

Vector Competence of Australian Mosquitoes for Yellow Fever Virus

Andrew F. van den Hurk,* Kate McElroy, Alyssa T. Pyke, Charles E. McGee, Sonja Hall-Mendelin, Andrew Day, Peter A. Ryan, Scott A. Ritchie, Dana L. Vanlandingham, and Stephen Higgs

Public Health Virology, Communicable Diseases Unit, Forensic and Scientific Services, Queensland Health, Coopers Plains, Australia;
Department of Pathology, University of Texas Medical Branch, Galveston, Texas; Queensland Institute of Medical Research, Brisbane, Australia;
School of Public Health, Tropical Medicine and Rehabilitation Sciences, James Cook University, Cairns, Australia;
Tropical Population Health Network, Queensland Health, Cairns, Australia

Abstract. The vector competence of Australian mosquitoes for yellow fever virus (YFV) was evaluated. Infection and transmission rates in Cairns and Townsville populations of *Aedes aegypti* and a Brisbane strain of *Ae. notoscriptus* were not significantly different from a well-characterized YFV-susceptible strain of *Ae. aegypti*. After exposure to $10^{7.2}$ tissue culture infectious dose (TCID₅₀)/mL of an African strain of YFV, > 70% of *Ae. aegypti* and *Ae. notoscriptus* became infected, and > 50% transmitted the virus. When exposed to $10^{6.7}$ TCID₅₀/mL of a South American strain of YFV, the highest infection (64%) and transmission (56%) rates were observed in *Ae. notoscriptus*. The infection and transmission rates in the Cairns *Ae. aegypti* were both 24%, and they were 36% and 28%, respectively, for the Townsville population. Because competent vectors are present, the limited number of travelers from endemic areas and strict vaccination requirements will influence whether YFV transmission occurs in Australia.

INTRODUCTION

Yellow fever is a hemorrhagic disease that was once a scourge of society in the Americas, Africa, and Europe.^{1,2} Periodic outbreaks occurred until the early 1900s in major cities such as New York, Memphis, and Philadelphia, resulting in hundreds of thousands of cases and tens of thousands of deaths.³ However, after it was established that mosquitoes transmit the virus between humans, comprehensive mosquito control programs and mass vaccination greatly reduced the disease burden, and the distribution of the virus became restricted to tropical areas of South America and Africa.⁴ Unfortunately, a breakdown in these mosquito control and vaccination activities, coupled with increased urbanization, has led to a resurgence of yellow fever, with an estimated 200,000 cases and 30,000 deaths worldwide per year.⁵ Indeed, there is concern that yellow fever will return to urban areas of Africa and South America, where concentrated human and mosquito population densities provide the ideal conditions for devastating outbreaks.⁴

Yellow fever virus (YFV) circulates in three ecologically distinct transmission cycles, each with its own suite of vectors.^{1,4} In both Africa and South America, the virus can exist in an urban cycle between humans and *Aedes aegypti*. In Africa, two additional transmission cycles have been recognized: the jungle cycle between monkeys and the canopy-dwelling *Ae. africanus* and the intermediate cycle or zone of emergence, where the virus circulates between monkeys and/or humans and mosquitoes, including *Ae. africanus*, *Ae. furcifer-taylori*, *Ae. luteocephalus*, and members of the *Ae. simpsoni* complex. In South America, the virus is maintained in a jungle cycle between monkeys and various species of *Haemagogus* (*Hg. janthinomys* and *Hg. leucocelaenus*) and *Sabethes chloropterus*.

Despite the current restricted geographical distribution of YFV, there is a risk of expansion through a viremic traveler, who may come in contact with local competent YFV vectors in a novel location. Considering that *Ae. aegypti* is prevalent in north Queensland,⁶ Australia is potentially receptive to

YFV transmission. Because it has previously been shown that different populations of *Ae. aegypti* exhibit different vector competence for YFV virus, a regionally specific investigation of Australian *Ae. aegypti* strains is warranted.^{7,8} Furthermore, if other mosquito species are able to transmit the virus, local transmission could occur in regions of Australia where *Ae. aegypti* is not distributed. Consequently, the objective of the current study was to undertake vector competence experiments to determine if selected Australian mosquito species were able to become infected with and transmit African and South American strains of YFV. In addition to *Ae. aegypti*, *Ae. notoscriptus* and *Ae. vigilax*, two species with a widespread distribution in urban areas of Australia, were also evaluated.

MATERIALS AND METHODS

Mosquitoes. Eggs of *Ae. aegypti* were collected in ovitrap buckets set at various locations in Cairns (16°55'S, 145°46'E) and Townsville (19°16'S, 146°49'E), north Queensland, Australia. *Ae. vigilax* eggs were from engorged females obtained in man-biting collections undertaken in Brisbane (27°30'S, 152°58'E), southeast Queensland. A colony of *Ae. notoscriptus* was initiated from larvae collected from Closeburn (27°19'S, 152°52'E), southeast Queensland, in 1995 and supplemented with Brisbane field material in 1997. Eggs were forwarded to the University of Texas Medical Branch (UTMB), Galveston, Texas, for vector competence experiments.

At UTMB, mosquitoes were reared in an insectary at 26°C, 70% relative humidity (RH), and a 12 hour to 12 hour light to dark lighting regimen. Larvae were maintained on equal parts of crushed TetraMin fish flakes (Tetra Werke, Melle, Germany) and RMH 2500 rodent diet (LabDiet; Purina Mills International, Richmond, IN). To increase numbers of *Ae. aegypti* and *Ae. vigilax*, females were allowed to feed on an anaesthetized Syrian hamster to stimulate egg production. The maintenance and care of experimental animals complied with the National Institutes of Health guidelines for the humane use of laboratory animals and was approved by the UTMB Animal Ethics Committee. The Townsville and Cairns *Ae. aegypti* strains were used at the F₂ and F₃ generation, respectively. Because of high egg mortality during transport and adults not readily mating in cages, the small number of F₀

*Address correspondence to Andrew F. van den Hurk, Public Health Virology, Communicable Diseases Unit, Queensland Health Forensic and Scientific Services, 39 Kessels Rd., Coopers Plains, Queensland, Australia 4108. E-mail: andrew_hurk@health.qld.gov.au

Ae. vigilax that survived from the original collections was exposed only to the wild-type African strain of YFV. The generation number of *Ae. notoscriptus* was > 50. As a positive control for all experiments, the YFV-susceptible RexD white-eye Higgs strain of *Ae. aegypti*, which originated from Rexville, Puerto Rico, was used.⁹⁻¹¹

Viruses. Three strains of YFV that had been characterized previously in mosquitoes were used for the experiments.¹²⁻¹⁴ These viruses were obtained from the National Institutes of Health-supported World Reference Center for Emerging Viruses at UTMB. An African strain of YFV, BA-55, was isolated from a fatal yellow fever case during an epidemic in Nigeria in 1987 and had been passaged three times in suckling mouse brains.¹³ The South American strain, Cinetrop 28 (OBS 7549), was isolated from a human case in Bolivia in 1999 and had been passaged two times in C6/36 (*Ae. albopictus*) cells. To produce freshly grown virus for mosquito exposure, confluent monolayers of C6/36 cells within 25-cm² flasks were inoculated with stock BA-55 or Cinetrop 28. Viruses were harvested at 96 hours post-inoculation for mosquito exposure.

To facilitate future YFV virulence studies with Australian mosquitoes, virus produced from an infectious clone of Asibi virus was also assessed for its ability to disseminate in *Ae. aegypti* and *Ae. notoscriptus*. Infectious clone-derived YFV Asibi was recovered from recombinant genome pYFV-As-RBZ IC, which contained the viral coding sequence derived from the parental YFV Asibi i.c. (pYFV-As IC)¹² and was modified to incorporate a Hepatitis Delta Rybozyme sequence. Briefly, pYFV-As-RBZ IC was linearized, purified, and transcribed *in vitro* using the mMessage mMachine SP6 kit (Ambion, Austin, TX) in accordance with manufacturer protocols. Viral RNAs were then LiCl-purified and directly electroporated into BHK-21 (baby hamster kidney) cells. Infectious virus was harvested from the supernatant at 72 hours post-electroporation for oral infection of mosquitoes.

Vector competence experiments. All mosquito virus manipulations were conducted in the Biosafety Level 3 (BSL-3) Insectary Facility located at the UTMB. The infectious blood meal was prepared by mixing harvested virus with an equal part of defibrinated sheep blood (Colorado Serum Company, Denver, CO). This blood/virus mixture was applied to the chamber of a Hemotek membrane feeding apparatus (Discovery Workshops, Accrington, Lancashire, United Kingdom) fitted with either hamster or mouse skin as the membrane. Within an isolation glove box, mosquitoes were allowed to feed on the blood/virus mixture for 1–2 hours. To determine the titer of the blood meal, pre- and post-feeding samples of the blood/virus mixture were obtained and immediately frozen at –80°C. After feeding, mosquitoes were sorted, and blood-engorged mosquitoes were placed in gauze-covered containers within an environmental growth cabinet set at 28°C, high humidity, and a 12:12 light to dark lighting regimen; 10% sucrose was provided as a nutrient source.

Infection, dissemination, and transmission rates were assessed for the BA-55 and Cinetrop 28 YFV 14–15 days after mosquitoes were exposed to the virus. The Asibi i.c. was only assessed for its ability to infect and disseminate in mosquitoes at day 14 post-exposure. Mosquitoes were chilled, and their legs and wings were removed. If virus is recovered from the legs and wings, then the mosquito has developed an infection,

where the virus has escaped through the mesenteron (midgut) and disseminated through the hemocoel.¹⁵

To assess transmission potential, the proboscis was inserted into a capillary tube containing Type B immersion oil, and the mosquito was allowed to expectorate for approximately 30 minutes. The contents of the capillary tube were expelled into 500 µL Leibowitz L-15 growth media (GM; Gibco-BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum, 10% tryptose phosphate broth, 100 U/mL penicillin, and 100 µg/mL streptomycin. The bodies and legs/wings were separately placed in a 2-mL round-bottomed microfuge tube containing 500 µL GM and a single 5-mm BB gun pellet (Daisy Outdoor Products, Rogers, AR). The bodies, legs/wings, and saliva expectorates were stored at –80°C to await analysis.

Virus assay. The blood/virus mixtures were titrated as serial 10-fold dilutions in 96-well microtiter plates that were then seeded with C6/36 cells. Mosquito bodies and legs/wings were homogenized in a TysseLyser mixer mill (Qiagen, Valencia, CA) and filtered using a 0.22-µm Supor membrane filter (Pall Corporation, Ann Arbor, MI); 100 µL filtered homogenate were inoculated in duplicate into wells of a microtitre plate, which were then seeded with C6/36 cells. After 5 days incubation at 28°C, plates were fixed in 3:1 cold acetone to phosphate-buffered saline (PBS) for 10 minutes before being stored at –20°C. Viral antigen in mosquito samples and the blood/virus mixture titrations was detected using a modification of the indirect immunofluorescence assay described previously.^{9,12} The YFV NS1-reactive monoclonal antibody 863 was used as the primary antibody,¹⁶ and Alexa Fluor 488-conjugated goat anti-mouse IgG (Invitrogen, Eugene, OR) was used as the secondary antibody. Samples were scored using an Olympus IX70 inverted epifluorescence microscope (Olympus, Tokyo, Japan) fitted with a fluorescein filter.

To facilitate detection of viral RNA in inactivated saliva, expectorates were shipped to the BSL-3 laboratories at Queensland Health Forensic and Scientific Services, Brisbane, Australia, and analyzed using a YFV-specific real-time TaqMan reverse transcriptase polymerase chain reaction (RT-PCR) assay; 200 µL TRIzol reagent (Invitrogen, Carlsbad, CA) was added to 200 µL saliva suspension before vortexing. The RNA was then extracted and purified using the QIAamp Viral RNA Mini Kit (Qiagen, Clifton Hill, Australia) as a modification of the method described in Moreira and others.¹⁷

The methods and conditions for the TaqMan RT-PCR assay incorporating synthetic oligonucleotide probe and primer positive controls have been previously described.¹⁸ Briefly, the TaqMan RT-PCR was performed using the ABI 7500 Fast Real-Time PCR System (PE Applied Biosystems, Foster City, CA), and cycling conditions (Fast Mode) consisted of one cycle at 50°C for 5 minutes, one cycle at 95°C for 2 minutes, and 40 cycles at 95°C for 3 seconds and 60°C for 30 seconds. A negative result indicating the absence of RNA detection corresponded to cycle threshold values of ≥ 40 cycles.

The flurogenic probe and primer pairs targeting the YFV 5' untranslated region (UTR) genomic region were based on respective sequences homologous to both BA-55 and Cinetrop 28 strains. Sequences for the TaqMan assay primers and dual-labeled probe were designed as follows: forward primer (YF-TaqFor primer), 5'-TGTGCTAATTGAGGTGCATTGG-3'; reverse primer (YFV wildrev), 5'-TCTCTGCTAATCGCTC

AACGAA-3'; probe (YFV-Prob), 5'-AATCGAGTTGCTA GGCAATAAACACATTTGGA-3'. The oligonucleotides used to produce the corresponding synthetic primer and probe controls contained the following sequences: primer control (forward sequence), 5'-TGTGCTAATTGAGGTGCATTGGAC AGAAGACTGTGGATGGCCCCTCATTCGTTGAGC GATTAGCAGAGA-3'; primer control (reverse sequence), 5'-TCTCTGCTAATCGCTCAACGAATGAGGGGCC ATCCACAGTCTTCTGTCCAATGCACCTCAATTAGC ACA-3'; probe control (forward sequence), 5'-TGCACCACC AACTGCTTAGAAATCGAGTTGCTAGGCAATAAA CACATTTGGAAGAACATCATCCCTGCATCC-3'; probe control (reverse sequence), 5'-GGATGCAGGGATGATGTT CTTCCAAATGTGTTTATTGCCTAGCAACTCGA TTTCTAAGCAGTTGGTGGTGA-3'.

Analysis. For each virus strain, infection, dissemination, and transmission rates within and between species were compared using Fisher's exact test with the α -value adjusted for multiple comparisons.

RESULTS

At day 14 post-exposure, all three species were susceptible to infection with YFV BA-55, with infection rates ranging from 19% to 88% for *Ae. vigilax* and *Ae. notoscriptus*, respectively (Table 1). There was no significant difference in infection, dissemination, and transmission rates between the three strains of *Ae. aegypti* or between these strains and *Ae. notoscriptus*. In contrast, rates of infection, dissemination, and transmission in *Ae. vigilax* were lower and in many cases, significantly lower than those rates of the other species tested. After the virus disseminated from the mesenteron, transmission occurred in > 70% of mosquitoes tested, irrespective of the species.

Fifteen days after exposure to YFV Cinetrop 28, the three strains of *Ae. aegypti* and *Ae. notoscriptus* became infected, developed a disseminated infection, and transmitted the virus (Table 2). There was no significant difference in infection, dissemination, and transmission rates between the three strains of *Ae. aegypti*. The highest infection, dissemination, and transmission rates were observed in *Ae. notoscriptus*, although these rates were not significantly different from the three strains of *Ae. aegypti*. Transmission occurred in \geq 88% of mosquitoes that developed a disseminated infection.

Fourteen days after exposure to the YFV Asibi i.c., there was no significant difference in infection and dissemination rates between the three strains of *Ae. Aegypti* or between these strains and *Ae. notoscriptus* (Table 3). The highest dis-

semination rate of 96% was observed in the Townsville strain of *Ae. aegypti*.

DISCUSSION

In the current study, we have shown that Australian mosquitoes readily become infected with and can transmit representative African and South American strains of YFV virus. Importantly, for both Australian strains of *Ae. aegypti* and *Ae. notoscriptus* that were tested, the infection, dissemination, and transmission rates were not significantly different from the RexD white-eye Higgs colony strain, which has been used in previous YFV susceptibility studies.^{9,14} Although our laboratory studies have incriminated Australian mosquitoes as potential YFV vectors, other factors could also influence whether a species will be involved in virus transmission cycles.

Considering the status of *Ae. aegypti* as the primary urban vector of YFV in endemic areas, the relatively high vector competence of Australian populations of this species for YFV in our study was not surprising. The vector status of *Ae. aegypti* is further enhanced by its affiliation with humans, including anthropophilic feeding tendencies,¹⁹ peridomestic larval habitats,²⁰ and indoor feeding and resting behavior.²¹ Indeed, these behavioral and biological traits facilitate regular outbreaks of dengue viruses (DENVs) in northern Queensland.^{22,23} However, because the distribution of *Ae. aegypti* encompasses only northern Queensland,⁶ the area of Australia where this species could serve as a vector would be confined to this region.

Interestingly, the only previous examination of Australian mosquitoes and YFV was by Russell and others,²⁴ who showed that *Ae. notoscriptus* was refractory to infection with a Ugandan strain of YFV. This finding contrasts with the current study, which revealed that the southeast Queensland colony of *Ae. notoscriptus* was highly susceptible to all strains of YFV tested and transmitted the BA-55 and Cinetrop 28 viruses at rates that were not significantly different from the RexD Higgs white-eye strain of *Ae. aegypti*. A number of factors may account for the variation in results observed in the current experiments and those findings by Russell and others,²⁴ including differences in mosquito and virus strains, number of virus passages before infection, number of mosquito generations in colony since field collection, method of virus assay, artificial feeding technique used, and titer of the infectious blood meal.^{25,26} Regardless, the results of all three of our experiments clearly show that *Ae. notoscriptus* could serve as a potential vector of YFV in Australia.

TABLE 1

Infection, dissemination, and transmission rates in mosquitoes exposed to a blood meal containing 10^{7.2} TCID₅₀/mL of a Nigerian strain of YFV (BA-55)

Mosquito species	Origin	% Infection*	% Dissemination†	% Transmission‡	% Transmission/dissemination§
<i>Ae. aegypti</i>	Cairns	80 (20/25)a	72 (18/25)a	52 (13/25)a,b	72 (13/18)a
<i>Ae. aegypti</i>	Townsville	72 (18/25)a	60 (15/25)a	60 (15/25)a	100 (15/15)a
<i>Ae. aegypti</i>	RexD¶	82 (9/11)a	64 (7/11)a,b	64 (7/11)a,b	100 (7/7)a
<i>Ae. notoscriptus</i>	Brisbane	88 (22/25)a	68 (17/25)a	56 (14/25)a,b	82 (14/17)a
<i>Ae. vigilax</i>	Brisbane	19 (5/26)b	19 (5/26)b	19 (5/26)b	100 (5/5)a

Values within each category that share the same letter are not significantly different at $\alpha = 0.05$ after adjusting for multiple comparisons.

* Percentage of mosquitoes containing virus in their bodies (number positive per number tested).

† Percentage of mosquitoes containing virus in their legs and wings (number positive per number tested).

‡ Percentage of mosquito exectorates in which virus was detected (number of positive exectorates per number tested).

§ Percentage of mosquitoes with a disseminated infection in which virus was detected in the exectorate (number of positive exectorates per number disseminated).

¶ RexD white-eye Higgs strain of *Ae. aegypti*, originally from Puerto Rico.

TABLE 2

Infection, dissemination, and transmission rates in mosquitoes exposed to a blood meal containing 10^{6.7} TCID₅₀/mL of a Bolivian strain of YFV (Cinetrop 28)

Mosquito species	Origin	% Infection*	% Dissemination†	% Transmission‡	% Transmission/dissemination§
<i>Ae. aegypti</i>	Cairns	24 (6/25)a	24 (6/25)a	24 (6/25)a	100 (6/6)a
<i>Ae. aegypti</i>	Townsville	36 (9/25)a	32 (8/25)a	28 (7/25)a	88 (7/8)a
<i>Ae. aegypti</i>	RexD	40 (10/25)a	36 (9/25)a	32 (8/25)a	89 (8/9)a
<i>Ae. notoscriptus</i>	Brisbane	64 (16/25)a	64 (16/25)a	56 (14/25)a	88 (14/16)a

Values within each category that share the same letter are not significantly different at $\alpha = 0.05$ after adjusting for multiple comparisons.
 * Percentage of mosquitoes containing virus in their bodies (number positive per number tested).
 † Percentage of mosquitoes containing virus in their legs and wings (number positive per number tested).
 ‡ Percentage of mosquito excretorates in which virus was detected (number of positive excretorates per number tested).
 § Percentage of mosquitoes with a disseminated infection in which virus was detected in the excretorate (number of positive excretorates per number disseminated).

In contrast to the restricted distribution of *Ae. aegypti*, *Ae. notoscriptus* has a broad geographical range across much of Australia.²⁷ This range would suggest that larger areas of Australia could be receptive to local transmission of YFV. *Ae. notoscriptus* is also prevalent in domestic environments, using natural and manmade containers as oviposition sites, and in some urban locations, it is the dominant container-inhabiting mosquito species.^{28,29} Although *Ae. notoscriptus* is more opportunistic in its host-feeding patterns than *Ae. aegypti*,^{19,30} it will readily attack humans, underlining its ability to be involved in urban transmission of YFV in areas of Australia where *Ae. aegypti* does and does not occur.

Despite all infected *Ae. vigilax* transmitting the BF-55 strain of YFV, the infection and transmission rates were lower than the other species tested. However, *Ae. vigilax* is a major pest species in coastal areas of Australia, and it has the capability to disperse at least 100 km from its brackish water larval habitats.^{31,32} In contrast to *Ae. aegypti*, the ecology of *Ae. vigilax* is not as intrinsically tied to humans, because it opportunistically feeds on a number of different mammals and is not overtly endophagic and/or endophilic.^{19,30,31} Notwithstanding, the relatively high population densities in urban areas and increased development in close proximity to larval habitats indicate that *Ae. vigilax* could play a secondary or supplemental role in transmission of YFV in Australia.

Although there are undoubtedly efficient vectors that could facilitate local transmission, anthropogenic influences will determine the risk of YFV emerging as a public health problem in Australia. Infected travelers have triggered numerous outbreaks of dengue in north Queensland²² and recent chikungunya virus (CHIKV) epidemics in Africa, the Indian Ocean, India, and Italy,³³ showing the ease with which arboviruses can be introduced into a region by a viremic traveler. At present, YFV mainly circulates in sylvan and/or rural areas of Africa and South America, which limits the number of people who come into contact with the virus.⁴ A resurgence of yellow fever in urban areas would increase the rate of human exposure to

YFV and thus, produce a larger pool of infected people with the potential to disseminate the virus to receptive locations around the world, including Australia.^{34,35} However, the number of people entering Australia from YFV-endemic areas is relatively low compared with those people who arrive from DENV- or CHIKV-endemic areas.³⁶

To protect Australia from potential YFV transmission, it is imperative that any travelers who could have been exposed to the virus are recognized by quarantine and public health authorities. Strict quarantine requirements currently necessitate that travelers who have stayed in a country with endemic YFV within 6 days before arriving in Australia must have a current international vaccination certificate.³⁷ If a person does not possess a vaccination certificate, they will be interviewed by quarantine personnel and if necessary, be required to sign a document that states that they agree to consult a medical practitioner should they develop symptoms consistent with YFV infection.

In Australia, the high-quality health care system, underpinned by rigorous disease surveillance, should also reduce the risk of local YFV transmission. This access to high-quality health care and diagnostics should also minimize the potential of imported cases triggering an explosive outbreak. Indeed, YFV is often included in the panel of tests requested by the attending physician for febrile patients who have arrived from YFV-endemic areas, regardless of their vaccination status. Although such tests are mostly negative for YFV infection in serological assays, on a recent occasion, a patient returning from Columbia displayed immunoglobulin M (IgM) reactivity to both YFV and DENVs (Taylor CT, personal communication). Unfortunately, difficulty in separating these closely related flaviviruses using current serological tests prevented a definitive diagnosis being made in this instance. Considering the presence of competent vectors, this finding highlights the need for continued vigilance from quarantine and public health authorities to help prevent local transmission of YFV in Australia. Importantly, the development of specific and

TABLE 3

Infection and dissemination rates in mosquitoes exposed to a blood meal containing 10^{8.0} TCID₅₀/mL of the Asibi infectious clone of YFV

Mosquito species	Origin	% Infection*	% Dissemination†	% Dissemination/infection‡
<i>Ae. aegypti</i>	Cairns	92 (23/25)a	80 (20/25)a	87 (20/23)a
<i>Ae. aegypti</i>	Townsville	96 (24/25)a	96 (24/25)a	100 (24/24)a
<i>Ae. aegypti</i>	RexD	96 (24/25)a	68 (17/25)a	71 (17/24)a
<i>Ae. notoscriptus</i>	Brisbane	76 (19/25)a	64 (16/25)a	84 (16/19)a

Values within each category that share the same letter are not significantly different at $\alpha = 0.05$ after adjusting for multiple comparisons.
 * Percentage of mosquitoes containing virus in their bodies (number positive per number tested).
 † Percentage of mosquitoes containing virus in their legs and wings (number positive per number tested).
 ‡ Percentage of infected mosquitoes that developed a disseminated infection (number of positive per number infected).

sensitive diagnostic assays to aid in case recognition, and the ability to rapidly procure supplies of vaccine will greatly enhance outbreak response capabilities.

Received January 30, 2011. Accepted for publication June 1, 2011.

Acknowledgments: The authors thank Robert Tesh for providing the wild-type YFV isolates. The authors thank Nicole Hausser, Jing Huang, and Judith Northill for technical assistance. We also thank Kay Marshall for assistance with mosquito colony maintenance, Silvie Moravek and Petrina Johnson for mosquito collections, and Mary-Anne Burns for shipping mosquito eggs to the United States. Finally, valuable insights into aspects of this study were provided by Craig Williams, Cassie Jansen, and Richard Russell.

Financial support: Andrew van den Hurk was supported by a Queensland International Fellowship. Both Jing Huang and Nicole Hausser were supported by National Institutes of Health Grant UC7 AI070083. Charles McGee was supported by Centers for Disease Control Grant for Public Health Research Dissertation (R36) PAR07-231. Funding for this study was also provided by the Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease.

Authors' addresses: Andrew F. van den Hurk, Alyssa T. Pyke, Sonja Hall-Mendelin, and Andrew Day, Public Health Virology, Communicable Diseases Unit, Queensland Health Forensic and Scientific Services, Coopers Plains, Queensland, Australia, E-mails: andrew_hurk@health.qld.gov.au, alyssa_pyke@health.qld.gov.au, sonja_hall-mendelin@health.qld.gov.au, and drew_day@health.qld.gov.au. Kate McElroy, Charles E. McGee, Dana L. Vanlandingham, and Stephen Higgs, Department of Pathology, University of Texas Medical Branch (UTMB), Galveston, TX, E-mails: kmhorne@utmb.edu, cemcgee@email.unc.edu, dlvanlan@utmb.edu, and sthiggs@utmb.edu. Peter A. Ryan, School of Biological Sciences, University of Queensland, Brisbane, Queensland, Australia, E-mail: peter.ryan@uq.edu.au. Scott A. Ritchie, School of Public Health, Tropical Medicine and Rehabilitative Services, James Cook University, Smithfield, Queensland, Australia, E-mail: scott.ritchie@jcu.edu.au.

REFERENCES

- Monath TP, 1988. Yellow fever. Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*, Vol. 5. Boca Raton, FL: CRC, 139–231.
- Monath TP, 2001. Yellow fever: an update. *Lancet Infect Dis* 1: 11–20.
- Vainio J, Cutts F, 1998. *Yellow fever*. Geneva, Switzerland: World Health Organization.
- Barrett ADT, Higgs S, 2007. Yellow fever: a disease that has yet to be conquered. *Annu Rev Entomol* 52: 209–229.
- World Health Organization, 2009. *Yellow Fever. Fact Sheet No. 100*. Available at: <http://www.who.int/mediacentre/factsheets/fs100/en/>. Accessed January 13, 2011.
- Russell RC, Currie BJ, Lindsay MD, Mackenzie JS, Ritchie SA, Whelan PI, 2009. Dengue and climate change in Australia: predictions for the future should incorporate knowledge from the past. *Med J Aust* 190: 265–268.
- Aitken TH, Downs WG, Shope RE, 1977. *Aedes aegypti* strain fitness for yellow fever virus transmission. *Am J Trop Med Hyg* 26: 985–989.
- Tabachnick WJ, Wallis GP, Aitken TH, Miller BR, Amato GD, Lorenz L, Powell JR, Beaty BJ, 1985. Oral infection of *Aedes aegypti* with yellow fever virus: geographic variation and genetic considerations. *Am J Trop Med Hyg* 34: 1219–1224.
- McGee CE, Tssetsarkin K, Vanlandingham DL, McElroy KL, Lang J, Guy B, Decelle T, Higgs S, 2008. Substitution of wild-type yellow fever Asibi sequences for 17D vaccine sequences in ChimeriVax-dengue 4 does not enhance infection of *Aedes aegypti* mosquitoes. *J Infect Dis* 197: 686–692.
- Miller BR, Mitchell CJ, 1991. Genetic selection of a flavivirus-refractory strain of the yellow fever mosquito *Aedes aegypti*. *Am J Trop Med Hyg* 45: 399–407.
- Wendell MD, Wilson TG, Higgs S, Black WC IV, 2000. Chemical and gamma-ray mutagenesis of the *white* gene in *Aedes aegypti*. *Insect Mol Biol* 9: 119–125.
- McElroy KL, Tssetsarkin KA, Vanlandingham DL, Higgs S, 2005. Characterization of an infectious clone of the wild-type yellow fever virus Asibi strain that is able to infect and disseminate in mosquitoes. *J Gen Virol* 86: 1747–1751.
- Miller BR, Monath TP, Tabachnick WJ, Ezike VI, 1989. Epidemic yellow fever caused by an incompetent mosquito vector. *Trop Med Parasitol* 40: 396–399.
- Mutebi JP, Gianella A, Travassos da Rosa A, Tesh RB, Barrett ADT, Higgs S, 2004. Yellow fever virus infectivity for Bolivian *Aedes aegypti* mosquitoes. *Emerg Infect Dis* 10: 1657–1660.
- Turell MJ, Gargan TP II, Bailey CL, 1984. Replication and dissemination of Rift Valley fever virus in *Culex pipiens*. *Am J Trop Med Hyg* 33: 176–181.
- Gould EA, Buckley A, Cammack N, Barrett ADT, Clegg JCS, Ishak R, Varma MGR, 1985. Examination of the immunological relationships between flaviviruses using yellow fever virus monoclonal antibodies. *J Gen Virol* 66: 1369–1382.
- Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM, Rocha BC, Hall-Mendelin S, Day A, Riegler M, Hugo LE, Johnson KN, Kay BH, McGraw EA, van den Hurk AF, Ryan PA, O'Neill SL, 2009. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. *Cell* 139: 1268–1278.
- van den Hurk AF, Hall-Mendelin S, Pyke AT, Smith GA, Mackenzie JS, 2010. Vector competence of Australian mosquitoes for chikungunya virus. *Vector Borne Zoonotic Dis* 10: 489–495.
- Jansen CC, Webb CE, Graham GC, Craig SB, Zborowski P, Ritchie SA, Russell RC, van den Hurk AF, 2009. Blood sources of mosquitoes collected from urban and peri-urban environments in eastern Australia with species-specific molecular analysis of avian blood meals. *Am J Trop Med Hyg* 81: 849–857.
- Montgomery BL, Ritchie SA, 2002. Roof gutters: a key container for *Aedes aegypti* and *Ochlerotatus notoscriptus* (Diptera: Culicidae) in Australia. *Am J Trop Med Hyg* 67: 244–246.
- Perich MJ, Davila G, Turner A, Garcia A, Nelson M, 2000. Behavior of resting *Aedes aegypti* (Culicidae: Diptera) and its relation to ultra-low volume adulticide efficacy in Panama City, Panama. *J Med Entomol* 37: 541–546.
- Hanna JN, Ritchie SA, 2009. Outbreaks of dengue in north Queensland, 1990–2008. *Commun Dis Intell* 33: 32–33.
- Ritchie SA, Long S, Smith G, Pyke A, Knox TB, 2004. Entomological investigations in a focus of dengue transmission in Cairns, Queensland, Australia, by using the sticky ovitraps. *J Med Entomol* 41: 1–4.
- Russell RC, Mukwaya LG, Lule M, 1977. Laboratory studies on the transmission of yellow fever virus by *Aedes (Finlaya) notoscriptus* (Dipt., Culicidae). *Aust J Exp Biol Med Sci* 55: 649–651.
- Knox TB, Kay BH, Hall RA, Ryan PA, 2003. Enhanced vector competence of *Aedes aegypti* (Diptera: Culicidae) from the Torres Strait compared with mainland Australia for dengue 2 and 4 viruses. *J Med Entomol* 40: 950–956.
- Lourenço de Oliveira R, Vazeille M, Bispo de Filippis AM, Failloux AB, 2003. Large genetic differentiation and low variation in vector competence for dengue and yellow fever viruses of *Aedes albopictus* from Brazil, the United States, and the Cayman Islands. *Am J Trop Med Hyg* 69: 105–114.
- Lee DJ, Hicks MM, Griffiths M, Russell RC, Marks EN, 1982. *The Culicidae of the Australasian Region*, Entomology Monograph No. 2, Vol. 2. Canberra, Australia: Australian Government Publishing Service Press.
- Kay BH, Watson TM, Ryan PA, 2008. Definition of productive *Aedes notoscriptus* (Diptera: Culicidae) habitats in western Brisbane, and a strategy for their control. *Aust J Entomol* 47: 142–148.
- Williams CR, Kokkinn MJ, Gilbert KS, 1999. Spatial heterogeneity in oviposition preference of the mosquito *Aedes notoscriptus* (Skuse) (Diptera: Culicidae) in Adelaide, South Australia. *Aust J Entomol* 38: 354–358.
- Kay BH, Boyd AM, Ryan PA, Hall RA, 2007. Mosquito feeding patterns and natural infection of vertebrates with Ross River and Barmah Forest viruses in Brisbane, Australia. *Am J Trop Med Hyg* 76: 417–423.

31. Lee DJ, Hicks MM, Griffiths M, Russell RC, Marks EN, 1984. *The Culicidae of the Australasian Region*, Entomology Monograph No. 2, Vol. 3. Canberra, Australia: Australian Government Publishing Service Press.
32. Hamlyn-Harris R, 1933. Some ecological factors involved in the dispersal of mosquitos in Queensland. *Bull Entomol Res* 24: 229–232.
33. Pialoux G, Gaüzère BA, Jauréguiberry S, Strobel M, 2007. Chikungunya, an epidemic arbovirosis. *Lancet Infect Dis* 7: 319–327.
34. Monath TP, 1999. Facing up to re-emergence of urban yellow fever. *Lancet* 353: 1541.
35. Gardner CL, Ryman KD, 2010. Yellow fever: a reemerging threat. *Clin Lab Med* 30: 237–260.
36. Australian Bureau of Statistics, 2010. 3401.0—*Overseas Arrivals and Departures, Australia, Jan 2010*. Available at: <http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/3401.0Jan%202010?OpenDocument>. Accessed November 16, 2010.
37. Australian Government Department of Health and Aging, 2010. *Yellow Fever—General Fact Sheet*. Available at: <http://www.health.gov.au/internet/main/publishing.nsf/content/health-publhlth-strateg-communic-factsheets-yellow.htm>. Accessed November 16, 2010.