Establishing a safe and effective model system for evaluating the Rift Valley Fever vector competence of North American mosquitoes

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Introduction

- Rift Valley Fever (RVF) is a viral zoonotic disease with a very broad host range. Native to Africa, periodic RVF epidemics kill large numbers of domesticated livestock animals and cause potentially fatal disease in humans.[1]
- The RVF virus can be transmitted by contact with body fluids of infected animals. Mosquitoes can also transmit the virus during blood feeding, and may also play a key role in ecology of the RVF virus by serving as a natural reservoir of the pathogen in the environment.[1]
- Due to the potential impact on livestock industries and human health resuting from an inadvertent or intentional introduction of the virus into North America (NA), RFV represents a potential agent of bioterrorism which may pose a threat to our nation's health and economy.
- Some African mosquito species that are known to vector the RVF virus are also native to NA. However, the ability of most of the over 170 NA mosquito species/subspecies to transmit the virus is not known.
- Vector competence research involving the use of virulent strains of RVF virus in mosquitoes must be carried out in BSL4 facilities with the highest levels of biological containment available. With as few as three such facilities available in the US, a bottleneck to progress thus exists in regards to assessing the bioterrorism threat of RVF in NA.
- Studies of African vectors of RVF virus have shown that MP-12, an attenuated vaccine strain of the RFV virus, is transmitted in a manner similar to virulent strains of RVF virus. MP-12, a BSL2 agent, has been used safely in humans and a variety of livestock animals.[2]
- We will use MP-12 to carry out safe and effective vector competence studies of NA mosquitoes. The establishment of a BSL2 model system for vector competence studies will increase the number of researchers that can contribute meaningful biologically relevant data for the assessment of the risk of RVF as an agent of bioterrorism in NA.

Materials and methods

We will examine both vertical and horizontal transmission of the RVF virus in nine species of naturally occurring species of NA mosquitoes -- eight native species and one introduced species. Horizontal and vertical transmission are represented in Figure 1.

The species used in our study were selected based on phylogenetic relatedness to known African vectors of the RVF virus. The species selected are as follows:
Culex quinquefasciatus, Cx. tarsalis, Aedes aegypti, Ae. dorsalis, Ae. melanimon, Ae. triseriatus, Ae. atropalpus, and Ae. epactius, and *Ae. albopictus* (Asian)

Horizontal transmission occurs during blood feeding. Experiments examining horizontal transmission will evaluate both acquisition of the virus by a naive mosquito via an infected blood meal and transmission of the virus during blood feeding to a naive host by an infected mosquito.

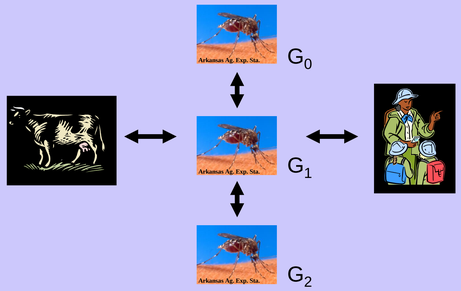

Figure 1. Horizontal transmission of a zoonotic pathogen like RVF virus can consist of transmission between individuals within a given species and/or between members of different species, as depicted. Vertical transmission comprises transmission to offspring during egg production, and is also known as transovarial (TO) transmission, as the virus must pass from the ovarian tissue of the female to the developing embryo. While infection of mammalian hosts occurs as a result of horizontal transmission, vertical transmission can allow the virus to persist in the environment during periods when no epidemic activity exists[3,4], an important consideration in the assessment of risks and countermeasures to RVF in NA.

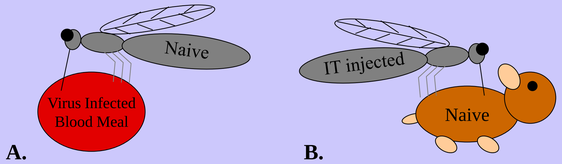

Figure 2. Horizontal transmission will be evaluated in two separate sets of experiments. **A.** Oral acquisition of virus will be evaluated by feeding an artificial blood meal consisting of a sheep's blood and MP-12 mixture. Mosquito heads will be analyzed for the presence of virus using reverse transcription-polymerase chain reaction (RT-PCR) at 14 days post blood feeding. The presence of MP-12 RNA in the heads will serve as evidence of viral dissemination. **B.** Transmission of virus from mosquito to host will be evaluated by allowing female mosquitoes previously infected via intrathoracic (IT) injection of MP-12 tissue culture supernatant to feed on newborn Syrian hamsters (< 1 week old), the most susceptible mammalian host known. Brain tissue of hamsters showing signs of illness will be analyzed for the presence of MP-12 RNA using RT-PCR. The presence of MP-12 RNA in the brains of hamsters fed upon by infected mosquitoes will serve as evidence of horizontal transmission of the virus to the mammalian host during blood feeding. Combination experiments will be performed for all species found to be capable of both oral acquisition and transmission to the mammalian host via blood feeding in order to investigate whether salivary gland barriers exist in those species that may act to prevent natural horizontal transmission.

Vertical transmission of MP-12 will be evaluated by providing an artificial blood meal to female mosquitoes previously infected by IT injection of MP-12. A blood meal is necessary for female mosquitoes to produce eggs. Eggs will be collected from IT infected females and discarded. Artificial blood feeding and egg collection will be repeated for the G_0 adults, and the G_1 offspring will be reared to adulthood. Artificial blood feeding and egg collection will be repeated again for G_1 to obtain an additional subsequent generation, G_2. 50% of the resulting G_1 and G_2 adult females will be analyzed in pools of five each using RT-PCR to detect the presence of MP-12 RNA. The presence of MP-12 RNA in the bodies of carcasses of G_1 and G_2 adult females will serve as evidence of TO transmission of MP-12 in G_0 females (see Figure 1).

Preliminary Results

Preliminary studies performed during our Summer Team Project demonstrated that MP-12 RNA could be detected in IT infected *Ae. aegypti* females using RT-PCR with a variety of primers (Figure 3A). MP-12 RNA could also be detected in the carcass and salivary glands of orally infected *Cx. quinquefasciatus* females at 14 days post infection from pools of glands from 20 females using RT-PCR (Figure 3B).

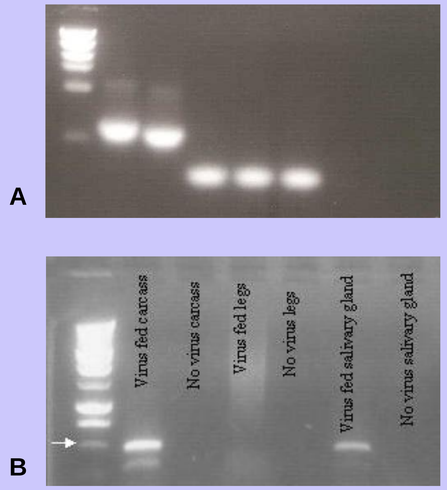

Figure 3. The MP-12 virus was detected in the bodies of IT and orally infected mosquitoes by RT-PCR. **A.** Single mosquito samples of *Ae. aegypti* females that were IT injected 14 days previously with MP-12 tissue culture supernatant (titer = 10^7) were analyzed by RT-PCR for the presence of MP-12 genomic RNA in the carcasses using three different primer sets for PCR amplification (lanes 2-4 for primer sets A-C respectively). MP-12 RNA was also detected in the carcasses of *Cx. quinquefasciatus* females at 14 days post IT infection (lane 5). Positive control (lane 6) is RT-PCR product from MP-12 RNA isolated from tissue culture supernatant. Negative controls for primer set C (used in lanes 4-6) were performed for uninfected *Ae. aegypti* and *Cx. quinquefasciatus* (lanes 7 and 8 respectively). **B.** MP-12 RNA was detected by RT-PCR using PCR primer set #3 in the carcasses (lane 2) and salivary glands (lane 6) of *Cx. quinquefasciatus* females that fed upon a mixture of sheep's blood and MP-12 tissue culture supernatant. Viral RNA could not be detected in the legs of infected females (lane 4), and no virus could be detected in the carcasses, legs, or salivary glands of uninfected females (lanes 3, 5, 7 respectively). Analysis performed 14 day post feeding.

Conclusions

- The detection of viral RNA in the salivary glands of orally infected mosquitoes using RT-PCR demonstrates the high degree of sensitivity with which results can be obtained using this technique, and support its use as a diagnostic tool to determine viral infection in mosquitoes.
- Preliminary data demonstrate that a viremic artificial blood meal administered at the virus titers used can provide an amount of virus particles sufficient to result in a disseminated infection in mosquitoes. Thus, data from preliminary oral infection study using MP-12 support the use of such an experimental approach to assess the ability of other mosquito species to acquire RVF virus as a result of blood feeding as would occur during natural horizontal transmission.
- The persistence of MP-12 RNA in the carcasses of mosquitoes at 14 days post IT infection demonstrates the high efficiency of IT injection of MP-12 tissue culture supernatant as a means of artificially inducing a disseminated MP-12 infection in mosquitoes for the purpose of evaluating the ability of mosquitoes to transmit RVF virus to a mammalian host during blood feeding as would occur in natural horizontal transmission.
- The persistence of MP-12 RNA in the carcasses of IT infected mosquitoes also supports the use of IT injection to artificially induce virus dissemination for the purpose of evaluating the ability of mosquitoes to vertically transmit RVF virus via TO transmission to developing embryos.
- Data from vector competence studies of NA mosquitoes using the MP-12 model system described herein will aid BSL4 facilities in establishing taxonomic and geographical priorities for further testing using virulent strains of RVF virus based on the known geographic ranges of the mosquitoes evaluated combined with other available data, such as human or livestock population density, proximity to major routes of transportation, and potential access to such areas by individuals intent on acts of bioterrorism.

Literature Cited

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