of virus that had been passaged twice in C6/36 cells prior to the infectious feed. This method suggests the use of a recently prepared blood meal.

Roundy et al. (50) noted that the criterion (iii) proposed by Barnett (51) for incrimination of an arthropod vector (repeated demonstration of natural infection of the vector) has only been fulfilled for Ae. aegypti and Ae. albopictus. Furthermore, as can be seen in our review, only one study (11) demonstrated ZIKV transmission to other species (criteria (iv) for incrimination of an arthropod (51)). However that study used a ZIKV strain not tested in any other work found for this review, and it did not include Ae. aegypti positive control tests. Thus, the defining evidence for Culex spp. as a ZIKV vector is still lacking.

Some authors (32, 38, 40) agree that the focus on prevention of ZIKV disease should remain on population control of the genus Aedes. Indeed, the probability of a Culex mosquito biting two humans in a sequence and transmitting the Zika virus is small, according to its preference for feeding on avian hosts (52). Nevertheless, Culex spp. are widespread in urban centers and also feed on human blood. Considering the results of studies with collected mosquitoes, when the Culex spp. was infected, the Aedes species was infected (30, 42, 43, 45). Only one study (15) presented data that showed neither Culex spp. nor Aedes mosquitoes infected with ZIKV. Conversely, in four papers (16, 24, 25, 26), there was no Culex mosquito infected while the official vector Ae. aegypti was infected. Two articles (25, 26) used the same collection of mosquitoes from the field with different methodologies to investigate viral ZIKV RNA. All these results with field-caught mosquitoes showed that Culex spp. and Ae. aegypti may use identical hosts (but not always). In fact, in a study with mosquitoes from field collections in Thailand (53), in Cx. quinquefasciatus there were mixed blood meals, with 7.84% from humans or monkeys, 47.06% from dogs, and 33.33% from other hosts. Comparatively, in Ae. aegypti, there were also several blood meals: 70.0% from humans plus monkeys or 13.33% only from monkeys and 10.0% from other kinds of hosts. These data demonstrate that variations in favorite hosts from place to place influence the infection rates of Culex spp. and Aedes mosquitoes. Additionally, as argued by Kaufman et al. (8), especially regarding the work from Pernambuco, Brazil (43), Cx. quinquefasciatus may serve as a secondary vector in places with abundant ZIKV infection in humans.

Additional experimental studies that use identical strains, experimental conditions, methodologies, with positive controls in Ae. aegypti and/or Ae. albopictus, and that utilize tests to prove the possibility of ZIKV transmission to other species from Culex spp., could be very decisive for discarding or confirming the contribution of these mosquitoes as a competent or incompetent ZIKV vector. According to the studies we have investigated, we feel that, besides well-implemented sanitation, the main strategies for the prevention and control of ZIKV should remain on the genus Aedes.

Conclusions

This work demonstrated the accumulation of evidence to prove the capacity of the ZIKV to infect Culex spp. However, only 7 studies out of the 36 identified for this review demonstrated the infection of Cx. restuans, Cx. quinquefasciatus, Cx. tarsalis, and Cx. coronator salivary glands. Furthermore, only 1 study showed the capacity of transmission to mice. Considering the records found here, Cx. quinquefasciatus remains the most widely studied species with confirmed salivary glands infected by ZIKV.

Additionally, only Asian or American ZIKV strains were able to infect the salivary glands of Culex mosquitoes: THA/2014/SV0127-14, SZ01, BRPE243, and PRVABC59. The MR766 and DAK AR 41525 African strains were unable to infect Culex spp. Further experimental studies that utilize the same strains, experimental conditions, use a positive Aedes control, and test ZIKV transmission to other species via Culex spp. are still needed to confirm the contribution of the Culex mosquitoes in ZIKV transmission. We believe that strategies for ZIKV control should stay focused on the genus Aedes, but responsible authorities should continue to monitor Culex spp. mosquitoes, especially regarding their ability to transmit ZIKV to other species. This surveillance should not be limited to determining whether their salivary glands are infected.

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REFERENCES

1. Waggoner JJ and Pinskey A. Zika Virus: diagnostics for an emerging pandemic threat. J Clin Microbiol. 2016;54:860-7.
2. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. Trans R Soc Trop Med Hyg. 1952;46:509-20.
3. Benelli G, Romano D. Mosquito vectors of Zika virus. Entomol Gen. 2017;36(4):309-18.
4. Boyer S, Calvez E, Chouin-Carneiro T, Diallo D, Failloux A-B. An overview of mosquito vectors of Zika virus. Microbes Infect [Internet]. 2018;20(11-12):646-60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29481568
5. Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau V-M. Potential sexual transmission of Zika virus. Emerg Infect...
Viveiros-Rosa et al. • Vector competence of Culex mosquitoes in Zika virus transmission

Dis [Internet]. 2015;21(2):359–61. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4313657 Accessed on November 8 2019.

Abushouk AI, Negjia A, Ahmed H. An updated review of Zika virus. J Clin Virol [Internet]. 2016;84:53-8. Available from: http://dx.doi.org/10.1016/j.jcv.2016.09.012 Accessed on November 8 2019.

Kuss S, Cohen JF. Full-length sequencing and genome characterization of Bagaza, Kedougou, and Zika viruses. Arch Virol. 2007;152(4):687-96.

Kauffman EB, Kramer LD. Zika Virus Mosquito Vectors: Competence, Biology, and Vector Control. J Infect Dis. 2017;216(Suppl 10):S976-90.

Gutiérrez-Bugallo G, Piedra LA, Rodriguez M, Bisset JA, Lourenço-de-Oliveira R, Weaver SC, et al. Vector-borne transmission and evolution of Zika virus. Nat Ecol Evol. 2019;3(4):561-9. Available at: https://doi.org/10.1038/s41559-019-0836-z Accessed on November 8 2019.

Marcondes CB, Contigiani M, Reimen W. Emergent and reemerging arboviruses in South America and the Caribbean: Why so many and why now? J Med Entomol. 2017;54(3):509-32.

Guo XX, Li CX, Deng YQ, Xing D, Liu QM, Wu Q, et al. Emergent and reemergent arboviruses in South America and the Caribbean: Why so many and why now? J Med Entomol. 2017;54(3):509-32.

Duchemin JB, Mee PT, Lynch SE, Vedururu R, Trinidad L, Parad-Grenier B, et al. Assessment of Local Mosquito Species Incriminated as Vectors due to the Potential of Zika Virus in Australia. PLoS Negl Trop Dis [Internet]. 2016;10(9):1-14. Available at: http://dx.doi.org/10.1371/journal.pntd.0004599 Accessed on November 8 2019.

Chaves MM, Alonso A, Leal CG, Lourenço-de-Oliveira R, Vazeille M, Failloux AB. Culex mosquitoes are experimentally unable to transmit zika virus. Eurosurveillance. 2016;21(35):1-4.

Boccolini D, Toma L, Di Luca M, Severini F, Romi R, Remoli ME, et al. Experimental investigation of the susceptibility of Italian Culex pipiens mosquitoes to Zika virus infection. Eurosurveillance. 2016;21(35):pii=30328. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.35.30328

Diallo D, Sall AA, Diagne CT, Faye OO, Faye YO, Ba Y, et al. Zika virus emergence in mosquitoes in Southeastern Senegal. PLoS One. 2014;9(4):10-11.

Franca RFO, Neves MHL, Ayres CFJ, Melo-Neto OP, Filho SPB. Zika virus detection, isolation and genome sequencing through Culicidae sampling during the epidemic in Brazil. Virus. PLoS Negl Trop Dis. 2016;10(9):e0004993. doi:10.1371/journal.pntd.0004993

Weger-Lucarelli J, Rueckert C, Chotiwat N, Nguyen C, Garcia Luna SM, Fauer JR, et al. Vector Competence of American Mosquitoes for Three Strains of Zika Virus. PLoS Negl Trop Dis. 2016;10(10):1-14. e0005101. doi:10.1371/journal.pntd.0005101

Suzuki S, Song S, Liu H, Li Y, Li X, Gao X, et al. Zika virus isolated from mosquitoes: a field and laboratory investigation in China, 2016. Sci China Life Sci. 2017;60(12):1364-71. doi:10.1007/s11427-017-9166-8

Amraoui F, Atyame-Nten C, Vega-Rúa A, Lourenço-De-Oliveira R, Failloux AB. Culex mosquitoes in the Americas. J Infect Dis. 2016;224(9):1349-56.

Huang Y-JS, Ayers VB, Lyons AC, Unlu I, Alto BW, Cohnstaedt LW, Pizer WR, et al. Entomological surveillance of Zika virus in Sardinia, Italy, 2016. Emerg Microbes Infect. 2019;8(1):699-706.

Heitmann A, Jansen S, Lühken R, Leggewie M, Schmidt-Pluskota B, et al. Experimental transmission of zika virus by mosquitoes from Asia. Sci Rep. 2015;5:10051. doi:10.1038/srep10051

Fernandes RS, Campos SS, Ferreira-de-Brito A, Miranda RM de, Barbosa da Silva KA, Castro MG de, et al. Culex quinquefasciatus from the northeast of Brazil is not competent to transmit the Local Zika Virus. PLoS Negl Trop Dis. 2016;10(9):e0004993. doi:10.1371/journal.pntd.0004993

Heitmann A, Jansen S, Lühken R, Leggewie M, Badusche M, Pluskota B, et al. Experimental transmission of zika virus by mosquitoes from central europe. Eurosurveillance. 2017;22(1-1): pii=30473. doi:10.2807/1560-7917.ES.2017.22.2.30473.

Amraoui F, Atyame-Nten C, Vega-Rúa A, Lourenço-De-Oliveira R, Failloux AB. Culex mosquitoes in the Americas. J Infect Dis. 2016;224(9):1349-56.

Heitmann A, Jansen S, Lühken R, Leggewie M, Badusche M, Pluskota B, et al. Experimental transmission of zika virus by mosquitoes from central europe. Eurosurveillance. 2017;22(1-1): pii=30473. doi:10.2807/1560-7917.ES.2017.22.2.30473.

Heitmann A, Jansen S, Lühken R, Leggewie M, Badusche M, Pluskota B, et al. Experimental transmission of zika virus by mosquitoes from central europe. Eurosurveillance. 2017;22(2):1-17. pii=30437. doi:10.2807/1560-7917.ES.2017.22.2.30437.

Heitmann A, Jansen S, Lühken R, Leggewie M, Badusche M, Pluskota B, et al. Experimental transmission of zika virus by mosquitoes from central europe. Eurosurveillance. 2017;22(2):1-17. pii=30437. doi:10.2807/1560-7917.ES.2017.22.2.30437.

Heitmann A, Jansen S, Lühken R, Leggewie M, Badusche M, Pluskota B, et al. Experimental transmission of zika virus by mosquitoes from central europe. Eurosurveillance. 2017;22(2):1-17. pii=30437. doi:10.2807/1560-7917.ES.2017.22.2.30437.

Heitmann A, Jansen S, Lühken R, Leggewie M, Badusche M, Pluskota B, et al. Experimental transmission of zika virus by mosquitoes from central europe. Eurosurveillance. 2017;22(2):1-17. pii=30437. doi:10.2807/1560-7917.ES.2017.22.2.30437.

Heitmann A, Jansen S, Lühken R, Leggewie M, Badusche M, Pluskota B, et al. Experimental transmission of zika virus by mosquitoes from central europe. Eurosurveillance. 2017;22(2):1-17. pii=30437. doi:10.2807/1560-7917.ES.2017.22.2.30437.

Heitmann A, Jansen S, Lühken R, Leggewie M, Badusche M, Pluskota B, et al. Experimental transmission of zika virus by mosquitoes from central europe. Eurosurveillance. 2017;22(2):1-17. pii=30437. doi:10.2807/1560-7917.ES.2017.22.2.30437.

Heitmann A, Jansen S, Lühken R, Leggewie M, Badusche M, Pluskota B, et al. Experimental transmission of zika virus by mosquitoes from central europe. Eurosurveillance. 2017;22(2):1-17. pii=30437. doi:10.2807/1560-7917.ES.2017.22.2.30437.
Competencia de los mosquitos Culex (Diptera: Culicidae) como vectores en la transmisión del virus del Zika: una revisión integradora

RESUMEN

Objetivo. Identificar estudios sobre la competencia de los mosquitos Culex como vectores de la transmisión del virus del Zika en todo el mundo.

Métodos. Se realizó una revisión integradora para identificar artículos relevantes sobre experimentos específicos dirigidos a determinar si los mosquitos Culex son vectores del virus del Zika. Se emplearon fuentes obtenidas a partir del portal electrónico de revistas CAPES (MEDLINE/PubMed, ScienceDirect Journals, Nature Publishing Group, SciELO, Springer Link, y otras 250 bases de datos) y la literatura gris.

Resultados. Se identificaron 344 estudios, 36 de los cuales fueron considerados para esta revisión. En 8 estudios se detectó infección en las glándulas salivales de Culex quinquefasciatus, Culex restuans, Culex tarsalis y Culex coronator. Cx. quinquefasciatus fue la especie más estudiada entre las confirmadas como potenciales vectores del virus del Zika, y solo las cepas de linajes asiáticos (THA/2014/SV0127-14; SZ01 [2016]) y americanos (BRPE243 [2015]; PRVABC59 [2015]) pueden infectar las glándulas salivales de los mosquitos Culex. Las cepas africanas analizadas (MR766 y DAK AR 41525) no fueron capaces de infectar las glándulas salivales.

Conclusiones. Aunque faltan pruebas convincentes que indiquen que las especies de Culex spp. son un vector competente del virus del Zika, estas deben seguir monitorizándose mediante estudios adicionales, especialmente respecto de su capacidad para transmitir el virus del Zika a otras especies. Esta vigilancia no debería limitarse solamente a determinar la infección en las glándulas salivales.

Palabras clave. Salud pública; virus Zika; mosquitos vectores; Culex.
Competência vetorial de mosquitos *Culex* (Diptera: Culicidae) na transmissão do vírus Zika: revisão integrativa

**Resumo**

**Objetivo.** Identificar estudos sobre a competência dos mosquitos *Culex* como vetores da transmissão do vírus Zika em todo o mundo.

**Métodos.** Uma revisão integrativa foi realizada para identificar artigos relevantes sobre experimentos específicos para determinar se os mosquitos *Culex* são vetores do vírus Zika. As fontes utilizadas na pesquisa foram o portal eletrônico CAPES (MEDLINE/PubMed, ScienceDirect Journals, Nature Publishing Group, Scielo, Springer Link, e outras 250 bases de dados) e a literatura cinza.

**Resultados.** Foram identificados 344 artigos, dos quais 36 foram considerados para esta revisão. Oito artigos relataram infecção nas glândulas salivares de *Culex quinquefasciatus*, *Culex restuans*, *Culex tarsalis* e *Culex coronator*. *Culex quinquefasciatus* foi a espécie mais estudada entre as confirmadas como vetores potenciais do vírus Zika. Apenas as linhagens asiáticas (THA / 2014 / SV0127-14; SZ01 [2016]) e americanas (BRPE243 [2015]; PRVABC59 [2015]) podem infectar as glândulas salivares dos mosquitos *Culex*. As cepas africanas analisadas (MR766 e DAK AR 41525) não foram capazes de infectar as glândulas salivares.

**Conclusões.** Ainda não há evidências convincentes para indicar que os mosquitos *Culex* são um vetor competente do vírus Zika. Contudo, estudos adicionais de monitoramento devem ser realizados, especialmente no que diz respeito à transmissão do vírus Zika para outras espécies de mosquitos. Além disso, os estudos não devem se limitar a estudar a infecção nas glândulas salivares.

**Palavras-chave** Saúde pública; Zika virus; mosquitos vetores; *Culex*.