

from day 76 performed at the national arbovirus reference laboratory indicated a C_t value of 35.82). Semen samples collected on days 99 and 117 tested negative for Zika virus RNA. Attempts at virus isolation from the semen sample collected on day 23 failed to cultivate infectious particles.

It is very unlikely that transmission of Zika virus infection to patient 2 occurred through a mosquito bite. Although occasional interceptions of exotic mosquito species have occurred at international ports of entry into New Zealand, neither of the *Aedes* species of mosquito capable of transmitting Zika virus infection is established in the country (10). This case report and results of research into the duration of infectivity of Zika virus in semen can inform the evolving guidelines concerning the recommended duration of abstinence from sexual intercourse and the practice of barrier protection methods to prevent sexual transmission of Zika virus infection.

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Culex pipiens and *Aedes triseriatus* Mosquito Susceptibility to Zika Virus

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DOI: <http://dx.doi.org/10.3201/eid2210.161082>

To the Editor: Zika virus, genus *Flavivirus*, has spread nearly uncontrolled since its introduction into the Western Hemisphere; autochthonous spread has occurred in ≥ 39 countries and territories, including several US territories. Transmission of Zika virus is usually by the bite of infected mosquitoes, and potential for emergence in areas with competent mosquito vectors is high (1). Future spread of Zika virus is unpredictable; however, eventual local spread in the United States is possible. As of July 13, 2016, a total of 1,306 travel-associated cases had been reported (ArboNET, <https://www.cdc.gov/zika>); substantial populations of *Aedes (Stegomyia) aegypti* (Linnaeus) mosquitoes exist in ≥ 16 states in the eastern, southeastern, and southwestern United States; and *Ae. (Stegomyia) albopictus* (Skuse) mosquitoes inhabit ≥ 28 states and continued expansion throughout the northern United States is probable (2). Mosquitoes of these 2 species have demonstrated the ability to transmit Zika virus (1).

The recent epidemic spread of Zika virus suggests that *Ae. aegypti* mosquitoes are the main vector; however, information about the role of other species in driving and maintaining Zika virus transmission is lacking. Of particular concern this summer (2016) is emergence and establishment of Zika virus in previously unaffected geographic areas; with the advent of mosquito season commencing in most of the continental United States, the likelihood of mosquito-borne transmission of Zika virus in states without populations of *Ae. aegypti* and *Ae. albopictus* mosquitoes remains unknown. To understand the potential risk for spread of Zika virus in temperate US states, we compared the relative abilities of *Culex pipiens* and *Ae. triseriatus* mosquitoes to transmit Zika virus in the laboratory. We used *Ae. aegypti* and *Ae. albopictus* mosquitoes as positive controls.

Laboratory colonies of mosquitoes used in this study were maintained at the University of Wisconsin–Madison, and vector competence for Zika virus was evaluated by using established procedures (3,4). Mosquitoes from each group were incapacitated (exposed to trimethylamine); legs were removed and collected. Salivary secretions were collected in capillary tubes containing a 1:1 ratio of fetal bovine serum and 50% sucrose. Mosquitoes were then placed

Table. Competence of mosquitoes, by species, as Zika virus vectors, 14 days after peroral infection, United States*

Mosquito species	No. virus-positive/no. tested (%)								
	Biological replicate 1, mean 6.02 log ₁₀ PFU/mL ± SD 0.67			Biological replicate 2, mean 4.74 log ₁₀ PFU/mL ± SD 0.06			Biological replicate 3, mean 6.83 log ₁₀ PFU/mL ± SD 0.45		
	I	D	T	I	D	T	I	D	T
<i>Culex pipiens</i> †	0/20 (0)	0/20 (0)	0/20 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/30 (0)	0/30 (0)	0/30 (0)
<i>Aedes triseriatus</i> ‡	ND	ND	ND	0/20 (0)	0/20 (0)	0/20 (0)	4/13 (31)	0/4 (0)	0/4 (0)
<i>Ae. albopictus</i> §	9/9 (100)	6/9 (67)	2/9 (22)	1/6 (17)	0/1 (0)	0/1 (0)	ND	ND	ND
<i>Ae. aegypti</i> ¶	ND	ND	ND	ND	ND	ND	17/17 (100)	12/17 (71)	4/17 (24)

*Zika virus strain PRVABC59 (GenBank accession no.KU501215) was originally isolated from a traveler to Puerto Rico in December 2015. I, infected; D, disseminated; ND, no data; T, transmitted.
†Originated from egg rafts collected in Iowa in 2002 and colonized at the Iowa State University Medical Entomology Laboratory.
‡Originated from eggs collected in Iowa in 2002 and 2003 and colonized at the Iowa State University Medical Entomology Laboratory.
§Originated from eggs collected in Missouri in 2002 and colonized at the Illinois Natural History Survey.
¶Black-eyed Liverpool strain.

in individual tubes; their bodies and legs were homogenized, clarified by centrifugation, and screened for virus infection. Dissemination was indicated by virus-positive legs, and transmission potential was indicated by virus-positive salivary secretions. All samples were screened by plaque assay on Vero cells. Mosquitoes were exposed to Asian lineage Zika virus strain PRVABC59 (GenBank accession no. KU501215) (5) by feeding on Zika virus-infected *Ifnar*^{-/-} mice (4). Mice (n = 4/replicate) yielded infectious blood meal concentrations of 6.02 log₁₀ PFU/mL ± 0.67 (mean ± SD; biological replicate no. 1), 4.74 log₁₀ PFU/mL ± 0.06 (replicate no. 2), and 6.83 log₁₀ PFU/mL ± 0.45 (replicate no. 3). Blood meal concentrations in mice were consistent with viremia concentrations of humans in the field (4).

All samples from *Cx. pipiens* mosquitoes and all replicates were negative for Zika virus by plaque assay (Table). In contrast, *Ae. triseriatus* mosquitoes were susceptible to infection when exposed to mice with the highest viremia concentrations (Table). However, none of these infected mosquitoes disseminated virus and none were capable of transmitting the virus. Data from *Ae. albopictus* and *Ae. aegypti* mosquitoes that had been exposed to the same mice demonstrated that the viremia concentrations used could productively infect mosquitoes. Of note, *Ae. albopictus* mosquito infection rates were dose dependent (i.e., infection rates increased with blood meal titer). Furthermore, data generated from exposure to the same mice demonstrated productive mosquito infection with these viremia concentrations (4). It therefore seems likely that if Zika virus circulation in the United States occurs, it will be driven by *Ae. albopictus* or *Ae. aegypti* mosquitoes (6). However, we cannot rule out that anthropophilic mosquitoes of other species in this country could be competent vectors.

These data argue for continued studies (experimental and epidemiologic) assessing interactions between differing mosquito–Zika virus combinations in the United States because of geographic variations that may exist in oral susceptibility of mosquitoes of the same or different species. The few vector competence studies conducted to

date have focused primarily on *Ae. aegypti* and *Ae. albopictus* mosquitoes (8), but mosquitoes of other species may be vectors, depending on geographic location. We focused on *Cx. pipiens* mosquitoes because they are ubiquitous (7), they are considered one of the principal vectors of West Nile virus in the northern half of the United States, and a recent report from Brazil suggests *Cx. quinquefasciatus* mosquitoes as potential Zika virus vectors (8). We chose *Ae. triseriatus* mosquitoes because they are the natural vector and overwintering host of La Crosse virus, they are extremely tolerant to a range of temperatures, they are distributed from Florida to eastern Canada (9), and they have been implicated as potential enzootic vectors for West Nile virus (10). To determine the risk for Zika virus transmission in the United States, surveillance of different human-biting mosquito species will be paramount. Although we expected that *Cx. pipiens* and *Ae. triseriatus* mosquitoes would not be competent Zika virus vectors, our experimental verification helps exclude uncertainties surrounding the potential vectors of this emerging pathogen.

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Yellow Fever—More a Policy and Planning Problem than a Biological One

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DOI: <http://dx.doi.org/10.3201/eid2210.160875>

To the Editor: The recent and ongoing outbreak (epidemic) of yellow fever (YF) in Angola is cause for concern, not only in West Africa, but also in contiguous and other nearby countries (1). As of June 21, 2016, the World Health Organization (WHO) had reported 3,137 cases (847 laboratory-confirmed) and 350 deaths (2), but at the present time, it is not cause for panic or for extravagant claims of an “impending global health threat” (3). As long as the Angola Ministry of Health reports that there have been <400 deaths from YF since it declared the outbreak 4 months ago, and because there is an effective vaccine against this disease, it is difficult to understand dire warnings of a global threat.

WHO considers the situation of high concern because of the inadequate surveillance system in Angola, one that is incapable of identifying new foci or areas where cases might emerge (2). Such an inability suggests that many more cases and deaths have already occurred (4). What is needed now, without further unconscionable delays, is a

proper vaccination campaign, one that has been unachievable to now. We here reaffirm what has been suggested by the WHO Strategic Advisory Group of Experts on Immunization and by others, but as of June 20, 2016, not applied by WHO: that the YF vaccine administered at one fifth of the regular dose could be used until the epidemic ends (5).

Despite vaccination campaigns in various provinces in Angola, circulation of YF virus (YFV; family *Flaviviridae*, genus *Flavivirus*) in some districts persists. Attempts to control this epidemic are being made by application of the effective YFV 17D vaccine that has been used for many decades worldwide. Whereas recognition of cases of YF has decreased in Angola, cases continue to occur there, and isolated cases have been detected in persons who have visited Angola as tourists or for business purposes. Furthermore, cases in nearby Democratic Republic of the Congo have increased. Because YF is not endemic to Asia, such patients have the potential to serve as primary sources of YFV and as index sources for subsequent clusters, outbreaks, or epidemics, not only in China, but elsewhere in Asia, which is a nightmare scenario.

The principal mosquito vector of YFV is *Aedes aegypti*, which is found in southern China and elsewhere in Asia, as are *Ae. albopictus* mosquitoes (6), which can also transmit YFV and serve as a bridging vector between jungle and urban cycles of YFV in a variety of ecosystems (7). These mosquitoes feed on humans and are found peridomestically. Mosquitoes of these species also are capable of transmitting dengue viruses, chikungunya virus, Zika virus, and other human pathogens. Their presence should serve as a warning to local health authorities of potential arbovirus disease outbreaks and, therefore, to maintain or initiate mosquito vector control programs. Most industrialized countries are aware of these warnings; the 40 YF-endemic countries, predominantly tropical areas in Africa and Central and South America (≈90% of cases reported every year occur in sub-Saharan Africa), maintain diagnostic competence and surveillance systems, including clinical findings, testing of sick nonhuman primates and arthropods, and other indicators. Four countries that produce YF vaccine have purchased stocks or have arrangements in place to obtain sufficient doses in instances of immediate need.

Because destinations of an increasing number of travelers include YF-endemic areas, national and international regulations require a recent (<10 years) verified history of vaccination against this virus; China does not have such regulations. If a person traveling to a recognized YF-endemic area is not required to be vaccinated in advanced, then they are essentially on their own with regard to self-protection, but the greater threat is to their own country, if and when they return.

Of ostensibly great concern has been 11 unvaccinated YF-infected Chinese residents and workers who returned