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Publication Date

2016

DOI

10.12688/f1000research.9839.1

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OPINION ARTICLE

Zika mosquito vectors: the jury is still out [version 1; referees: 5 approved]

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v1 First published: 20 Oct 2016, 5:2546 (doi: [10.12688/f1000research.9839.1](https://doi.org/10.12688/f1000research.9839.1))
 Latest published: 20 Oct 2016, 5:2546 (doi: [10.12688/f1000research.9839.1](https://doi.org/10.12688/f1000research.9839.1))

Abstract

After a 40-year hiatus, the International Congress of Entomology (ICE 2016) convened in Orlando, Florida (September 25-30, 2016). One of the symposia at ICE 2016, the Zika Symposium, covered multiple aspects of the Zika epidemic, including epidemiology, sexual transmission, genetic tools for reducing transmission, and particularly vector competence. While there was a consensus among participants that the yellow fever mosquito, *Aedes aegypti*, is a vector of the Zika virus, there is growing evidence indicating that the range of mosquito vectors might be wider than anticipated. In particular, three independent groups from Canada, China, and Brazil presented and discussed laboratory and field data strongly suggesting that the southern house mosquito, *Culex quinquefasciatus*, also known as the common mosquito, is highly likely to be a vector in certain environments.



This article is included in the [Zika & Arbovirus Outbreaks](#) channel.

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| published 20 Oct 2016 | report | report | report | report |
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How to cite this article: Leal WS. **Zika mosquito vectors: the jury is still out [version 1; referees: 5 approved]** *F1000Research* 2016, 5:2546 (doi: [10.12688/f1000research.9839.1](https://doi.org/10.12688/f1000research.9839.1))

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Grant information: My research on vector biology is supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award numbers R01AI095514 and R21AI128931.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: W.S.L. has a long-term collaboration with FIOCRUZ-PE and is a co-author of the paper by Guedes *et al.* 21 , currently under consideration for publication.

First published: 20 Oct 2016, 5:2546 (doi: [10.12688/f1000research.9839.1](https://doi.org/10.12688/f1000research.9839.1))

Background

The International Congresses of Entomology (ICEs) are the “Olympics of Entomology,” which started in 1910 in Brussels, Belgium. It is only the third time that ICE has been held in the United States of America (USA), the last conference being 40 years ago when delegates convened in Washington, DC for XV ICE. This time delegates from 102 countries convened in Orlando, Florida (September 25–30, 2016) for a historic event titled “Entomology Without Borders.” With 6,682 delegates, ICE 2016 was undoubtedly the largest gathering of scientists in the history of entomology.

This Opinion article is based mainly on the current literature and the Zika Symposium at ICE 2016, which was organized by Dr. Constância Ayres, Oswaldo Cruz Foundation (FIOCRUZ-PE), Recife, Brazil, and Dr. Adriana Costero, National Institutes of Health, Bethesda, Maryland. The symposium featured the following speakers (Figure 1): Dr. Celina Martelli, Centro de Pesquisas Aggeu Magalhães, FICRUZ-PE, Brazil; Dr. Stephen Higgs, Kansas State University, Manhattan, Kansas; Dr. Brian D. Foy, Colorado State University, Fort Collins, Colorado; Dr. Constância Ayres, FICRUZ PE, Dr. Duschinka Guedes, FIOCRUZ-PE; Dr. Luciano Moreira, FIOCRUZ-MG; Dr. Anthony A. James, University of California, Irvine; Dr. Fiona F. Hunter, Brock University, Canada; and Dr. Tang-yan Zhao, Institute of Microbiology and Epidemiology, Beijing, China; and was attended by hundreds of delegates.

Zika history

At the time we were preparing to submit a bid on behalf of the Entomological Society of America to host ICE 2016 in Orlando

and during the first years of preparation for the conference, Zika was not part of the vocabulary of a large majority of entomologists, myself included. The Zika virus (ZIKV) was isolated first from a febrile monkey and later from the mosquito *Aedes africanus* about the time our predecessors were preparing to host VIII ICE in Stockholm¹. After Sweden in 1948, ICE convened in Amsterdam, Montreal, Vienna, London, Moscow, Canberra, Washington, Kyoto, Hamburg, Vancouver, Beijing, Florence, Iguacu Falls, Brisbane, Durban, and Daegu (South Korea), and yet ZIKV was not part of our jargon. During these years while ICE delegates were travelling throughout the world to advance the field of entomology, ZIKV was silently making headway out of the Zika forest (Entebbe, Uganda), hitchhiking on humans, and conquering new habitats. As it reached new environments, the virus was likely being transmitted locally by native species of mosquitoes and/or other “illegal immigrants” as, for example, the notorious yellow fever mosquito, *Aedes aegypti*. ZIKV was isolated from humans for the first time in 1954 during an outbreak of jaundice suspected of being yellow fever. The virus was isolated from one of the three patients examined, and the other two exhibited high titers of serum antibodies against the virus². As Dr. Stephen Higgs pointed out in his presentation, outbreaks occurred in Asia between 1954 and 2007, and up to that point when the virus reached Yap Island only 14 human cases had been identified, so no one paid much attention to the virus. However, on Yap Island, Federal State of Micronesia, it was estimated that 73% of residents 3 years of age or older had been infected with ZIKV³, which is characterized by rash, conjunctivitis, and arthralgia. In an attempt to identify the mosquito vector, they collected adults from the field and found that *Aedes hensilli* and *Culex quinquefasciatus*, 41.2 and 28.1%, respectively, were the predominant species.



Figure 1. Snapshot of the Zika Symposium at the 2016 International Congress of Entomology in Orlando, FL. From the left: Dr. Adriana Costero; Dr. Celina Martelli; Dr. Duschinka Guedes (back row); Dr. Tang-yan Zhao; Dr. Anthony A. James (back row); Dr. Stephen Higgs; Dr. Constância Ayres; the author (back row); Dr. Brian D. Foy; Dr. Luciano Moreira; and Dr. Fiona F. Hunter.

Because the virus was not found in any field-collected *Aedes* mosquitoes, they conducted laboratory studies to determine the vector competence of *Ae. hensilli* to transmit ZIKV. About 80% of the mosquitoes fed through the Hemotek® feeding system on ZIKV-containing sheep blood became infected, but only 13–23% developed dissemination infections. By contrast, 60% of the mosquitoes fed blood-containing Chikungunya virus (CHIKV) became infected and 80% disseminated. Their findings supported the possibility that *Ae. hensilli* served as a vector during the Zika outbreak on Yap Island.

Preparing for Chikungunya and Zika major outbreaks

Up until 2013, ZIKV infection was considered a mild infection, but in French Polynesia the symptoms were complicated by Guillain-Barré syndrome⁴, a rare neurological disorder identified 100 years ago. It has been estimated that between September 2013 and March 2014 as many as 28,000 patients were affected, i.e., ca. 11% of the Polynesian population⁵. The next and most notorious ZIKV outbreak occurred in Brazil, the locale from which the virus has been moving rapidly up toward North America and back to Africa. Apparently, ZIKV went under the “radar screen” soon after the arrival of CHIKV. Brazil was expecting an outbreak of CHIKV; primary care physicians (PCPs) had been trained at least two years in advance on how to distinguish CHIKV symptoms from those elicited by the Dengue virus (DENV). The first local transmission of CHIKV in Brazil was reported on September 16, 2014⁶. Epidemiologists and PCPs created a network using WhatsApp, “Chickv – the mission,” to share information and get better prepared for the epidemic. While working on CHIKV, they started observing cases sometimes referred to as “dengue fraca” (weak dengue), which was characterized by low fever and an intense allergic reaction. The detective work of medical doctors prompted epidemiologists to consider the possibility of another virus outbreak. ZIKV was detected in Brazil for the first time in April/May 2015. According to an investigative account by professor of bioethics, Debora Diniz⁶, the first publication on the occurrence of ZIKV in Brazil⁷ was authored by the second group to isolate the virus. The first group to report their findings to the media (on April 29, 2015) published their findings in a peer-reviewed journal⁸ five months after the first scientific report⁷.

Microcephaly

During the Zika Symposium, Dr. Celina Martelli reported that in August–September 2015, neurologists, neonatologists, and epidemiologists became more aware of neonates with microcephaly, and in October clinical data and brain imaging suggested a congenital infection. Then, they noticed an important correlation between the major circulation of ZIKV in the Northeast region of Brazil and the time of the first gestational months of mothers. Thereafter, ZIKV was detected in amniotic fluid⁹, and it is now well established that ZIKV causes microcephaly¹⁰. It was this teratogenic effect, unique for a flavivirus¹¹ and arboviruses in general, coupled with its rapid dissemination in Latin America that led the World Health Organization on February 1, 2016 to declare ZIKV an international public health emergency.

Sexual transmission

ZIKV is the only arbovirus currently known to also be sexually transmitted. Speaking at ICE 2016, Dr. Brian Foy suggested that sexual transmission is underestimated in epidemiological data. He pointed out that there are two main causes for this underestimation. It is difficult to decouple sexual from mosquito-borne transmission in outbreak areas and questionnaires regarding patients’ sex life tend to be inaccurate for various reasons. The case study of Dr. Foy and his wife in the United States was the first report to suggest sexual transmission of ZIKV. Five to six days after returning from field research in Senegal, Dr. Foy had typical symptoms of arbovirus infection, and a few days later his wife started developing similar symptoms, although her headaches and photophobia were more intense and arthralgia remained for months. There were no infected mosquito vectors in the area, their children did not get sick, and Dr. Foy had no reason to “lie on a questionnaire.” He immediately suspected sexual transmission and the serological data supported his hypothesis¹². It was the first documented case of sexual transmission of ZIKV, but it garnered little attention from authorities, because no one could envision that ZIKV would cause such an epidemic eight years later. Dr. Foy suggested that sexual transmission is a matter of major concern, particularly now that there is growing evidence that ZIKV remains active in seminal fluid for six months¹³ and localizes to and replicates very well in tissues of the urogenital tract¹⁴ after both mosquito bite and sexual transmission. In short, the epidemic is unlikely to be controlled only by interrupting/terminating transmission by mosquito vectors.

Genetically-based vector control strategies

Dr. Luciano Moreira discussed his laboratory findings indicating that a bacterium isolated from the southern house mosquito, *Cx. quinquefasciatus*, *Wolbachia* sp (*wMel_Br* strain), blocks transmission of ZIKV by the yellow fever mosquito, *Ae. aegypti*, and could be a useful tool for decreasing ZIVK transmission. Dr. Anthony James gave an overview of all genetic tools currently available in vector control. He explained that the strategy for population suppression is a genetic tool analogous to the development of an insecticide in the sense that the ultimate goal is to reduce or eliminate populations of vectors. Another strategy is population alteration (formerly known as population replacement) in which the ability of the mosquito to transmit a virus is changed, i.e., a gene engineered into the mosquito’s genome impacts the vector competence and, consequently, the virus does not replicate and is not transmitted. This is analogous to the *Wolbachia* strategy reported by Dr. Moreira. The two techniques have quite different approaches for an ultimate goal of reducing transmission of vector-borne diseases. The former is aimed at reducing mosquito populations and, consequently, reducing mosquito bites and transmission of viruses. On the other hand, with population alteration mosquitoes still bite, but no longer transmit the target virus. Lastly, Dr. James discussed a technique called gene drive, which allows a gene to move quickly into a population. This would have a long-term effect, so he emphasized the need for laboratory tests and contained facility experiments before full implementation in a

vector control program. He concluded by suggesting that the best scenario is cases involving one single pathogen causing a disease and one single mosquito vector. Later, in the discussion he gave a specific example when answering questions from Dr. Thomas Scott, University of California-Davis. He suggested that these genetic tools might not be the best strategies for ZIKV given that at this point there seem to be multiple vectors not only at the species but also at the population level. The current genetic technologies would not be appropriately applied to such complex systems. He would not recommend any specific genetic strategy for ZIKV at this point. By contrast, he indicated that DENV might be a good candidate, particularly in areas where clearly there is only one vector species. Dr. James noted that in the *Aedes*-DENV case replacement might be the best alternative given that it is difficult to achieve population suppression of *Aedes* mosquitoes.

Zika vectors

Dr. Fiona Hunter examined closely the phylogeny of ZIKV-related viruses and showed that ZIKV belongs to a transition point between classical *Culex*-associated and *Aedes*-associated viruses. Her analysis suggested that ZIKV belongs to a clade (supported 99%) of neurotropic viruses, including West Nile Virus (WNV) and Saint Louis Encephalitis (SLE) virus, which are typically transmitted by *Culex* mosquitoes. ZIKV does not belong to a clade of hemorrhagic viruses, such as DENV and yellow fever, which are typically transmitted by *Aedes* mosquitoes. In short, there is apparently a dichotomy between the mosquito vector species vis-à-vis the taxonomy of the virus per International Committee on Taxonomy of Viruses. She suggested that we should keep an open mind, because ZIKV might have a larger range of vectors. In her field studies in the Dominican Republic, she collected one ZIKV-infected *Culex* mosquito, but was not able to identify the mosquito to the species level because of the damage caused by her trapping system. Additionally, she reported preliminary data on vector competence of *Cx. pipiens* collected in Canada by RT-qPCR and plaque assays showing at least 2% ZIKV transmission with her ongoing analysis. In summary, her taxonomic analysis along with field and laboratory findings support her hypothesis for a wider range of ZIKV vectors.

Dr. Constância Ayres suggested that *Cx. quinquefasciatus* is being held to a different standard than *Ae. aegypti* with regard to ZIKV transmission and its potential role as a ZIKV vector has been overlooked. She started her presentation by stressing the textbook¹⁵ criteria for incrimination of arthropods as vectors of humans and other animals. In particular, she emphasized that in multiple reported cases no data were available in the urban environment regarding criteria #3 and #4, i.e., “repeated demonstration that suspected vectors, collected under natural conditions, harbor the identifiable, infective stage of the pathogen” and “a biological association between clinical cases and infected mosquitoes in time and space,” respectively. For example, no infected mosquitoes were collected from Yap States and French Polynesia during the outbreaks. They studied vector competence under laboratory conditions and assumed that *Aedes* species were vectors, although they never fulfilled the above criteria. She stressed the fact that

there are significant populations of *Cx. quinquefasciatus* in these locations, but they were not analyzed for ZIKV infection, and vector competence was not studied under laboratory conditions. Given this scenario, Dr. Ayres wrote a position paper¹⁶ arguing the need to determine unambiguously whether *Cx. quinquefasciatus* is a ZIKV vector. Ever since, various studies have suggested that *Culex* species are not ZIKV vectors, whereas evidence from other studies (see below) strongly suggests that *Cx. quinquefasciatus* might be a significant vector. Of note, Aliota and collaborators¹⁷ fed mosquitoes from laboratory colonies on mice previously infected with Asian lineage ZIKV strain PRVABC59. All samples from *Cx. pipiens* mosquitoes and all replicates were negative for ZIKV by plaque assay, 14 days post-infection (dpi). By contrast, at least one replicate for each, *Ae. aegypti* and *Ae. albopictus*, showed at least a 22% transmission rate. Huang *et al.*¹⁸ studied infection and dissemination rates of 7 and 14 dpi and demonstrated that *Cx. pipiens* and *Cx. quinquefasciatus* (Vero Beach strain) were refractory to ZIKV, although they did not report a positive control. Fernandes¹⁹ and collaborators achieved a remarkable feat. In a short period of time, they were able to capture *Cx. quinquefasciatus* from various suburbs in Rio de Janeiro and conduct vector competence studies under laboratory conditions using F1 generations. Although survival rate, yield of blood meals, and other parameters were not reported, their data showed zero transmission rates for *Cx. quinquefasciatus* at 7, 14, and 21 dpi. Their publication was widely highlighted in the press and social media, but a report from Guo *et al.*²⁰ that appeared the next day got absolutely no coverage. As discussed (see also below), this report provides clear evidence of infection, dissemination, replication in salivary glands and transmission to infant mice by *Cx. pipiens quinquefasciatus*. As it stands now, we should take Dr. Hunter's advice and keep in mind that the jury is still out regarding ZIKV vectors. It might as well be that virus strains and/or mosquito populations account for the discrepancies. Dr. Ayres pointed out in her presentation that one should not forget the socioeconomic and ecologic factors, environment, and behavior of *Cx. quinquefasciatus* in Recife when considering the full range of vectors.

Dr. Duschinka Guedes presented solid evidence demonstrating that ZIKV was detected in midgut, salivary glands, and saliva of *Cx. quinquefasciatus* from Recife, Brazil, which were artificially infected with a strain of the ZIKV isolated from a local patient. In addition to conventional vector competence studies under laboratory conditions, Dr. Guedes showed that both *Cx. quinquefasciatus* and *Ae. aegypti* expectorated the virus into honey-soaked filter papers, 9–12 dpi. Additional evidence of replication in the salivary glands was demonstrated by transmission electron microscopy data. ZIKV-infected acinar cells showed signs of cytopathic disruptions, and mature ZIKV particles were clearly observed. Lastly, she reported that multiple pools of *Cx. quinquefasciatus* mosquitoes collected from urban areas with a high incidence of microcephaly in Recife were infected with ZIKV²¹. Dr. Guedes' presentation strongly suggests that in Recife, Brazil, both *Ae. aegypti* and *Cx. quinquefasciatus* are ZIKV vectors. Working independently in China, Dr. Tang-yan Zhao reached similar conclusions. Dr. Zhao performed conventional vector competence

studies, which demonstrated ZIKV replication in midgut and salivary glands²⁰. Additionally, she placed 91-day-old infant mice in a cage with *Cx. quinquefasciatus* mosquitoes previously infected with ZIKV. The infant mice developed red blotches on the skin, and at 10 dpi eight of the nine mice had high titers of viral RNA in their brains. These two independent studies discussed at the Zika Symposium are complementary in nature and they both support the notion that *Cx. quinquefasciatus* might be a ZIKV vector. It is, therefore, advisable that vector management programs aimed at mitigating ZIKV transmission do not ignore *Cx. quinquefasciatus*, unless new and unambiguous evidence will show that a target population of the common mosquito is not a vector. For the time being, it is prudent to consider that vector competence may vary among different populations of the same species and/or the strains of the virus.

Zika discussion

During the discussion at the end of the symposium, the forum was opened for questions and comments. “Is anyone looking for the virus in birds?” asked Dr. Scott Ritchie, James Cook University, Australia. This question captures the sentiment that both questions were thought provoking, and we still do not have all or many answers when it comes to ZIKV. Hopefully, we will be better prepared when convening in Finland for ICE 2020. Wouldn’t it be wonderful to report in Helsinki that mosquito vector populations

have been reduced or eliminated, the Zika and other epidemics were contained, vaccines have been made available, and entomologists are ready to further improve the human condition by tackling other problems than the Zika epidemic?

Competing interests

W.S.L. has a long-term collaboration with FIOCRUZ-PE and is a co-author of the paper by Guedes *et al.*²¹, currently under consideration for publication.

Grant information

My research on vector biology is supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award numbers R01AI095514 and R21AI128931.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

The author would like to thank Drs. Constância Ayres and Adriana Costero for organizing the symposium, all speakers for sharing the most recent data, and ICE 2016 delegates who attended the Zika Symposium, particularly those who asked question and shared their thoughts.

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Version 1

Referee Report 09 November 2016

doi:[10.5256/f1000research.10608.r17269](https://doi.org/10.5256/f1000research.10608.r17269)



Jeffery K. Tomberlin

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The title and abstract are very appropriate as they give the reader a quick overview of the subject matter discussed as related to Zika. This opinion piece provides a brief background of the International Congress of Entomology with an emphasis on a symposium exploring what is, and not, known about Zika. The article summarizes presentations given by a number of globally recognized researchers in the field of medical entomology and disease ecology. The article does an excellent job providing an overview of where society, and research, stands today with regards to Zika and its potential spread globally. However, as they discuss, additional research is needed to fully understand the ecology of the pathogen and vector capacity as related to disease manifestations and frequency of occurrence. I would like to stress the outbreak of Zika is a demonstration of the need to continue exploring the mechanisms regulating mosquito, and other arthropod vectors, attraction to, and blood-feeding on, hosts as the development of methods to disrupt such interactions could prove vital in protecting human populations and their associated livestock globally.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Report 09 November 2016

doi:[10.5256/f1000research.10608.r17437](https://doi.org/10.5256/f1000research.10608.r17437)



Andre Freire Furtado

Centro de Pesquisas Aggeu Magalhães , Recife, Brazil

In this opinion article, Dr. Walter S. Leal presents not only the discrepant results in the literature concerning the vector or vectors mosquitoes of Zika (ZIKV) – (Aedes, Culex), but gives an up-to-date knowledge about the history of ZIKV infection and its consequence on the human health.

Dr. Leal clearly describes all the steps since the ZIKV isolation from a febrile monkey of Uganda forest, the isolation of ZIKV from human in 1954 and presents the results (opinions) of different groups of research, concerning the responsible vector of ZIKV transmission as well as the different concerns to explain the discrepancies. The paper also brings information about ZIKV epidemics in the world, the

sexual transmission, and a very helpful discussion on the genetically based vector control strategies. A disease considered as a mild infection in the beginning is now well established as causing microcephaly through congenital infection. This teratogenic effect led the World Health Organization (WHO) to declare ZIKV an international public health emergency.

I read this submission and my opinion is that it is worth to be indexed considering its high standard.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Report 08 November 2016

doi:[10.5256/f1000research.10608.r17268](https://doi.org/10.5256/f1000research.10608.r17268)



Mario Alberto Cardoso da Silva-Neto

Instituto de Bioquímica Medica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

I have read the MS "Zika mosquito vectors: the jury is still out" by Walter S. Leal Department of Molecular and Cellular Biology, University of California-Davis, Davis, USA. This MS nicely describes the scientific and academic atmosphere and major findings of International Congress of Entomology (ICE 2016) held in Orlando, Florida (September 25-30, 2016). The MS goes far beyond the simple description of the common routine in Meetings such like this. In fact due to our major and now global concern on arboviroses the MS provides a precise account of the most relevant issues discussed on this meeting. Also, it poses an ultimate perspective that should be urgently followed regarding the discussion of the true vectors of Zika. Such relevant points must be immediately addressed concerning the final definition of the molecular and genetic strategies to be used for its control which were also discussed in the meeting and reported in the present MS. I believe the scientific content and the present format of this MS are suitable for indexing.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Report 07 November 2016

doi:[10.5256/f1000research.10608.r17135](https://doi.org/10.5256/f1000research.10608.r17135)



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In summarizing a timely and well-attended symposium at the ICE2016 conference, Leal's opinion article also masterfully encapsulates the history of the Zika epidemic and glaring gaps in our understanding of Zika virus transmission. While still in its early phase, the Zika outbreak is a relatively "minor" public health

event on a global scale, compared to other infectious and arboviral diseases, such as Malaria. But because of its astonishingly rapid emergence, especially in Brazil, its unusual damaging effect on neurological development of the human neonatal brain, its spectacular evolution since it was first isolated, and its sexual transmission and stealthy existence in seminal fluid in humans, this virus has “gone viral”, especially during the 2016 summer Olympics in Rio de Janeiro. Leal’s summary highlights important needs in the research agenda to combat Zika. All public health interventions rally around “source/vector reduction”, but the full array of mosquito species that are competent to vector the Zika virus remains to be determined. Recent research has added genetic approaches to the tool-box for vector management, but it will be years before these powerful options can be broadly implemented.

A couple of corrections and editorial points:

1. Consider replacing “conquering” with “spreading into” in the sentence: “ZIKV was silently making headway out of the Zika forest (Entebbe, Uganda), hitchhiking on humans, and conquering new habitats” (p.2).
2. Change “91-” to “nine one-” in the sentence: “Additionally, she placed 91-day-old infant mice in a cage” (p.5).

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: I have co-authored papers with Walter Leal.

Referee Report 31 October 2016

doi:[10.5256/f1000research.10608.r17310](https://doi.org/10.5256/f1000research.10608.r17310)



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This opinion article is essentially a meeting report, where most of the speakers showed data indicating that *Culex* mosquitoes could be vectors of Zika. However, as pointed by Dr Leal, several other groups published independent reports with negative results for *Culex* infection with Zika. While the major claim of this paper (that the vector competence of *Culex* for Zika is still an open question) seems a fair statement, reading of the published papers do not provide an immediate explanation for the discrepant results from different groups. Of course, although experimental errors can not be excluded, data can be reconciled as differences in virus and or mosquito strains (or even symbiont microbiota) can explain the distinct findings from each group. If Zika transmission was merely an academic question, I would say that time would solve the question. However, this is a major threat for global public health, and transmission mediated by one or two species calls for very distinct control strategies. Therefore, I would add to this report that there this debate needs urgently to evolve in the experimental field, ideally as collaborative research, but at least with exchange of virus and mosquitoes strains, and rigorous comparisons of the methodology.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.
