Potential for North American Mosquitoes (Diptera: Culicidae) to Transmit Rift Valley Fever Virus

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ABSTRACT To determine which arthropods should be targeted for control should Rift Valley fever virus (RVFV) be detected in North America, we evaluated *Culex erraticus* (Dyar and Knab), *Culex erythrothorax* Dyar, *Culex nigripalpus* Theobald, *Culex pipiens L.*, *Culex quinquefasciatus* Say, *Culex tarsalis* Coquillett, *Aedes dorsalis* (Wiedemann), *Aedes vexans* (Meigen), *Anopheles quadrimaculatus* Say, and *Culicoides sonorensis* Wirth and Jones from the western, midwestern, and southern United States for their ability to transmit RVFV. Female mosquitoes were allowed to feed on adult hamsters inoculated with RVFV, after which engorged mosquitoes 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. Other specimens were inoculated intrathoracically, held for 7 d, and then allowed to feed on a susceptible hamster to check for a salivary gland barrier. When exposed to hamsters with viremias $\geq 10^{8.8}$ plaque-forming units/ml blood, *Cx. tarsalis* transmitted RVFV efficiently (infection rate = 93%, dissemination rate = 56%, and estimated transmission rate = 52%). In contrast, when exposed to the same virus dose, none of the other species tested transmitted RVFV efficiently. Estimated transmission rates for *Cx. erythrothorax*, *Cx. pipiens*, *Cx. erraticus*, and *Ae. dorsalis* were 10, 8, 4, and 2%, respectively, and for the remaining species were ≤1%. With the exception of *Cx. tarsalis* and *Cx. pipiens*, all species tested had moderate to major salivary gland barriers. None of the *C. sonorensis* became infected and none of the *An. quadrimaculatus* tested transmitted RVFV by bite, even after intrathoracic inoculation, indicating that these species would not be competent vectors of RVFV. Although *Ae. vexans* from Florida and Louisiana were relatively efficient vectors of RVFV, specimens of this species captured in Colorado or California were virtually incompetent, illustrating the need to evaluate local population for their ability to transmit a pathogen. In addition to laboratory vector competence, factors such as seasonal density, host feeding preference, longevity, and foraging behavior should be considered when determining the potential role that these species could play in RVFV transmission.

KEY WORDS Rift Valley fever, vector, transmission, North America

As illustrated by the introduction of West Nile virus (WNV) into the United States in 1999 and its subsequent spread across North America, exotic arboviruses have the potential to be introduced, become established in North America, and cause significant disease and economic disruption. Of particular concern is 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). 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 (Meegan 1979, Laughlin et al. 1979). The detection of RVFV on 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 to continue to spread (House et al. 1992).

Although Rift Valley fever is predominately a disease of domestic ruminants, where infection in pregnant animals usually results in abortion, and infection of newborn animals is nearly always fatal, humans are also susceptible to infection (Easterday 1965, Easter-

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day et al. 1962, Meegan and Bailey 1988, Bird et al. 2009). In humans, most infections result in an undifferentiated febrile disease; however, ≈1% of infections result in hemorrhagic complications, which are often fatal. Ocular sequellae that can cause retinal damage, including blindness, have also been documented (Schrire 1951, Siam and Meegan 1980, Al-Hazmi et al. 2005).

Although 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), this virus has been associated almost exclusively with mosquitoes in nature, with the virus isolated from at least 40 species in eight genera (Meegan and Bailey 1988, Fontenille et al. 1998). Because methods of control and degree of risk vary for different mosquito species, it is necessary to identify which species are competent vectors and might be involved in the natural transmission cycle so that the appropriate control measures can be employed.

To determine which mosquito species in the midwestern and western United States might serve as potential vectors should RVFV be introduced into North America, we evaluated mosquitoes from Colorado and California for their potential to serve as natural vectors of RVFV. We also tested additional specimens from Louisiana to confirm results of an earlier study (Turell et al. 2008). We selected the western and midwestern regions as they would allow us to examine mosquitoes not tested during earlier studies (Gargan et al. 1988, Turell et al. 2008) and that are abundant in the major beef cattle-rearing states. RVFV is a select agent and requires biological safety level-3 agriculture facilities with vaccination or a biological safety level-4 facility for experimental study.

Materials and Methods

Mosquitoes and Biting Midges. Mosquitoes were captured in California, Colorado, or Louisiana; placed in screen-topped containers; and shipped to United States Army Medical Research Institute of Infectious Diseases (USAMRIID). Overall, eight species were collected in sufficient numbers for evaluation and two additional species were obtained from colonies (Table 1). Upon 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.

Viruses and Virus Assay. The ZH501 strain of RVFV, isolated in 1977 from the blood of a 10-yr-old Egyptian girl who had a fatal RVFV infection (Meagan 1979), was used throughout this study. 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 and biting midge specimens were triturated in 1 ml of diluent (10% heat-inactivated fetal bovine serum in medium 199 with Earle's salts [Invitrogen, Carlsbad, CA] 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.

Table 1. Source and colonization history of mosquitoes evaluated for their vector competence for RVFV

Species	Location	Year	Generation
Ae. vexans (Meigen)	CA and CO	2008	P_o/F_1
Ae. dorsalis (Wiedemann)	CA and CO	2008	P_o/F_1
An. quadrimaculatus Say	Alachua Co., FL	2007	unk
C. sonorensis Wirth and Jones	Boulder Co., CO	2003	unk
Cx. erraticus (Dyar and Knab)	Calcasieu Parish, LA	2008	P_o
Cx. erythrothorax Dyar	Riverside Co., CA	2008	P_o
Cx. nigripalpus Theobald	Calcasieu Parish, LA	2008	P_o
Cx. quinquefasciatus Say	CA and LA	2008	P_o/F_1
Cx. pipiens L.	CA and CO	2008	P_o/F_1
Cx. tarsalis Coquillett	CA and CO	2008	P_o/F_1

P_o, field-collected mosquitoes; F_1, first generation progeny of field-collected mosquitoes; unk, unknown from a colony.

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 50–100 mosquitoes. To feed the biting midges, an anesthetized hamster was placed inside of a Plexiglas cage (with a stocking net sleeve) and the flies were allowed to feed on the hamster. Immediately after mosquito or biting midge 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 or biting midge feeding. After exposure to the viremic hamsters, nonengorged mosquitoes or biting midges were removed and destroyed by placing them in a freezer at −20°C. Apple slices, or a 10% sucrose solution, were provided as a carbohydrate source, and mosquitoes and biting midges 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 blood meal, moist toweling or a water dish was added to each cage to stimulate oviposition. Eggs obtained from several species were hatched and larvae reared to provide an F_1 generation that was also tested for their susceptibility to RVFV, as described above.

To determine whether the mosquitoes or biting midges could transmit virus by bite, they were allowed to feed on susceptible hamsters either individually or in small groups of two to five flies each. Because RVFV infection consistently is fatal to hamsters, we considered death or euthanasia (when moribund) of these hamsters to indicate virus transmission. Presence of virus was verified by isolating virus from brain tissue

from a subset of the dead or euthanized hamsters (data not shown). Immediately after each transmission trial, mosquitoes or biting midges 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 suspensions, the mosquito was considered to have a disseminated infection (Turell et al. 1984). 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 (only occurred three times in this study), data from that pool were not used to calculate the transmission rate, regardless of hamster survival.

The infection rate was the percentage of mosquitoes or biting midges feeding on the original viremic hamsters that contained virus. The dissemination rate was the percentage of mosquitoes or biting midges feeding on the original viremic hamsters (regardless of their infection status) that contained virus in their legs, and the transmission rate was the percentage of mosquitoes or biting midges feeding on the original viremic hamsters that refed (regardless of their infection status) that transmitted virus by bite. We used the modified Wald method of calculating 95% confidence intervals (Agresti and Coull 1998).

Inoculated Mosquitoes and Biting Midges. We also inoculated some of the mosquitoes and biting midges (Rosen and Gubler 1974) to produce a cohort of flies with a known disseminated infection. These flies 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).

Results

Hamster Viremia. Viremias in the 10 hamsters used to expose mosquitoes to RVFV ranged from $10^{7.2}$ to $10^{10.6}$ PFU/ml ($10^{4.7}$ to $10^{8.1}$ PFU of virus ingested per mosquito, respectively). Because the viremias in two of the hamsters, $10^{7.2}$ and $10^{7.4}$, were similar, the data for mosquitoes feeding on either of these hamsters were combined and used to represent mosquitoes feeding on an animal with a moderate natural viremia level. Viremias in the remaining eight hamsters ranged from $10^{8.8}$ to $10^{10.6}$ PFU/ml, and the data from mosquitoes feeding on these hamsters were combined and used to represent mosquitoes feeding on an animal with a high natural viremia. Viremias in lambs and calves are as high as $10^{10.2}$ and $10^{9.2}$ mouse intracranial 50% lethal dose, respectively (Easterday et al. 1962, McIntosh et al. 1973).

Table 2. Infection, dissemination, and transmission rates for mosquitoes and biting midges (*Culicoides*) orally exposed to RVFV

Species	No. tested	Inf. rate[a]	Dissem. rate[b]	Trans. rate[c]	Estimated trans. rate[d]
Infectious dose = $10^{7.3 \pm 0.1}$ PFU/ml					
Cx. erraticus	46	9 (3–21)	4 (<1–15)	0 (20)	1
Cx. nigripalpus	34	0 (0–9)	0 (0–9)	0 (15)	<1
Cx. tarsalis	71	58 (46–69)	10 (5–19)	6 (18)	9
Ae. dorsalis	7	57 (25–84)	0 (0–32)	0 (5)	<1
Ae. vexans	19	11 (2–33)	0 (0–15)	0 (8)	<1
Infectious dose = $\geq 10^{8.8}$ PFU/ml					
Cx. erraticus	53	36 (24–89)	13 (6–25)	5 (21)	4
Cx. erythrothorax	33	70 (53–83)	30 (17–47)	7 (14)	10
Cx. nigripalpus	49	49 (36–63)	4 (<1–14)	4 (27)	1
Cx. pipiens	25	52 (34–70)	8 (1–26)	25 (4)	8
Cx. quinquefasciatus	13	15 (3–43)	0 (0–20)	0 (2)	<1
Cx. tarsalis	43	93 (81–98)	56 (41–71)	31 (13)	52
Ae. vexans	204	30 (24–37)	3 (1–6)	2 (94)	1
An. quadrimaculatus	11	64 (35–85)	0 (0–23)	0 (6)	<1
Ae. dorsalis	46	78 (64–88)	33 (21–47)	0 (31)	2
C. sonorensis	17	0 (0–16)	0 (0–16)	0 (1)	<1

[a] Infection rate = percentage of mosquitoes containing virus in their bodies (95% confidence interval).

[b] Dissemination rate = percentage of mosquitoes, regardless of infection status, containing virus in their legs (95% confidence interval).

[c] Transmission rate = percentage of refeeding mosquitoes, regardless of infection status, that transmitted virus (no. feeding).

[d] Estimated transmission rate = percentage of mosquitoes with a disseminated infection × percentage of mosquitoes with a disseminated infection that transmitted virus by bite (Table 3).

Susceptibility to Infection. When exposed to a moderate viremia of $10^{7.3 \pm 0.1}$ PFU/ml, infection rates were low, except for *Aedes dorsalis* (Wiedemann) (57%) and *Culex tarsalis* Coquillett (58%) (Table 2). When mosquitoes fed on hamsters with viremias $\geq 10^{8.8}$ PFU/ml, all of the mosquito species tested became infected; however, virus was not detected in any of the 17 *Culicoides sonorensis* Wirth and Jones tested. In addition to the lack of infection in the 17 orally exposed *C. sonorensis*, RVFV also was not detected in any of 14 *C. sonorensis* that had been inoculated with RVFV 6 d previously, indicating that this species was not susceptible to infection with RVFV.

Viral Dissemination. As with infection, dissemination rates were highest in *Cx. tarsalis* (10%) when exposed to a viremia $10^{7.3 \pm 0.1}$ PFU/ml. However, when fed on a hamster with a viremia $\geq 10^{8.8}$ PFU/ml, disseminated infections were detected in all mosquito species tested, except *Anopheles quadrimaculatus* Say and *Culex quinquefasciatus* Say (Table 2). Again, dissemination rates were highest in *Cx. tarsalis* (56%), followed by *Ae. dorsalis* (33%) and *Culex erythrothorax* Dyar (30%).

Viral Transmission. With the exception of *An. quadrimaculatus* and *C. sonorensis*, all 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. Transmission rates were similar in groups with a disseminated infection after either oral exposure or intrathoracic inoculation, indicating that the route of infection did not affect the presence of a salivary gland barrier.

Table 3. Transmission of RVFV by mosquitoes and biting midges (*Culicoides*) with a disseminated infection after either oral exposure or intrathoracic inoculation

Species	Route of infection				Totals	
	Oral[a]		Inoculated			
	N[b]	T.R.[c]	N[b]	T.R.[c]	N[b]	T.R.[c]
Cx. erraticus	4	25	n.t.		4	25
Cx. erythrothorax	4	25	2	0	6	17
Cx. nigripalpus	3	33	n.t.		3	33
Cx. pipiens	1	100	n.t.		1	100
Cx. tarsalis	6	83	8	100	14	93
Ae. vexans	5	40	14	29	19	32
An. quadrimaculatus	n.t.		9	0	9	0
Ae. dorsalis	11	0	10	10	21	5
C. sonorensis	n.t.		9	0	9	0

n.t., Not tested.

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

[b] Number of mosquitoes that fed on a susceptible hamster.

[c] Percentage of mosquitoes that transmitted RVFV by bite.

Discussion

The spread of RVFV to Egypt and the Arabian Peninsula (Meegan 1979, Laughlin et al. 1979, Jupp et al. 2002, Shoemaker et al. 2002, Balkhy and Memish 2003, Madani et al. 2003, Bird et al. 2009), the recent outbreaks of Rift Valley fever in eastern Africa (Bird et al. 2008), and the successful invasion of North America by WNV all indicate the potential for RVFV to spread to and become established in North America. As shown in this and previous studies (Gargan et al. 1988, Turell et al. 1988, 2008), competent vectors of RVFV exist in North America and, if introduced, local vectors and vertebrate amplifying hosts would allow the virus to spread and cause significant economic damage (Lupton et al. 1982, House et al. 1992).

It is important to identify which species should be targeted for control and which ones could be ignored should RVFV be introduced into North America. Based on the laboratory studies reported in this work, *Cx. tarsalis* should be considered to be a highly capable vector of RVFV. Not only is it highly competent, but it also feeds frequently on mammals, including cattle (Tempelis and Washino 1967, Nelson et al. 1976), and thus could acquire and transmit RVFV to domestic ungulates.

Studies identifying a species as a potential vector in one geographic area may not extend to members of that same species from a different geographic area. For example, *Aedes vexans* (Meigen) captured in Louisiana and Florida were competent vectors and readily transmitted RVFV by bite with an estimated transmission rate of 27% (Turell et al. 2008). However, the *Ae. vexans* in this study, captured in Colorado and California, were virtually incompetent with an estimated transmission rate of only 1%, despite being tested under virtually identical conditions. This variation in vector competence appeared to be the result of both a midgut infection and a midgut escape barrier (Kramer et al. 1981). Although infection and dissemination rates were 91 and 67%, respectively, in the *Ae. vexans* captured in Florida or Louisiana (Turell et al. 2008), these rates were only 30 and 3%, respectively, for *Ae. vexans* captured in Colorado or California, although they fed on hamsters with similar viremias and were handled in the same manner. However, there did not appear to be a difference in salivary gland barriers as the transmission rates for *Ae. vexans* with a disseminated infection were similar for the two studies, 32 and 40%. This indicates the need to evaluate geographical populations of a mosquito species for their ability to transmit a particular virus.

Similarly, the relative inability of *Cx. quinquefasciatus* to transmit RVFV is surprising because of the close relationship between this species and *Culex pipiens* L., the incriminated vector during the outbreak in Egypt in 1977–79 (Meegan et al. 1980). However, several other populations of *Cx. quinquefasciatus* also have been shown to be inefficient vectors of RVFV, including those from Australia, the southeastern United States, Kenya, and South Africa (McIntosh et al. 1980, Turell and Kay 1998, Turell et al. 2008). The identity of some of the *Cx. pipiens/quinquefasciatus* specimens captured in California could be separated based on the location where they were captured. When these mosquitoes were allowed to feed on the same hamsters and handled in the same manner, infection rates were significantly higher in *Cx. pipiens* (52%) than in *Cx. quinquefasciatus* (15%) (Fisher exact test, $P = 0.039$) providing additional information that these two species, although genetically similar, may not respond similarly to virus infection.

Although the actual means by which WNV was introduced into North America will probably never be known, it is most likely that it was transported to the New York City area in an infected mosquito that was transported in an aircraft arriving from a region with ongoing WNV transmission. The extremely low viremias that occur in humans infected with WNV make it unlikely that WNV was transported to North America in a viremic human. In contrast, viremia levels in humans infected with RVFV can exceed 10^8 PFU/ml (Meegan 1979). Therefore, not only could RVFV be introduced by an infected mosquito transported into North America on an aircraft, but it could also be brought here in an infected human (e.g., a returning ecotourist or visitor), greatly increasing the potential for introduction.

The viremias used in this study, $10^{7.2 - 10.6}$ PFU/ml, are consistent with viremias determined for natural infections with RVFV, in which viremias in lambs and calves were as high as $10^{10.2}$ and $10^{9.2}$ mouse intracranial 50% lethal dose, 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. Further studies are required to evaluate other potential vectors of RVFV in North America, determine the potential for North American animals (particularly 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) on the transmission of this pathogen.

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