

## Vector Competence of Selected African Mosquito (Diptera: Culicidae) Species for Rift Valley Fever Virus

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**ABSTRACT** Outbreaks of Rift Valley fever (RVF) in Egypt, Yemen, and Saudi Arabia have indicated the potential for this disease to spread from its enzootic areas in sub-Saharan Africa. Because little is known about the potential for most African mosquito species to transmit RVF virus (family *Bunyaviridae*, genus *Phlebovirus*, RVFV), we conducted studies to determine the vector competence of selected African species of mosquitoes for this virus. All eight species tested [*Aedes palpalis* (Newstead), *Aedes mcintoshi* Huang, *Aedes circumluteolus* (Theobald), *Aedes calceatus* Edwards, *Aedes aegypti* (L.), *Culex antennatus* (Becker), *Culex pipiens* (L.), and *Culex quinquefasciatus* Say], were susceptible to infection, and all except *Ae. calceatus*, *Ae. aegypti* and *Cx. quinquefasciatus* transmitted RVFV by bite after oral exposure. Estimated transmission rates for mosquitoes that successfully transmitted RVFV by bite ranged from 5% for *Ae. mcintoshi* to 39% for *Ae. palpalis* for mosquitoes that fed on a hamster with a viremia  $\geq 10^8$  plaque-forming units of virus/ml. We did not recover RVFV from any of 3,138 progeny of infected female mosquitoes. RVFV is unusual among arboviruses in that it has been isolated in nature from a large number of species and that numerous mosquitoes and other arthropods are able to transmit this virus in the laboratory. The recent introduction and spread of West Nile virus into the Americas and the spread of RVFV to the Arabian Peninsula illustrates the potential for viruses, once enzootic in Africa, to spread to other parts of the world.

**KEY WORDS** Rift Valley fever virus, transmission, mosquito, Africa, vector

Rift Valley fever virus (family *Bunyaviridae*, genus *Phlebovirus*, RVFV) has been associated with numerous outbreaks of severe disease in domestic ruminants in sub-Saharan Africa over the past 70 yr (Meegan and Bailey 1988, Gerdes 2004). However, the recent movement of RVFV out of Africa into 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 (RVF) is predominately a problem in domestic ruminants, where infections in pregnant an-

imals usually results in abortion and infection of newborn animals is nearly always fatal, humans are also susceptible to infection (Easterday et al. 1962, Meegan and Bailey 1988). In humans, most infections result in an undifferentiated febrile disease; however,  $\approx 1\%$  of the infections result in hemorrhagic complications, which are often fatal. In addition, ocular sequelae occur that can cause retinal damage, including blindness (Siam and Meegan 1980, Al-Hazmi et al. 2005).

Although RVFV is a member of the genus *Phlebovirus* and transmission by sand flies is known to occur in the laboratory (Hoch et al. 1984, Turell and Perkins 1990, Dohm et al. 2000), RVFV has been associated almost exclusively with mosquitoes in nature. It has been isolated from at least 40 species of mosquitoes in eight genera (Meegan and Bailey 1988, Fontenille et al. 1998). Laboratory studies have indicated that numerous species of mosquitoes are susceptible to oral infection and are able to transmit RVFV by bite (McIntosh et al. 1973b, 1980; Meegan and Bailey 1988, Gargan et al. 1988, Turell et al. 1996). However, some of these studies have focused on mosquitoes from areas where RVF is not enzootic in an attempt to determine the risk of local transmission of this virus, should it be introduced into a region where the mosquitoes are found (Gargan et al. 1988, Turell et al.

The opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army.

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| 14. ABSTRACT<br><b>Outbreaks of Rift Valley fever (RVF) in Egypt, Yemen, and Saudi Arabia have indicated the potential for this disease to spread from its enzootic areas in sub-Saharan Africa. Because little is known about the potential for most African mosquito species to transmit RVF virus (RVFV), we conducted studies to determine the vector competence of selected African species of mosquitoes for this virus. All eight species tested (Aedes palpalis (Newstead), Aedes mcintoshi Huang, Aedes circumluteolus (Theobald), Aedes calceatus Edwards, Aedes aegypti (L.), Culex antennatus (Becker), Culex pipiens (L.), and Culex quinquefasciatus Say, were susceptible to infection and all except Ae. calceatus, Ae. aegypti and Cx. quinquefasciatus transmitted RVFV by bite after oral exposure. Estimated transmission rates for mosquitoes that successfully transmitted RVFV by bite ranged from 5% for Ae. mcintoshi to 39% for Ae. palpalis for mosquitoes that fed on a hamster with a viremia &gt;108 plaque-forming units of virus/ml. We did not recover RVFV from any of 3,138 progeny of infected female mosquitoes. RVFV is unusual among arboviruses in that it has been isolated in nature from a large number of species and that numerous mosquitoes and other arthropods are able to transmit this virus in the laboratory. The recent introduction and spread of West Nile virus into the Americas and the spread of RVFV to the Arabian Peninsula illustrates the potential for viruses, once enzootic in Africa, to spread to other parts of the world.</b> |                                    |                                     |                                          |                                                              |                                 |
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**Table 1.** Source and colonization history of mosquitoes evaluated for their vector competence for Rift Valley fever virus

| Species                     | Location                 | Yr               | Generation                                  |
|-----------------------------|--------------------------|------------------|---------------------------------------------|
| <i>Ae. aegypti</i>          | Kenya                    | 1982             | P <sub>0</sub> <sup>a</sup>                 |
| <i>Ae. calceatus</i>        | Kenya                    | 1982             | P <sub>0</sub> <sup>a</sup>                 |
| <i>Ae. circumluteolus</i>   | Kenya                    | 1985, 1989       | P <sub>0</sub> /F <sub>1</sub> <sup>b</sup> |
| <i>Ae. mcintoshi</i>        | Kenya                    | 1985, 1986, 1988 | P <sub>0</sub> /F <sub>1</sub> <sup>b</sup> |
| <i>Ae. palpalis</i>         | Central African Republic | 1985             | P <sub>0</sub> /F <sub>1</sub> <sup>b</sup> |
| <i>Cx. antennatus</i>       | Kenya                    | 1985             | P <sub>0</sub> <sup>b</sup>                 |
| <i>Cx. pipiens</i>          | Egypt                    | 1980             | F <sub>0</sub> <sup>b</sup>                 |
| <i>Cx. quinquefasciatus</i> | Kenya                    | 1983             | P <sub>0</sub> <sup>b</sup>                 |

<sup>a</sup> Collected as eggs in Africa and reared to adults at USAMRIID.<sup>b</sup> Captured as adults.

1988a, Turell and Kay 1998). In addition, some of the studies with mosquitoes from areas where RVF is enzootic used large pools of mosquitoes (Smithburn et al. 1949; McIntosh et al. 1973b, 1980). Although these studies can determine whether a particular species is competent, they are unable to differentiate a highly efficient vector from a vector that is only marginally competent.

In our study, conducted during the 1980s, we examined eight species of mosquitoes collected in RVF enzootic areas for their susceptibility to oral infection and their subsequent ability to transmit RVFV by bite. Several of these species also were tested for their ability to vertically transmit RVFV to their progeny.

#### Materials and Methods

**Mosquitoes.** The mosquito species evaluated for their vector competence for RVFV and colonization histories are listed in Table 1. Mosquitoes were captured in Africa and transported to a biological safety level-3 laboratory (with HEPA-filtered exhaust air, treated sewage, and a 100% clothing change) at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). They were then provided apple slices as a carbohydrate source and held at 26°C for 7–10 d until either exposed to viremic hamsters or allowed to feed on an uninfected hamster to stimulate egg production. In addition to the field-collected female mosquitoes, first-generation progeny of some of these mosquitoes also were used in these studies. All larvae were reared under standard conditions at 26°C (Gargan et al. 1983).

In addition to the species tested for vector competence, *Eretmapodites quinquevittatus* Theobald, derived from specimens collected in South Africa, were tested for their ability to transmit RVFV vertically to their progeny.

**Viruses and Virus Assays.** Three strains of RVFV: ZH501, isolated in 1977 from the blood of a 10-yr-old Egyptian girl who had a fatal RVFV infection (Meegan 1979); Zinga (DakArB1976), isolated from *Mansonia africana* (Theobald) mosquitoes captured in the Central African Republic in 1969; and a Kenyan strain (21445) isolated from *Aedes mcintoshi* Huang in 1983 were used throughout this study.

Individual specimens (mosquito larvae, pupae, or adults) were triturated in 1 ml of diluent (10% heat-inactivated fetal bovine serum in Medium 199 (Invitrogen, Carlsbad, CA) with Hanks' salts and antibiotics) and frozen at –70°C until tested for infectious virus by a plaque-assay on Vero cell monolayers. Serial 10-fold dilutions of each specimen were tested on 12-well plates as described by Gargan et al. (1983). Virus titers were expressed as log<sub>10</sub> plaque-forming units (PFU) per specimen.

**Determination of Vector Competence.** To provide a source of viremic blood, adult female Syrian hamsters were inoculated intraperitoneally with 0.2 ml of a suspension containing ≈10<sup>4</sup> PFU of RVFV. These hamsters were anesthetized 1 or 2 d later and placed individually (i.e., one per cage) on the top of cages containing 50–150 mosquitoes. Immediately after mosquito feeding, 0.2 ml of blood was obtained from each hamster by cardiac puncture, and it was added to 1.8 ml of diluent. The blood suspensions were frozen at –70°C until assayed on Vero cell monolayers to determine the viremia at the time of mosquito feeding. In addition to the blood sample, three mosquitoes from each replicate were triturated individually in 1 ml of mosquito diluent immediately after feeding. These suspensions were tested by plaque assay to determine the actual virus dose ingested. After exposure to the viremic hamsters, engorged mosquitoes were transferred to 3.8-liter screen-topped cardboard cages. Apple slices, or a 7% 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 rates. Approximately 1 wk after the infectious bloodmeal, moist toweling or a water dish was added to each cage to stimulate oviposition.

To determine whether the mosquitoes could transmit virus by bite, mosquitoes 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 death or euthanasia (when moribund) of these animals to indicate virus transmission. Presence of virus was verified by isolating virus from brain tissue from a subset of the dead hamsters. Immediately after each transmission trial, mosquitoes were killed by freezing at –20°C for 5 min, identified to species, and their legs and bodies triturated separately in 1 ml of diluent. These suspensions then were frozen at –70°C until tested for virus.

Mosquito infection was determined by recovering virus from its body tissue suspension. If virus was recovered from its body, but not its legs, the mosquito was considered to have a nondisseminated infection limited to its midgut. In contrast, if virus was recovered from both body and leg suspensions, the mosquito was considered to have a disseminated infection (Turell et al. 1984). The dissemination rate was the percentage of orally exposed mosquitoes that contained virus in their legs. Because some of the mosquitoes were tested for transmission in small pools, it was not always possible to determine which mosquito in a pool actually

Table 2. Infection and dissemination rates for mosquitoes orally exposed to Rift Valley fever virus

| Species                                        | Virus strain | Days of extrinsic incubation |                   |                   |       |                   |                   |     |                   |                   |        |                   |                   |
|------------------------------------------------|--------------|------------------------------|-------------------|-------------------|-------|-------------------|-------------------|-----|-------------------|-------------------|--------|-------------------|-------------------|
|                                                |              | 3-10                         |                   |                   | 11-16 |                   |                   | ≥18 |                   |                   | Totals |                   |                   |
|                                                |              | N                            | I.R. <sup>a</sup> | D.R. <sup>b</sup> | N     | I.R. <sup>a</sup> | D.R. <sup>b</sup> | N   | I.R. <sup>a</sup> | D.R. <sup>b</sup> | N      | I.R. <sup>a</sup> | D.R. <sup>b</sup> |
| Infectious dose = 10 <sup>5.5-6.8</sup> PFU/ml |              |                              |                   |                   |       |                   |                   |     |                   |                   |        |                   |                   |
| <i>Ae. circumluteolus</i>                      | K-21445      | 10                           | 70a               | 10a               | 19    | 58a               | 21ab              | 29  | 66a               | 34a               | 58     | 64a               | 26a               |
| <i>Ae. mcintoshi</i>                           | K-21445      | 83                           | 35b               | 13a               | 57    | 23b               | 7c                | 70  | 23c               | 10b               | 210    | 28b               | 10b               |
| <i>Ae. palpalis</i>                            | Zinga        | NT                           | NT                | NT                | 19    | 58a               | 42a               | 34  | 56ab              | 41a               | 53     | 57a               | 42a               |
| <i>Cx. antennatus</i>                          | K-21445      | NT                           | NT                | NT                | 17    | 18b               | 6bc               | 23  | 35bc              | 0b                | 40     | 28b               | 3b                |
| Infectious dose = 10 <sup>7.0-7.8</sup> PFU/ml |              |                              |                   |                   |       |                   |                   |     |                   |                   |        |                   |                   |
| <i>Ae. aegypti</i>                             | ZH501        | 2                            | 100ab             | 0bc               | 3     | 33b               | 0c                | NT  | NT                | NT                | 5      | 60bcd             | 0b                |
| <i>Ae. calceatus</i>                           | ZH501        | 13                           | 92a               | 15bc              | 20    | 65ab              | 30bc              | 11  | 91ab              | 55a               | 44     | 80ab              | 32ab              |
| <i>Ae. circumluteolus</i>                      | ZH501        | 10                           | 20c               | 10bc              | 10    | 30b               | 0c                | 6   | 17c               | 17a               | 26     | 23d               | 8b                |
| <i>Ae. mcintoshi</i>                           | K-21445      | 20                           | 60b               | 45ab              | 10    | 30b               | 20bc              | 30  | 60bc              | 43a               | 60     | 55c               | 40a               |
| <i>Cx. quinquefasciatus</i>                    | ZH501        | 12                           | 67ab              | 8c                | 39    | 33b               | 5c                | NT  | NT                | NT                | 51     | 41cd              | 6b                |
| <i>Cx. pipiens</i>                             | ZH501        | 50                           | 74ab              | 8c                | 20    | 85a               | 45ab              | NT  | NT                | NT                | 70     | 77ab              | 19b               |
| Infectious dose = 10 <sup>8.5-9.0</sup> PFU/ml |              |                              |                   |                   |       |                   |                   |     |                   |                   |        |                   |                   |
| <i>Ae. aegypti</i>                             | ZH501        | 41                           | 85a               | 39ab              | 4     | 75abc             | 75ab              | NT  | NT                | NT                | 45     | 84b               | 42b               |
| <i>Ae. calceatus</i>                           | ZH501        | 13                           | 100a              | 3ab               | 20    | 100a              | 50ab              | 11  | 100a              | 36b               | 44     | 100a              | 43b               |
| <i>Ae. circumluteolus</i>                      | K-21445      | 11                           | 73ab              | 36ab              | 22    | 82ab              | 59ab              | 9   | 67abc             | 44abc             | 42     | 76bc              | 50b               |
| <i>Ae. mcintoshi</i>                           | K-21445      | 115                          | 55bc              | 34b               | 126   | 54c               | 43b               | 114 | 42c               | 18bc              | 355    | 50de              | 35b               |
| <i>Ae. palpalis</i>                            | Zinga        | 23                           | 87a               | 61a               | 80    | 83a               | 70a               | 66  | 91a               | 77a               | 169    | 86b               | 72a               |
| <i>Cx. antennatus</i>                          | K-21445      | 25                           | 52bc              | 8cd               | 40    | 63bc              | 15c               | 70  | 62b               | 19c               | 135    | 60cd              | 16c               |
| <i>Cx. pipiens</i>                             | ZH501        | 15                           | 93a               | 33ab              | 34    | 91a               | 29bc              | 15  | 87ab              | 47b               | 64     | 91ab              | 34b               |
| <i>Cx. quinquefasciatus</i>                    | ZH501        | 9                            | 22c               | 11bc              | 13    | 31bc              | 0c                | NT  | NT                | NT                | 22     | 27e               | 5c                |

NT, not tested; N, number tested.

<sup>a</sup> Infection rate, percentage of mosquitoes containing virus in their bodies. Infection rates in the same virus dose group followed by the same letter are not significantly different at  $\alpha = 0.05$ .<sup>b</sup> Dissemination rate, percentage of mosquitoes containing virus in their legs. Dissemination rates in the same virus dose group followed by the same letter are not significantly different at  $\alpha = 0.05$ .

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.

**Inoculated Mosquitoes.** We also inoculated some of the mosquitoes (Rosen and Gubler 1974) to produce a cohort of mosquitoes with a known disseminated infection. These mosquitoes were then tested individually on susceptible hamsters to examine for the presence of a salivary gland barrier (Kramer et al. 1981, Turell and Bailey 1987).

**Vertical Transmission.** To test for the potential for vertical transmission, adult female mosquitoes of selected species were inoculated with RVFV, held for 7 d at 26°C, and then allowed to feed, en masse, on an anesthetized, naïve hamster. An oviposition dish was added 5 d later and eggs collected. Seven days after the first bloodmeal, the mosquitoes were provided a second naïve hamster, and eggs were collected as described above. In some cases, a third ovarian cycle of eggs was collected. Eggs from these mosquitoes with known disseminated infections were hatched and reared at 26°C. The progeny were tested either as pools of up to 25 fourth-stage larvae or pupae, or they were reared to the adult stage and then tested separately in pools of up to 25 males or females. All pools were triturated in 2 ml of diluent and then tested for RVFV by plaque assay.

This research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations in force at the time the work was done and adhered to the principles stated in the Guide for the Care and Use of Laboratory Animals, 1978 or 1985. The

facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

## Results

**Vector Competence.** Viremias in the 39 hamsters used to expose mosquitoes to RVFV ranged from  $10^{5.8}$  to  $10^{10.0}$  PFU/ml of blood ( $10^{3.3}$ - $10^{7.5}$  PFU ingested per mosquito, respectively). Viremias induced by each of the three strains of RVFV were similar, with hamsters inoculated with the ZH501, K-21445, and Zinga strains of RVFV, each producing a mean viremia of  $10^{8.3}$  PFU/ml of blood. Because infection rates tended to increase with increasing virus dose ingested, we arbitrarily grouped the mosquitoes into those exposed to low ( $10^{5.8-6.8}$  PFU/ml), moderate ( $10^{7.0-7.8}$  PFU/ml), or high ( $\geq 10^8$  PFU/ml) viremias.

All eight species were susceptible to infection after ingesting RVFV, even at the lowest dose that a particular species was exposed (Table 2). Although all species became infected, different "barriers" were present in different species (Table 3). These ranged from a midgut infection barrier associated with low infection rates, midgut escape barrier in which only a small percentage of infected mosquitoes developed a disseminated infection, or a salivary gland barrier in which only a small percentage of those mosquitoes with a disseminated infection transmitted virus by bite when allowed to refeed on a susceptible vertebrate host.

The two *Ae. (Stegomyia)* species tested, *Aedes aegypti* (L.) and *Aedes calceatus* Edwards, were both

**Table 3.** Vector competence for mosquitoes fed on hamsters with RVFV viremia  $\geq 10^{3.0}$  PFU/ml

| Species                     | No. tested | Infection rate <sup>a</sup> | Dissemination rate <sup>b</sup> | Dissemination (I) rate <sup>c</sup> | Transmission (D) rate <sup>d</sup> | Estimated transmission rate <sup>e</sup> |
|-----------------------------|------------|-----------------------------|---------------------------------|-------------------------------------|------------------------------------|------------------------------------------|
| <i>Ae. aegypti</i>          | 45         | 84                          | 42                              | 50 (38)                             | 14 (7)                             | 6                                        |
| <i>Ae. calceatus</i>        | 44         | 100                         | 43                              | 43 (44)                             | 0 (21)                             | <2                                       |
| <i>Ae. circumluteolus</i>   | 42         | 76                          | 50                              | 66 (21)                             | 21 (48)                            | 10                                       |
| <i>Ae. mcintoshi</i>        | 355        | 50                          | 35                              | 70 (177)                            | 14 (171)                           | 5                                        |
| <i>Ae. palpalis</i>         | 159        | 86                          | 72                              | 82 (137)                            | 55 (33)                            | 39                                       |
| <i>Cx. antennatus</i>       | 135        | 60                          | 16                              | 27 (81)                             | 84 (38)                            | 13                                       |
| <i>Cx. pipiens</i>          | 64         | 91                          | 34                              | 38 (58)                             | 100 (28)                           | 34                                       |
| <i>Cx. quinquefasciatus</i> | 22         | 27                          | 5                               | 17 (6)                              | NT                                 | <5                                       |

NT, not tested.

<sup>a</sup> Infection rate = percentage of mosquitoes containing virus in their bodies.<sup>b</sup> Dissemination rate = percentage of mosquitoes containing virus in their legs.<sup>c</sup> Dissemination (I) rate = percentage of infected mosquitoes containing virus in their legs (no. of infected mosquitoes) (i.e., lack of a midgut escape barrier).<sup>d</sup> Transmission (D) rate = percentage of refeeding mosquitoes with a disseminated infection that transmitted RVFV by bite (no. with a disseminated infection that fed) (i.e., lack of a salivary gland barrier) (from Table 5).<sup>e</sup> The estimated transmission rate for mosquitoes feeding on a viremia  $\geq 10^3$  PFU/ml = the percentage of mosquitoes which developed a disseminated infection with RVFV multiplied by the transmission rate for those individuals with a disseminated infection.

highly susceptible to infection and virus dissemination. However, a salivary gland barrier existed in these species as only one of 28 of these mosquitoes with a disseminated infection transmitted virus when fed on a susceptible hamster (Table 3).

All three *Ae. (Neomelaniconion)* species tested, *Aedes circumluteolus* (Theobald), *Ae. mcintoshi*, and *Ae. palpalis* (Newstead), were moderately susceptible to infection and virus dissemination, with at least 50% of each species becoming infected after ingesting blood containing  $\geq 10^3$  PFU/ml of RVFV, and at least 50% of the infected mosquitoes developing a disseminated infection (Tables 2 and 3). Of these three species, *Ae. palpalis* consistently had higher infection and dissemination rates than the other two species at each of the virus doses tested. As with the *Ae. (Stegomyia)* spp. tested, there was evidence of a salivary gland barrier (Tables 3 and 4). However, these ranged from a major barrier with *Ae. mcintoshi* (only 14% of the mosquitoes with a disseminated infection transmitted virus by bite) to a more moderate one for *Ae. palpalis*, 55% transmitted) (Table 4).

All three *Culex* species tested, *Culex antennatus* (Becker), *Culex pipiens* (L.), and *Culex quinquefas-*

*ciatus* Say, were susceptible to RVFV. However, *Cx. pipiens* were significantly more susceptible to infection ( $\chi^2 > 19.3$ ,  $df = 1$ ,  $P < 0.001$ ) and to virus dissemination ( $\chi^2 > 8.2$ ,  $df = 1$ ,  $P < 0.01$ ) than were either of the other two *Culex* spp. tested. We were not able to determine whether *Cx. quinquefasciatus* could transmit virus by bite because none of the ones with a disseminated infection fed on a susceptible host. However, there was little evidence of a salivary gland barrier in either *Cx. antennatus* or *Cx. pipiens*, because 84 and 100%, respectively, of the refeeding mosquitoes with a disseminated infection of these two species, transmitted virus by bite.

**Virus Titer Recovered from Mosquitoes.** For all species tested, the mean titers of virus recovered from specimens with a nondisseminated infection were between 10- and 1,000-fold lower than those recovered from specimens of the same species with a disseminated infection (i.e., with virus detected in their legs) (Table 5). For nearly all species tested, more virus was recovered from the legs of mosquitoes with a disseminated infection, almost always  $\approx 10^{4.3}$  PFU per leg sample, than was recovered from the entire body of those individuals with a nondisseminated infection.

**Table 4.** Transmission rates for mosquitoes with a disseminated infection with RVFV after either oral exposure or intrathoracic inoculation

| Species                   | Route of infection |                       |                |                       |                |                       |
|---------------------------|--------------------|-----------------------|----------------|-----------------------|----------------|-----------------------|
|                           | Oral <sup>a</sup>  |                       | Inoculated     |                       | Totals         |                       |
|                           | N <sup>b</sup>     | T.R. (N) <sup>c</sup> | N <sup>b</sup> | T.R. (N) <sup>c</sup> | N <sup>b</sup> | T.R. (N) <sup>c</sup> |
| <i>Ae. aegypti</i>        | 4                  | 0 (0)                 | 3              | 33 (1)                | 7              | 14 (1)                |
| <i>Ae. calceatus</i>      | 21                 | 0 (0)                 | NT             | NT                    | 21             | 0 (0)                 |
| <i>Ae. circumluteolus</i> | 17                 | 18 (3)                | 31             | 23 (7)                | 48             | 21 (10)               |
| <i>Ae. mcintoshi</i>      | 97                 | 12 (12)               | 74             | 16 (12)               | 171            | 14 (24)               |
| <i>Ae. palpalis</i>       | 26                 | 54 (14)               | 7              | 57 (4)                | 33             | 55 (18)               |
| <i>Cx. antennatus</i>     | 5                  | 60 (3)                | 33             | 88 (29)               | 38             | 84 (32)               |
| <i>Cx. pipiens</i>        | 8                  | 100 (8)               | 20             | 100 (20)              | 28             | 100 (28)              |

T.R., transmission rate; NT, not tested.

<sup>a</sup> Mosquitoes with a disseminated infection (virus in their legs) after oral exposure to RVFV.<sup>b</sup> Number of mosquitoes that fed.<sup>c</sup> Percentage of mosquitoes that fed that transmitted virus (no. that transmitted virus).

Table 5. Viral titers in mosquitoes orally infected with RVFV (assayed  $\geq 7$  d after oral exposure)

| Species                   | Nondisseminated |                        | Disseminated |           |           |            |              |            |                 |
|---------------------------|-----------------|------------------------|--------------|-----------|-----------|------------|--------------|------------|-----------------|
|                           | No. tested      | Body                   | No. tested   | Body      | Legs      | No. tested | Transmitters | No. tested | Nontransmitters |
| <i>Ae. aegypti</i>        | 5               | 4.1 (0.3) <sup>a</sup> | 5            | 5.6 (0.3) | 4.3 (0.4) | 0          | NA           | 3          | 5.6 (0.4)       |
| <i>Ae. calceatus</i>      | 44              | 4.8 (0.5)              | 35           | 5.6 (0.3) | 4.3 (1.2) | 0          | NA           | 21         | 5.7 (0.3)       |
| <i>Ae. circumluteolus</i> | 41              | 3.5 (1.0)              | 47           | 5.7 (0.6) | 4.3 (0.7) | 3          | 5.9 (0.3)    | 17         | 5.6 (0.6)       |
| <i>Ae. mcintoshi</i>      | 63              | 2.7 (0.8)              | 144          | 5.6 (0.6) | 4.3 (0.6) | 6          | 5.8 (0.6)    | 60         | 5.7 (0.5)       |
| <i>Ae. palpalis</i>       | 34              | 3.9 (0.7)              | 137          | 5.4 (0.4) | 4.4 (0.5) | 14         | 5.6 (0.2)    | 11         | 5.7 (0.4)       |
| <i>Cx. antennatus</i>     | 60              | 2.7 (0.7)              | 21           | 5.5 (0.7) | 4.0 (0.9) | 3          | 6.2 (0.9)    | 3          | 5.0 (0.9)       |
| <i>Cx. pipiens</i>        | 21              | 2.9 (0.7)              | 18           | 5.0 (0.3) | 3.9 (1.0) | 8          | 5.5 (0.2)    | 0          | NA              |

NA, not applicable.

<sup>a</sup> Mean (SD) of the log<sub>10</sub> PFU per specimen.

For mosquitoes with a disseminated infection for each species tested, virus titers of mosquitoes transmitting virus by bite were not significantly different than those that failed to transmit virus by bite ( $t \leq 1.6$ ,  $df \geq 4$ ,  $P > 0.21$ ) (Table 5).

**Vertical Transmission Studies.** Despite testing >3,138 progeny of mosquitoes inoculated with RVFV, we did not detect evidence of vertical transmission in these specimens (Table 6). Testing a sample of the inoculated adult females indicated that all of them were infected with RVFV.

### Discussion

All eight mosquito species tested in these studies were susceptible to infection with RVFV, and all except *Ae. calceatus*, *Ae. aegypti*, and *Cx. quinquefasciatus* transmitted RVFV by bite after oral exposure. Although all of the species were susceptible to infection, different "barriers" (i.e., midgut infection, midgut escape, and salivary gland; Kramer et al. 1981) seemed to be the determining factor of the vector competence for the various species. *Cx. quinquefasciatus* had a major midgut infection barrier as only 27% became infected, even at the highest viremia levels tested ( $\geq 10^5$  PFU/ml). This is consistent with other studies that found that this species is a relatively poor vector of RVFV (Turell and Kay 1998; McIntosh et al. 1980; M.J.T., unpublished data). All of the other species tested were generally susceptible to oral infection, with infection rates  $\geq 50\%$  when they fed on a hamster with a viremia  $\geq 10^5$  PFU/ml. At this exposure dose, the *Aedes* species tested had only a moderate midgut escape barrier with virus disseminating to the hemocoel in 43–82% of the infected specimens tested (Table 3). However, there was a more severe midgut escape barrier in the three *Culex* species, with only 17–38% of the infected

specimens developing a disseminated infection. Therefore, a midgut escape barrier seemed to be the principal determinant of vector competency in the *Culex* species. This is similar to what has been reported for *Cx. pipiens* (Turell et al. 1984).

Our failure to demonstrate transmission of RVFV by *Ae. aegypti* and *Cx. quinquefasciatus* may have been due to the relatively small sample size tested as only four *Ae. aegypti* and no *Cx. quinquefasciatus* with a disseminated infection after oral exposure fed on a susceptible hamster. However, an inoculated *Ae. aegypti* mosquito in this study did transmit RVFV by bite, and orally exposed and inoculated *Ae. aegypti* and *Cx. quinquefasciatus* have been shown to be able to transmit RVFV (McIntosh et al. 1980, Turell and Bailey 1987, Turell and Kay 1998). Therefore, there does not seem to be an absolute salivary gland barrier in either of these species. However, previous studies (McIntosh et al. 1980, Turell and Bailey 1987, Gargan et al. 1988, Turell et al. 1988a), indicate that although *Aedes* (*Stegomyia*) spp. can become infected and develop a disseminated infection after oral exposure to RVFV, these species tend to be inefficient vectors due to a salivary gland barrier (Kramer et al. 1981). In our study, although the *Aedes* species tested were highly susceptible to infection and virus dissemination, these species were generally inefficient vectors due to a salivary gland barrier, with  $\leq 21\%$  of *Ae. aegypti*, *Ae. calceatus*, *Ae. circumluteolus*, and *Ae. mcintoshi* successfully transmitting RVFV by bite. However, virtually all of the *Cx. antennatus* and *Cx. pipiens* with a disseminated infection that fed on a susceptible hamster transmitted RVFV by bite. In addition to the two *Culex* species examined in the current study, other studies report essentially a lack of a salivary gland barrier in *Culex zombaensis* Theobald, *Culex tarsalis* Coquillett, and *Culex annulirostris* Skuse (Gargan et al. 1988; Turell and Kay 1998; M.J.T., unpublished data). Similarly, for most *Aedes* species, transmission rates for mosquitoes with a disseminated infection have generally been  $< 50\%$ . In addition to the ones in the current study, these include *Aedes albopictus* (Say), *Aedes canadensis* (Theobald), *Aedes triseriatus* (Say), *Aedes vexans* (Meigen), *Aedes sollicitans* (Walker), *Aedes taeniorhynchus* (Wiedemann), *Aedes fowleri* (Charmoy), *Aedes juppi* McIntosh, *Aedes caballus* (Theobald), *Aedes cantator* (Coquil-

Table 6. Lack of vertical transmission of RVFV by mosquitoes

| Species                    | Stage tested     |       |      |        | Totals |
|----------------------------|------------------|-------|------|--------|--------|
|                            | Larval           | Pupal | Male | Female |        |
| <i>Ae. aegypti</i>         | 495 <sup>a</sup> | 0     | 148  | 116    | 759    |
| <i>Ae. circumluteolus</i>  | 0                | 0     | 55   | 102    | 157    |
| <i>Ae. mcintoshi</i>       | 0                | 23    | 539  | 379    | 942    |
| <i>Er. quinquevittatus</i> | 728              | 0     | 334  | 218    | 1,280  |

<sup>a</sup> Number of progeny (by stage) of RVFV-infected mosquitoes tested.

lett), and *Aedes excrucians* (Walker) (McIntosh et al. 1980; Gargan et al. 1988; Jupp and Cornel 1988; Turell and Bailey 1987; Turell et al. 1988a, 1988b). Therefore, these studies suggest that a midgut escape barrier seems to be the principal determinant of vector competence in the *Culex* species, whereas a salivary gland barrier is the principal determinant in the *Aedes* species.

As mosquitoes were exposed to higher viral doses, not only were infection rates generally higher but also the percentage of infected individuals that developed a disseminated infection increased. Therefore, the midgut escape barrier seemed to be dose dependent, independent of the infection rate. Similar findings also have been reported for other mosquitoes exposed to RVFV (Turell et al. 1988b).

We examined the relationship between the amount of virus recovered from a mosquito and the various barriers. As expected, for each species, mosquitoes with a disseminated infection had significantly more virus than members of the same species without a disseminated infection. For most species, those with a disseminated infection contained at least 100- to 1,000-fold more virus than their infected, but nondisseminated, cage mates. In contrast, we did not find a difference between the titers of mosquitoes with a disseminated infection that did or did not transmit virus by bite. Therefore, although total body titer was an excellent predictor of virus dissemination beyond the midgut, it had no predictive value to determine which mosquito with a disseminated infection would be able to transmit virus by bite.

For each species tested, the transmission rate for mosquitoes with a disseminated infection after oral exposure was not significantly different ( $\chi^2 \leq 2.54$ ,  $df = 1$ ,  $P \geq 0.11$ ) from that in those with a disseminated infection after intrathoracic inoculation. This allowed us to use animals more efficiently to obtain data about a possible salivary gland barrier in these species because all of the inoculated specimens were known to have a disseminated infection, and feeding success was greater in those specimens that did not have to take an "infectious" bloodmeal before the transmission attempt. A similar lack of differences in the transmission rates for mosquitoes with a disseminated infection after oral exposure to RVFV compared with those inoculated with this virus also has been reported for *Ae. albopictus*, *Ae. fowleri*, *Aedes caspius* (Pallas), *Anopheles pharoensis* Theobald, and *Culex perexiguus* Theobald (Turell et al. 1988a, 1988b, 1996). This has allowed us to calculate an estimated transmission rate (i.e., percentage of mosquitoes with a disseminated infection that transmit virus by bite multiplied by the percentages of mosquitoes that develop a disseminated infection after oral exposure) that should be an accurate estimate of the vector competence of that particular mosquito species. Our estimated transmission rates for those mosquito species that successfully transmitted RVFV by bite and fed on a hamster with a viremia  $\geq 10^8$  PFU of virus/ml ranged from 39% for *Ae. palpalis* to 5% for *Ae. mcintoshi*. Our "high" dose in this study,  $\geq 10^8$  PFU/ml is consistent with viremias

determined for natural infections with RVFV, where viremias in lambs and calves were up to  $10^{10.2}$  and  $10^{9.2}$  mouse intracranial LD<sub>50</sub>, respectively (Easterday 1965, McIntosh et al. 1973a), and viremias in humans were up to  $10^{8.6}$  mouse intracranial LD<sub>50</sub> (Meegan 1979). Therefore, the results obtained in our study should apply to these mosquito species when exposed to RVFV-infected cattle or sheep in a natural outbreak of RVF.

We did not recover RVFV from any of 3,138 progeny of infected female mosquitoes. This is consistent with the results of several previous studies that also failed to find laboratory confirmation of vertical transmission of RVFV (McIntosh et al. 1980, Jupp and Cornel 1988, Turell et al. 1988b). However, isolating RVFV from both male and female *Ae. mcintoshi* [reported as *Aedes lineatopennis* (Ludlow)] reared from field-collected larvae (Linthicum et al. 1985) clearly demonstrates that vertical transmission of this virus can occur under natural conditions. Additional studies are needed to further evaluate the potential for various mosquito species to maintain this virus vertically. Various studies have isolated RVFV from a number of mosquito species (Meegan and Bailey 1988). These studies include detection of RVFV from *Ae. mcintoshi* (as *Ae. lineatopennis*) (McIntosh 1972, Linthicum et al. 1985), *Ae. circumluteolus* (Kokernot et al. 1957), *Ae. palpalis* (Meegan and Bailey 1988), *Cx. antennatus* (Lee 1979), and *Cx. pipiens* (Meegan et al. 1980).

The recent introduction and spread of West Nile virus into the Americas and the spread of RVFV to the Arabian Peninsula illustrates the potential for viruses, once enzootic in Africa, to spread to other parts of the world. Additional studies are needed to evaluate other potential vectors of RVFV and to determine the role of other factors (e.g., environmental temperature) on the transmission of this pathogen.

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