Potential for Mosquitoes (Diptera: Culicidae) From Florida to Transmit Rift Valley Fever Virus

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ABSTRACT We evaluated *Aedes atlanticus* Dyar and Knab, *Aedes infirmatus* Dyar and Knab, *Aedes vexans* (Meigen), *Anopheles crucians* Wiedemann, *Coquillettidia perturbans* (Walker), *Culex nigripalpus* Theobald, *Mansonia dyari* Belkin, Heinemann, and Page, and *Psorophora ferox* (Von Humboldt) from Florida to determine which of these species should be targeted for control should Rift Valley fever virus (RVFV) be detected in North America. Female mosquitoes that had fed on adult hamsters inoculated with RVFV were incubated for 7–21 d at 26°C, then allowed to refeed on susceptible hamsters, and tested to determine infection, dissemination, and transmission rates. We also inoculated mosquitoes intrathoracically, held them for 7 d, and then allowed them to feed on a susceptible hamster to check for a salivary gland barrier. When exposed to hamsters with viremias $\geq 10^{7.6}$ plaque-forming units per milliliter of blood, at least some individuals in each of the species tested became infected; however, *Cx. nigripalpus*, *An. crucians*, and *Ae. infirmatus* were essentially incompetent vectors in the laboratory because of either a midgut escape or salivary gland barrier. Each of the other species should be considered as potential vectors and would need to be controlled if RVFV were introduced into an area where they were found. Additional studies need to be conducted with other geographic populations of these species and to determine how environmental factors affect transmission.

KEY WORDS competence, vector, transmission, North America

The introduction of West Nile virus (WNV) into the United States in 1999 and its subsequent spread across North America illustrates the potential for an exotic arbovirus to be introduced and become established in North America and to cause significant disease and economic disruption. Rift Valley fever virus (RVFV), which has been responsible for numerous outbreaks of severe disease in ruminants and humans in sub-Saharan Africa over the past 80 yr (Meegan and Bailey 1988, Gerdes 2004, Bird et al. 2009) is of particular concern. Although originally limited to sub-Saharan Africa, an outbreak in Egypt in 1977 caused an estimated 200,000 human cases as well as having devastating effects on the sheep and cattle industry (Laughlin et al. 1979, Meegan 1979). Its continued spread to the Arabian Peninsula (Jupp et al. 2002, Shoemaker et al. 2002, Balkhy and Memish 2003, Madani et al. 2003) has raised very real concerns regarding the agricultural and medical impact this zoonotic disease agent might have if it were introduced into Europe or the Americas (House et al. 1992, Britch et al. 2007, Hartley et al. 2011).

Rift Valley fever (RVF) is predominately a disease of domestic ruminants (cattle, goats, and sheep), where infection in pregnant animals usually results in abortion, and infection of new-born animals is nearly always fatal (Easterday et al. 1962, Easterday 1965, Meegan and Bailey 1988, Bird et al. 2009). In humans, most infections result in an undifferentiated febrile illness. However, ≈1% of infections result in hemorrhagic complications, which are often fatal, and an additional 1% develop encephalitis from which the patient usually recovers. Ocular sequellae that can cause retinal damage, including blindness, have also been documented (Schrire 1951, Siam and Meegan 1980, Al-Hazmi et al. 2005).

RVFV is a member of the genus *Phlebovirus* in the family *Bunyaviridae* with known laboratory transmission by sand flies (Hoch et al. 1984, Turell and Perkins 1990, Dohm et al. 2000). However, this virus has been associated almost exclusively with mosquitoes in na-

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14. ABSTRACT

We evaluated Aedes atlanticus Dyar and Knab, Aedes infirmatus Dyar and Knab, Aedes vexans (Meigen), Anopheles crucians Wiedemann, Coquillettidia perturbans (Walker), Culex nigripalpus Theobald, Mansonia dyari Belkin, Heinemann, and Page, and Psorophora ferox (Von Humboldt) from Florida to determine which of these species should be targeted for control should Rift Valley fever virus (RVFV) be detected in North America. Female mosquitoes that had fed on adult hamsters inoculated with RVFV were incubated for 7?21 d at 26 C, then allowed to refeed on susceptible hamsters, and tested to determine infection, dissemination, and transmission rates.Wealso inoculated mosquitoes intrathoracically, held them for 7 d, and then allowed them to feed on a susceptible hamster to check for a salivary gland barrier. When exposed to hamsters with viremias 107.6 plaque-forming units per milliliter of blood, at least some individuals in each of the species tested became infected however, Cx. nigripalpus, An. crucians, and Ae. infirmatus were essentially incompetent vectors in the laboratory because of either a midgut escape or salivary gland barrier. Each of the other species should be considered as potential vectors and would need to be controlled if RVFV were introduced into an area where they were found. Additional studies need to be conducted with other geographic populations of these species and to determine how environmental factors affect transmission.

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Table 1. Mosquitoes collected in Polk County (November, December, and April), and Alachua County (July), Florida, and evaluated for their vector competence for Rift Valley fever virus by year and month of collection

Species	2011		2012	
Aedes atlanticus Dyar and Knab				July
Aedes infirmatus Dyar and Knab	Nov.	Dec.		July
Aedes vexans (Meigen)	Nov.	Dec.		July
Anopheles crucians Wiedemann	Nov.	Dec.	April	July
Coquillettidia perturbans (Walker)			April	
Culex nigripalpus Theobald	Nov.	Dec.		July
Mansonia dyari Belkin, Heinemann, and Page	Nov.	Dec.		
Psorophora ferox (Von Humboldt)		Dec.		July

ture, with the virus isolated from at least 40 species in eight genera (Meegan and Bailey 1988, Logan et al. 1991, Fontenille et al. 1998, Sang et al. 2010). Because methods of control and degree of risk vary for different mosquito species, it is necessary to identify which species from North America are competent vectors and might be involved in the natural transmission cycle so that the appropriate control measures can be used if RVFV was introduced into North America.

To determine which mosquito species in the southeastern United States might serve as potential vectors should RVFV be introduced into North America, we evaluated mosquitoes from two counties in Florida for their potential to serve as natural vectors of RVFV. In addition to confirming results from earlier studies (Gargan et al. 1988, Turell et al. 2008a) for three species, we also tested five species not evaluated previously, including the first ever results for members of the genera *Coquillettidia*, *Mansonia*, and *Psorophora.* As a select agent, RVFV requires biological safety level (BSL)-3 agriculture facilities with vaccination or a biosafety level-4 facility for experimental study.

Materials and Methods

Mosquitoes. Mosquitoes were trapped in Centers for Disease Control and Prevention (CDC) mini-type light traps (Bioquip, Rancho Dominguez, CA) baited with dry ice and black light LED or incandescent light at four locations in Polk County, FL (28.03609 N, 81.87908 W) and (28.06390 N, 81.88040 W) in November and December of 2011, and at (28.11682 N, 81.47509 W) and (28.25698 N, 81.68485 W) in April 2012. Mosquitoes were also collected at two locations in Alachua County, FL (29.634236 N, 82.359171 W) and (29.723136 N, 82.399252 W) in July 2012. Mosquitoes were collected directly into screen-topped 3.8-liter cardboard containers and shipped with water-saturated cotton balls and a honey-saturated sponge to the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). On arrival at USAMRIID, they were provided apple slices, placed in 3.8-liter cardboard containers, and then placed in an incubator maintained at 26°C and a photoperiod of 16:8 (L:D) h until tested for their susceptibility to RVFV. Overall, eight species were collected in sufficient numbers for evaluation (Table 1).

Viruses and Virus Assay. We used the ZH501 strain of RVFV, isolated in 1977 from the blood of a 10-yr-old Egyptian girl who had a fatal RVFV infection (Meegan 1979). This strain was passed twice in fetal rhesus monkey lung cells and once in Vero (African green monkey kidney) cells before use in this study.

Mosquito specimens were triturated in 1 ml of diluent [10% heat-inactivated fetal bovine serum in Medium 199 with Earle's salts (Invitrogen, Inc., Carlsbad, CA), $NaHSO_3$, and antibiotics] and then frozen at −70°C until tested for infectious virus by a plaque assay on Vero cell monolayers. Virus titers were expressed as Log_{10} plaque-forming units (PFU) per specimen.

Determination of Vector Competence. Adult female Syrian hamsters were inoculated intraperitoneally with 0.2 ml of a suspension containing between 10^4 and $10^{5.5}$ PFU of RVFV to provide a source of viremic blood. These hamsters were anesthetized 1 d later and placed individually (i.e., one per cage) on top of cages each containing 50–150 mosquitoes. Immediately after mosquito feeding, a blood sample was collected from the anesthetized hamsters by cardiac puncture, and the hamsters were then euthanized by CO_2 exposure. The blood suspensions (0.2 ml of blood added to 1.8 ml of diluent) were frozen at −70°C until assayed on Vero cell monolayers (as described above for the mosquito suspension) to determine viremias at the time of mosquito feeding. After exposure to the viremic hamsters, nonengorged mosquitoes were removed and either destroyed by placing them in a freezer at −20°C or inoculated intrathoracically with RVFV to examine for the presence of a salivary gland barrier (see below). Apple slices, or a 10% sucrose solution, were provided as a carbohydrate source, and mosquitoes were held at 26°C and a photoperiod of 16:8 (L:D) h until tested for infection, dissemination, and transmission. Approximately 5 d after the infectious bloodmeal, moist toweling or a water dish was added to each cage to stimulate oviposition for later studies.

To determine whether the mosquitoes could transmit virus by bite, they were allowed to feed on susceptible hamsters either individually or in small groups of two to five mosquitoes each. Because RVFV infection consistently is fatal to hamsters, we considered hamsters found dead or euthanized (when moribund) to indicate virus transmission. Presence of virus was verified by isolating virus from brain tissue from a subset of the hamsters that were euthanized or found dead (data not shown). Immediately after each transmission trial, mosquitoes were killed by freezing at −20°C for 5 min, identified to species, their feeding status confirmed, and their legs and bodies triturated separately in 1 ml of diluent. These suspensions were then frozen at −70°C until assayed for virus.

The extent of virus infection in mosquitoes was determined by assaying a mosquito's body separately from its legs. If virus was detected in its body, but not its legs, the mosquito was considered to have a nondisseminated infection limited to its midgut. In contrast, if virus was detected in both the body and leg

Table 2. Infection rates by day after feeding on a hamster infected with Rift Valley fever virus

Species	Day 7	Day 14	Day >21	Total
		Infectious dose = $10^{5.7}$ PFU/ml		
Cx. nigripalpus	13 (8, <1–49)[a]	13 (8, <1–49)	n.t.	13 (16, 2–38)
Ma. dyari	57 (7, 25–84)	0 (5, 0–40)	n.t.	33 (12, 14–61)
		Infectious dose = $10^{6.6}$ PFU/ml		
Cq. perturbans	40 (15, 20–64)	46 (39, 32–61)	63 (8, 30–87)	47 (62, 35–59)
		Infectious dose = $>10^{7.6 \pm 0.1}$ PFU/ml		
Ae. infirmatus	65 (17, 41–83)	54 (35, 38–70)	71 (21, 50–86)	62 (73, 50–72)
Ae. vexans	50 (2, 9–91)	54 (13, 29–77)	38 (13, 16–65)	46 (28, 30–64)
An. crucians	50 (8, 22–78)	44 (16, 23–67)	100 (1, 22–100)	48 (25, 30–67)
Cx. nigripalpus	8 (13, <1–35)	19 (62, 11–41)	13 (52, 6–26)	16 (127, 10–23)
Ma. dyari	69 (29, 51–83)	55 (42, 40–69)	71 (14, 45–89)	62 (85, 52–72)
		Infectious dose = $10^{8.3 \pm 0.2}$ PFU/ml		
Ae. atlanticus	40 (5, 12–77)	n.t.	73 (11, 43–91)	63 (16, 39–82)
Ae. infirmatus	59 (29, 41–75)	53 (36, 37–68)	66 (61, 53–76)	60 (126, 52–68)
Ae. vexans	n.t.	20 (5, 2–64)	46 (13, 23–71)	39 (18, 20–61)
An. crucians	55 (11, 28–79)	80 (10, 48–95)	100 (1, 22–100)	68 (22, 47–84)
Cx. nigripalpus	10 (29, 3–27)	24 (41, 14–40)	13 (30, 5–30)	17 (100, 11–26)
Ma. dyari	60 (48, 46–73)	47 (19, 27–68)	46 (11, 21–72)	55 (78, 44–66)
Ps. ferox	100 (6, 64–100)	89 (18, 66–98)	88 (16, 63–98)	90 (40, 76–97)
		Infectious dose = $>10^{9.5}$ PFU/ml		
Ae. atlanticus	n.t.	92 (13, 65–99)	100 (3, 47–100)	94 (16, 70–99)
Ae. infirmatus	n.t.	100 (16, 83–100)	n.t.	100 (16, 83–100)
Cq. perturbans	100 (27, 89–100)	100 (34, 91–100)	100 (13, 80–100)	100 (74, 96–100)

[a] Infection rate = percentage of mosquitoes containing virus in their bodies (number tested, 95% CI).
n.t., not tested.

suspensions, the mosquito was considered to have a disseminated infection (Turell et al. 1984b). Because some of the mosquitoes were tested for transmission in small pools, it was not always possible to determine which mosquito(es) in a pool actually transmitted virus by bite. Therefore, if more than one mosquito with a disseminated infection fed in a pool, data from that pool were not used to calculate the transmission rate, regardless of hamster survival.

The infection rate was the percentage of mosquitoes tested that contained virus after feeding on the original viremic hamsters. The dissemination rate was the percentage of mosquitoes tested that contained virus in their legs (regardless of their infection status) after feeding on the original viremic hamsters, and the transmission rate was the percentage of mosquitoes that refed that transmitted virus by bite (regardless of their infection status). We used the modified Wald method of calculating 95% CIs (Agresti and Coull 1998; http://www.measuringusability.com/wald.htm).

Inoculated Mosquitoes. To produce a cohort of mosquitoes known to have a disseminated infection, we inoculated some of the mosquitoes (Rosen and Gubler 1974) with 0.3 μl of a suspension containing $\approx 10^5$ PFU/ml ($\approx 10^{1.5}$ PFU per mosquito). These mosquitoes were then fed individually on susceptible hamsters to test for the presence of a salivary gland barrier (Kramer et al. 1981, Turell and Bailey 1987), but were not used to calculate either an infection or dissemination rate, as inoculation bypasses both the midgut infection and dissemination barriers.

Results

Hamster Viremia. Viremias in the 11 hamsters used to expose mosquitoes to RVFV ranged from $10^{5.7}$ to $10^{9.9}$ PFU/ml ($10^{3.2}$ to $10^{7.4}$ PFU of virus ingested per mosquito, respectively). Because infection and dissemination rates tend to be related to the amount of virus ingested, we combined the data for mosquitoes feeding on hamsters with similar viremias (Table 2). Therefore, we examined mosquitoes exposed to two low viremias ($10^{5.7}$ and $10^{6.6}$), two moderate viremias ($10^{7.6 \pm 0.1}$ and $10^{8.3 \pm 0.2}$), and to a high viremia ($10^{>9}$). Viremias in lambs and calves are as high as $10^{10.2}$ and $10^{9.2}$ mouse intracranial LD_{50}, respectively (Easterday et al. 1962, McIntosh et al. 1973).

Susceptibility to Infection. For each of the species tested, infection rates for mosquitoes that ingested a particular dose of RVFV were similar at each time point, and all of the species tested became infected after feeding on a hamster with a RVF viremia (Table 2). When exposed to a moderate viremia ($10^{7.6-8.5}$ PFU/ml) at least 40% of each of the species (except for *Culex nigripalpus* Theobald) became infected, and infection rates were similar for each species whether they fed on a hamster with a viremia of $10^{7.6}$ or $10^{8.5}$ PFU/ml (Table 2). With the exception of *Cx. nigripalpus*, virtually all of the specimens of the six species

Table 3. Transmission of Rift Valley fever virus by mosquitoes with a disseminated infection after either oral exposure or intrathoracic inoculation

Species	Route of infection		Totals
	Oral[a]	Inoculated	
	T.R. (*N*)[b]	T.R. (*N*)[b]	T.R. (95% CI)[c]
Ae. atlanticus	100 (1)	0 (1)	50 (2, 9–91)
Ae. infirmatus	0 (6)	7 (15)	5 (21, <1–24)
Ae. vexans	50 (2)	0 (1)	29 (42, 17–44)[d]
An. crucians	n.t.	0 (8)	0 (69, 0–5)[e]
Cx. nigripalpus	25 (12)	n.t.	25 (12, 8–54)
Cq. perturbans	100 (4)	63 (8)	75 (12, 46–92)
Ma. dyari	n.t.	38 (8)	38 (8, 13–70)
Ps. ferox	100 (2)	25 (8)	40 (10, 17–69)

[a] Mosquitoes with a disseminated infection after feeding on a viremic hamster.

[b] Transmission rate = percentage of mosquitoes that transmitted RVFV by bite (number of mosquitoes with a disseminated infection that fed on a susceptible hamster).

[c] Transmission rate = percentage of mosquitoes with a disseminated infection that transmitted RVFV by bite (number tested, 95% CI).

[d] Includes 39 mosquitoes tested from Turell et al. (2008a, 2010) and Iranpour et al. (2011).

[e] Includes 61 mosquitoes tested from Gargan et al. (1988).

n.t., not tested.

Table 4. Relative importance of midgut infection, midgut escape, and salivary gland barriers on the overall vector competence of selected North American mosquitoes for RVFV tested at least 14 d after ingesting about 10^8 PFU of RVFV/ml of blood

Mosquito species	Midgut infection barrier	Midgut escape barrier	Salivary gland barrier	Overall vector competence
Cq. perturbans	+[a]	+	+	Very high
Ps. ferox	–	++	++	High
Ae. atlanticus	+	+++	++[b]	Moderate
Ma. dyari	+	+++	++	Moderate
Ae. vexans	++	++	++	Moderate
Ae. infirmatus	+	+++	+++	Low
An. crucians	++	++++	++++	Very low
Cx nigripalpus	++++	++++	++	Very low

[a] Rating: –, essentially nonexistent, virus crosses this barrier in >80% of mosquitoes; +, minor, virus crosses this barrier in 60–80% of mosquitoes; ++, moderate, virus crosses this barrier in 40–60% of mosquitoes; +++, severe, virus crosses this barrier in 20–40% of mosquitoes; ++++, very severe, virus crosses this barrier in <20% of the mosquitoes.

[b] Questionable, based on small sample size.

that fed on hamsters with high viremias became infected.

Viral Dissemination. Dissemination rates increased with increasing viremia in the hamsters. Although infection rates were similar in mosquitoes that fed on hamsters with viremias of $10^{7.6}$ or $10^{8.5}$ PFU/ml, dissemination rates were generally twice as high in mosquitoes that fed on hamsters with viremias of $10^{8.5}$ than in those that fed on hamsters with viremias of $10^{7.6}$ PFU/ml (Table 4). With the exception of *Anopheles crucians* Wiedemann and *Cx. nigripalpus*, all of the other species developed moderate to high level of dissemination.

Viral Transmission. For each of the species tested, the transmission rates for mosquitoes with a disseminated infection after oral exposure or after intrathoracic inoculation were not significantly different (Fisher exact test; $P > 0.133$). With the exception of *An. crucians*, all of the other species successfully transmitted RVFV by bite (Table 3). However, there was evidence of a moderate to significant salivary gland barrier (Kramer et al. 1981) in several species, and transmission rates for mosquitoes with a disseminated infection ranged from 0% (*An. crucians*) to 75% (*Coquillettidia perturbans* (Walker)). The relative importance of midgut infection, midgut escape (for infected mosquitoes), and salivary gland (for mosquitoes with a disseminated infection) barriers for the species evaluated in these studies are shown in Table 4.

Discussion

This is the first report on the ability of members of the genera *Coquillettidia*, *Mansonia*, and *Psorophora* to transmit RVFV. In all, we evaluated five species that had not been examined previously and additional specimens from three others. Two of these species, *Cq. perturbans* and *Psorophora ferox* (Von Humboldt), were highly competent laboratory vectors of RVFV (Tables 4 and 5). Because of their preference for feeding on large mammals (Magnarelli 1977, Gingrich and Williams 2005, Molaei et al. 2008) and their occurrence in large numbers, they should be targeted for immediate control should RVFV be introduced into an area where these species are found. In addition, three species [*Aedes atlanticus* Dyar and Knab, *Aedes vexans* (Meigen), and *Mansonia dyari* Belkin, Heinemann, and Page] were moderately efficient vectors when exposed to virus by feeding on hamsters with moderate viremias and should also be considered for control. The *Ma. dyari* are particularly relevant, as numerous isolations of RVFV were made from field-collected pools of *Mansonia africana* (Theobald) and *Mansonia uniformis* (Theobald) in Kenya in 1989 and 2007 (Logan et al. 1991, Sang et al. 2010). The other species (*Aedes infirmatus* Dyar and Knab, *An. crucians*, and *Cx. nigripalpus*) were, at best, inefficient vectors in the laboratory. The results for *An. crucians* and *Cx. nigripalpus* were similar to those reported earlier (Gargan et al. 1988; Turell et al. 2008a, 2010) and confirm their relative inability to transmit RVFV. The results for *Ae. vexans* are interesting in that they are similar to those reported for this species captured in the southeastern United States (Turell et al. 2008a), which was found to be a moderately efficient laboratory transmitter of RVFV. However, two other studies (Turell et al. 2010, Iranpour et al. 2011), evaluating *Ae. vexans* captured in the northern and western United States (California, Colorado, and Wyoming) and near Winnipeg, Manitoba, Canada, found this species to be virtually incompetent at transmitting RVFV. Thus, there can be significant differences in the ability of regional populations of a mosquito species to transmit RVFV, and for those species with a wide geographical range, it may be necessary to evaluate populations from distinct geographic populations.

Table 5. Infection, dissemination, and estimated transmission rates for mosquitoes orally exposed to Rift Valley fever virus

Species	Infection rate[a]	Dissemination rate[b]	Estimated transmission rate[c]
Infectious dose = $10^{5.7}$ PFU/ml			
Cx. nigripalpus	13 (16, 2–38)	0 (8, 0–29)	<1
Ma. dyari	33 (12, 14–61)	0 (5, 0–40)	<1
Infectious dose = $10^{6.6}$ PFU/ml			
Cq. perturbans	47 (62, 35–59)	23 (47, 13–37)	17
Infectious dose = $>10^{7.6 \pm 0.1}$ PFU/ml			
Ae. infirmatus	62 (73, 50–72)	13 (56, 6–24)	<1
Ae. vexans	46 (28, 30–64)	15 (26, 5–34)	4
An. crucians	48 (25, 30–67)	0 (17, 0–16)	<1
Cx. nigripalpus	16 (127, 10–23)	3 (114, <1–8)	<1
Ma. dyari	62 (85, 52–72)	23 (56, 14–36)	9
Infectious dose = $10^{8.3 \pm 0.2}$ PFU/ml			
Ae. atlanticus	63 (16, 39–82)	18 (11, 4–49)	9
Ae. infirmatus	60 (126, 52–68)	26 (97, 18–35)	1
Ae. vexans	39 (18, 20–61)	28 (18, 12–51)	8
An. crucians	68 (22, 47–94)	9 (11, <1–40)	<1
Cx. nigripalpus	17 (100, 11–26)	1 (71, <1–8)	<1
Ma. dyari	55 (78, 44–66)	20 (30, 9–38)	8
Ps. ferox	90 (40, 76–97)	38 (34, 24–55)	16
Infectious dose = $>10^{9.5}$ PFU/ml			
Ae. atlanticus	94 (16, 70–99)	81 (16, 56–94)	40
Ae. infirmatus	100 (16, 83–100)	75 (16, 50–93)	4
An. crucians	100 (5, 60–100)	20 (5, 2–64)	<1
Cq. perturbans	100 (74, 96–100)	94 (47, 82–98)	71
Cx. nigripalpus	20 (5, 2–64)	0 (5, 0–40)	<1
Ps. ferox	100 (9, 73–100)	100 (6, 64–100)	40

[a] Infection rate = percentage of mosquitoes containing virus in their bodies (number tested, 95% CI) for mosquitoes tested 7–22 d after feeding on a viremic hamster.

[b] Dissemination rate = percentage of mosquitoes tested ≥14 d after the infectious bloodmeal, regardless of infection status, containing virus in their legs (number tested, 95% CI).

[c] Estimated transmission rate for mosquitoes ≥14 d after the infectious blood meal = percentage of mosquitoes with a disseminated infection times the percentage of mosquitoes with a disseminated infection that transmitted virus by bite from Table 3.

The viremias used in this study, $10^{5.7–9.9}$ PFU/ml, are consistent with viremias determined for natural infections with RVFV, where viremias in lambs and calves were as high as $10^{10.2}$ and $10^{9.2}$ mouse intracranial LD_{50}, respectively (Easterday et al. 1962, McIntosh et al. 1973). Therefore, the results obtained in our study should apply to the various mosquito species tested, should they feed on RVFV-infected cattle or sheep in a natural outbreak of RVF. As expected, infection, dissemination, and expected transmission rates increased with exposure to higher viremias for nearly all the species tested. However, these rates remained nearly constant for *Cx. nigripalpus* across all of the exposure doses tested (Table 4). This may indicate that a small percentage of the population is susceptible, while the vast majority of individual *Cx. nigripalpus* are completely refractory to oral infection.

Many of the species tested displayed significant midgut infection and midgut escape barriers (Kramer et al. 1981), indicating that they might not be extremely efficient vectors of RVFV if fed on an animal with a moderate viremia. However, even mosquito species with poor or moderate vector competence for transmission of RVFV could initiate or substantially contribute to an RVF epizootic or epidemic in North America if their population sizes are very large and sympatric with susceptible vertebrate amplifying hosts and populations of secondary insect vectors. This situation occurred during the RVF outbreak in northeastern Kenya in 2006–2007 when highly abundant *Aedes mcintoshi/circumluteolus* were important vectors of RVFV (Sang et al. 2010) despite the fact that *Ae. mcintoshi* is a very poor vector in the laboratory with an estimated transmission rate of only 5% (Turell et al. 2008b). Similarly, nearly incompetent *Ae. aegypti* vectors were responsible for an epidemic of yellow fever in Nigeria in 1987 (Miller et al. 1989, Nasidi et al. 1989). In addition, previous studies indicate that the presence of other organisms (e.g., filarial worms) may greatly affect the ability of mosquitoes to transmit an arbovirus. For example, the presence of microfilariae of *Brugia malayi* (Brug) in a viremic gerbil resulted in a more than fourfold increase in dissemination rates and a more than sixfold increase in transmission rates in *Aedes taeniorhynchus* (Wiedemann) as compared with those that fed on a gerbil with the same viremia but without microfilariae (Turell et al. 1984a). Similar examples of microfilarial enhancement have been shown with other mosquito and virus and filarial worm combinations (Turell et al. 1987, Vaughan et al. 1999). Similarly, the presence of malarial sporozoites enabled RVFV to bypass a salivary gland barrier in *Anopheles stephensi* Liston and turned an incompetent vector into a competent one (Vaughan and Turell 1996). In addition, environmental temperature has been shown to affect the ability of mosquitoes to transmit RVFV, with different effects for different species (Turell et al. 1985, Turell 1993, Brubaker and Turell 1998). Therefore, environmental factors or the presence of other pathogens may significantly affect the ability of the mosquitoes tested here to transmit RVFV, and even though some of them may have limited vector competence under laboratory conditions, when exposed to conditions in the real world, where filarial infections are common, they might become relatively efficient vectors and should be prioritized for control. Additional studies are needed to determine how these other factors may affect the ability of these mosquitoes to transmit RVFV.

The recent geographic range expansion of RVFV into the Arabian Peninsula (Balkhy and Memish 2003), combined with manifold nodes of connection among RVFV-endemic regions and North American sea and air ports (Kasari et al. 2008, Linthicum et al. 2008) and ecological infrastructure in selected regions of North America potentially hospitable to RVFV development and transmission during seasons when RVF activity has been observed in endemic regions (Linthicum et al. 2007), bring about scenarios in which RVFV could emerge in and spread through North America. The identification of potential North Amer-

ican mosquito vectors of RVFV is critical to predictive models being developed that relate historical changes in mosquito populations with climate to classify regions in the United States at elevated risk of RVFV transmission at times when RVF activity is high in endemic regions (Britch et al. 2008). Control of potential U.S. mosquito vectors of RVFV is central to strategies being developed for containment of the virus should it be detected (Britch et al. 2007), and results such as presented here will contribute to prioritizing limited vector control resources by U.S. mosquito and vector control districts. Further studies are required to evaluate other potential vectors of RVFV in North America, determine the potential for North American domestic ungulates and wild ungulates (particularly white-tailed deer) to produce a viremia with RVFV sufficiently high to infect potential vectors, and to determine the role of other factors (e.g., environmental temperature or presence of other organisms) on the transmission of this pathogen.

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