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SCIENTIFIC NOTE

POTENTIAL FOR STABLE FLIES AND HOUSE FLIES (DIPTERA: MUSCIDAE) TO TRANSMIT RIFT VALLEY FEVER VIRUS[1]

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ABSTRACT. Rift Valley fever (RVF), a disease of ruminants and humans, has been responsible for large outbreaks in Africa that have resulted in hundreds of thousands of human infections and major economic disruption due to loss of livestock and to trade restrictions. As indicated by the rapid spread of West Nile viral activity across North America since its discovery in 1999 and the rapid and widespread movement of chikungunya virus from Africa throughout the Indian Ocean Islands to Asia and Europe, an introduced exotic arbovirus can be rapidly and widely established across wide geographical regions. Although RVF virus (RVFV) is normally transmitted by mosquitoes, we wanted to determine the potential for this virus to replicate in 2 of the most globally distributed and common higher flies: house flies, *Musca domestica*, and stable flies, *Stomoxys calcitrans*. Neither species supported the replication of RVFV, even after intrathoracic inoculation. However, *S. calcitrans* was able to mechanically transmit RVFV to susceptible hamsters (*Mesocricetus auratus*) after probing on infected hamsters with high viral titers. Therefore, *S. calcitrans*, because of its close association with domestic animals that serve as amplifying hosts of RVFV, should be considered a possible mechanical vector of RVFV, and it may contribute to the rapid spread of a RVF outbreak. Other *Stomoxys* species present in Africa and elsewhere may also play similar roles.

KEY WORDS RVF, vector, mechanical transmission, North America, emerging disease potential

The introduction of West Nile virus into the United States in 1999 and its subsequent spread across North America, as well as the emergence of chikungunya virus in Africa, the Indian Ocean Islands, and much of southern Asia and Europe, illustrate the potential for exotic arboviruses to be introduced and become established in new regions of the world and to cause significant disease and economic disruption. Of particular concern is Rift Valley fever virus (RVFV) (genus *Phlebovirus*, family *Bunyaviridae*), which has been responsible for numerous outbreaks of severe disease in ruminants and humans in Africa over the past 80 years (Meegan and Bailey 1988, Gerdes 2004). 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 industries (Laughlin et al. 1979, Meegan 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 substantial concerns regarding the agricultural and medical impact that this zoonotic disease agent might have if it were to continue to spread (House et al. 1992). Recent RVFV activity has continued from 2006 to the present in various countries in Africa (Anyamba et al. 2009).

Although most other members of the genus *Phlebovirus* are associated with sand flies in nature, RVFV has been associated almost exclusively with mosquitoes, with the virus isolated from at least 40 species in 8 genera (Meegan and Bailey 1988, Fontenille et al. 1998). Laboratory studies indicate that numerous species of mosquitoes are susceptible to oral infection and are able to transmit RVFV by bite (McIntosh et al. 1973b, 1980, 1983; Meegan and Bailey 1988, Turell et al. 1996, 2007, 2008b), including some present in the United States (Gargan et al. 1988, Turell et al. 2008a, 2010). However, the potential for higher Diptera, e.g., house flies, *Musca domestica* L., and stable flies, *Stomoxys calcitrans* (L.), both of which are cosmopolitan in their distribution, has not been examined. Therefore, we evaluated the potential for these flies to become infected with and transmit RVFV after intrathoracic inoculation and for *S. calcitrans* to mechanically transmit RVFV after taking a partial blood meal on a viremic hamster (*Mesocricetus auratus* Waterhouse). RVFV is a select

[1] Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

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agent, and work with animals requires an agricultural biological safety level (ABSL)-3 facility with vaccination or a biological safety level (BSL)-4 facility.

Flies were provided as pupae from the US Department of Agriculture—Center for Medical Agricultural and Veterinary Entomology (CMAVE) and were from long-established colonies that were maintained by standard methods and rearing media (Hogsette 1992). Flies were allowed to emerge as adults at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) and provided a mixture of milk, sugar, and powdered egg (*M. domestica*) or a gauze pledget soaked in Gatorade (*S. calcitrans*) as a food source. Emerged adults were transferred to an ABSL-3 laboratory at USAMRIID and then exposed to RVFV.

To determine whether RVFV could replicate in either of these species, we inoculated individual flies intrathoracically (Rosen and Gubler 1974) with about 1 µl of the ZH501strain of RVFV. This strain was isolated in 1977 from the blood of a 10-year-old Egyptian girl who had a fatal RVFV infection (Meagan 1979) and passed twice in fetal rhesus monkey (*Macaca mulatta* Zimmerman) lung cells and once in Vero (African green monkey [*Chlorocebus sabaeus* L.] kidney) cells before use in this study. The inoculum contained about $10^{5.5}$ plaque-forming units (PFU)/ml ($10^{2.5}$ PFU/fly) of virus, and flies were placed in 0.9-liter cardboard cages in an incubator maintained at 27°C immediately after inoculation. House flies were triturated individually 7 days later in 1 ml of diluent (10% heat-inactivated fetal bovine serum in Medium 199 with Earle's salts [Invitrogen Inc., Carlsbad, CA] and antibiotics) and then 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 6- or 12-well plates as described by Gargan et al. (1983). Viral titers were expressed as $\log_{10}$ PFU per specimen. Stable flies were allowed to feed either individually, or in small groups, on naïve adult female Syrian hamsters (Harlan Sprague Dawley, Indianapolis, IN) 7 days after inoculation. Immediately after the feeding attempt, the flies were killed by freezing and triturated individually in 1 ml of diluent and then frozen at −70°C until tested for infectious virus as described above. Hamsters were observed for 21 days for evidence of infection.

In addition to the studies to determine whether RVFV could replicate in house or stable flies, we also determined the ability of a group of uninoculated stable flies to mechanically transmit RVFV from a viremic hamster to naïve ones. Hamsters were inoculated intraperitoneally with $10^{4.2}$ PFU in 0.1 ml of diluent and anesthetized with a ketamine, xylazine, and acepromazine suspension either 28 or 40 h later, when virus titers were $10^{6.9}$ and $10^{9.7}$ PFU/ml. Groups of uninfected stable flies were allowed to feed on the anesthetized hamsters. To simulate an interrupted feeding scenario, when we observed a stable fly that had probed for 15–30 sec, we used an aspirator to capture it and placed it in another cage. About 20 min later, we placed an anesthetized, naïve hamster in a 3.8-liter cage with screening over the top and added one of the "virus-exposed" stable flies to that cage and allowed it to continue feeding. A total of 13 flies were tested in this manner using 7 from the high- and 6 from the low-titer donor hamsters, respectively. After the stable flies had fed on the high-titered hamster and on the naïve hamsters we froze them immediately, triturated their proboscises and bodies separately in 1 ml of diluent, and froze these suspensions at −70°C until tested for infectious virus as above. The hamsters were observed daily. Because RVFV is virtually 100% fatal in hamsters, death, or euthanasia if moribund, of these hamsters was considered evidence of 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).

Table 1. Lack of replication in stable flies and house flies inoculated with $10^{2.2}$ plaque-forming units of Rift Valley fever virus.

Species	Number tested	Infection rate[1]
Stomoxys calcitrans	13	0
Musca domestica	8	0

[1] Percentage of the inoculated flies that contained infectious virus 7 days after inoculation.

None of the flies contained infectious RVFV when tested 7 days after intrathoracic inoculation (Table 1), and none of the 17 inoculated stable flies that fed on a hamster 7 days after inoculation transmitted virus by bite. Therefore, neither species of fly was able to support replication of RVFV, and neither species would be able to transmit RVFV biologically.

When allowed to probe on a hamster with a viremia of $10^{6.9}$ PFU/ml, none of the *S. calcitrans* transmitted virus mechanically; however, when initially exposed to a hamster with a viremia of $10^{9.7}$ PFU/ml, 4 of 7 (57%) flies transmitted the virus mechanically (Table 2). This confirms an

Table 2. Mechanical transmission of Rift Valley fever virus replication by stable flies.

Viremia in donor hamster	Transmission to recipient hamsters	
	Number tested	Transmission rate[1]
$10^{6.9}$ PFU/ml	6	0
$10^{9.7}$ PFU/ml	7	57

[1] Percentage of the exposed flies that mechanically transmitted Rift Valley fever virus.

earlier study by Hoch et al. (1985) that had demonstrated that stable flies and mosquitoes were able to mechanically transmit RVFV and that this transmission was dependant on the viremia of the initial infected animal. Because viremias in lambs and calves are as high as $10^{10.2}$ and $10^{9.2}$ mouse intracranial LD_{50}, respectively (McIntosh et al. 1973a), stable flies should be able to mechanically transmit virus if exposed to RVFV-infected lambs or calves in a natural outbreak of RVF. When tested after feeding on a naïve hamster about 20 min after they had probed on the hamster with a viremia of $10^{9.7}$ PFU/ml, we recovered RVFV from the bodies of about half of the flies. However there was no correlation between the amounts of virus detected, or even the presence of virus in the fly's body and mechanical transmission. Similarly, we only detected RVFV (1 PFU detected) from a single proboscis sample, and that was from a nontransmitting fly.

Even though they are not susceptible to infection and thus unable to transmit virus biologically, stable flies may play an important role in the explosive amplification of a RVFV outbreak. Rift Valley fever virus is extremely stable, and infectious titers remained nearly constant, even in samples held at ambient temperature (about 22°C) for 24 h (MJT, unpublished data). Similarly, stable fly bites are painful and often lead to the fly being unable to obtain a complete blood meal. This leads to interrupted feedings and greatly increases the likelihood of mechanical transmission. There have been 14 species of stable flies identified in Africa (Mihok et al. 1995), and populations of 200 *Stomoxys nigra* Macquart per cow have been recorded in Mauritius (Kunz and Monty 1976); populations of between 50 and 200 *S. calcitrans* per cow have been observed in the United States, Europe, South America, and Australia (JAH, unpublished data). Therefore, consideration should be given to control of these mechanical vectors of RVFV.

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