PREDICTING THE INTRODUCTION AND TRANSMISSION OF RIFT VALLEY FEVER VIRUS IN THE UNITED STATES

A Thesis

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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December 2014

Major Subject: Entomology

ABSTRACT

Rift Valley fever virus (RVFV) is a mosquito-borne virus in the family *Bunyaviridae* that has spread throughout continental Africa to Madagascar and the Arabian Peninsula. The establishment of RVFV in North America would have serious consequences for human and animal health in addition to a significant economic impact on the livestock industry. Specific objectives of this thesis are to identify high-risk regions involved in RVFV importation to the U.S., evaluate pathways of introduction, and theoretically quantify the relative importance of local vectors and vertebrate hosts to RVFV transmission should the virus reach the U.S.

To estimate the relative risk of RVFV introduction to the U.S., the number of infectious mosquitoes arriving in the U.S. was quantified for five pathways: infected mosquitoes arriving by airplane, infected mosquitoes arriving by boat, infected mosquitoes arriving through tire trade, infected humans arriving by flight, and the trade of infected mammals. Results suggest that mosquito transport by airplane, mosquito transport by ship, and human travel are important pathways for RVFV introduction to the U.S. New York, Houston, Washington D.C., and Atlanta are high-risk regions for RVFV introduction in the U.S. Further, Saudi Arabia, South Africa, Nigeria, Egypt, Senegal, Ethiopia, Yemen and Angola are identified as regions at-risk for importing RVFV to the U.S.

Published and unpublished data on RVFV vector competence, vertebrate host competence, and mosquito feeding patterns from the United States were combined to

quantitatively implicate mosquito vectors and vertebrate hosts that may be important to RVFV transmission in the United States. A viremia-vector competence relationship based on published mosquito transmission studies was used to calculate a vertebrate host competence index which was then combined with mosquito blood feeding patterns to approximate the relative contribution of a mosquito or vertebrate host to RVFV transmission. Results implicate several *Aedes* spp. mosquitoes and vertebrates in the order Artiodactyla as important hosts for RVFV transmission in the U.S. Moreover, this study identifies critical gaps in knowledge necessary to comprehensively evaluate the different contributions of mosquitoes and vertebrates to potential RVFV transmission in the U.S.

DEDICATION

I dedicate this work to my family and friends who are tremendously supportive and patient while I continue to piece together my understanding of life, death and everything in-between.

ACKNOWLEDGEMENTS

First and foremost, I want to thank my committee for their guidance and support. I want to thank my committee chair, Dr. Gabe Hamer, for his perpetual enthusiasm and expertise in the field of disease ecology. I want to thank all the members of the Texas A&M Hamer lab for their availability, constructive feedback, and enthusiasm for disease ecology. I thank Dr. Gabe Hamer, Dr. Michael Turell, Dr. Desiree LaBeaud, and Dr. Rebekah Kading for their time and expertise to develop and publish Chapter III. I want to thank Samantha Casas for consolidating data from published literature and Dr. John Morrill for providing unpublished data. I want to thank the National Science Foundation Graduate Research Fellowship program #1252521 and the National Science Foundation/National Institute of Health Ecology of Infectious Disease program #EF-0840403 for funding. Last of all, I want to thank my family and friends who help keep what matters most in perspective.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Globalization and the movement of people and goods worldwide is reshaping global ecosystems and facilitating the spread of pathogens (Tatem and Tatem 2006, Hatcher et al. 2012). With the discovery of 100 new viral diseases in the past 30 years, pathogen dispersal is moving to the forefront of the public health arena (Daszak et al. 2000, Gubler 2002, Apperson et al. 2004). Biological invasions are associated with a variety of adverse affects and are often irreversible once established (Simberloff 2005). For this reason, it is important to take proactive approaches to prevent pathogen introduction. The objective of this thesis is to quantitatively evaluate important transmission hosts and routes of introduction to the United States for Rift Valley fever virus (RVFV), a mosquito-borne virus (arbovirus) recognized as a potential threat to the United States (U.S.) due to its effect on human and animal health and demonstrated ability to spread geographically.

The invasion of the new world by West Nile virus (WNV) demonstrates that developed nations such as the United States (U.S.) are vulnerable to zoonotic diseases. Once WNV was introduced to New York in 1999, it quickly spread through the contiguous U.S. resulting in more than 30,000 cases of human illness and over 1,500 deaths (CDC 2014b). During the initial epidemics of WNV in the U.S. in 2002 and 2003, many mosquito control programs did not have a strong focus on *Culex* spp. mosquitoes. As knowledge of the WNV transmission system increased, vector control

has improved by targeting *Culex* species to reduce human exposure events. The delay of *Culex* spp. vector control might have allowed more human WNV disease and may have contributed to the rapid spread of the virus across the U.S. highlighting the importance of *a priori* response strategies for potential viral threats.

Rift Valley fever virus (RVFV) is an emerging infectious disease in Africa and the Middle East. If introduced to North America, RVFV is capable of serious health and socioeconomic consequences potentially incapacitating large numbers of humans, susceptible farm animals, and instigating heavy restrictions on livestock trade (Weaver and Reisen 2010, Hartley et al. 2011). Although transmission of the virus can occur through aerosol inhalation or direct tissue-tissue contact by handling of infected organisms, an enzootic cycle between mosquito vectors and domestic or wild animals has been repeatedly proposed as a main mechanism of transmission (Meegan and Bailey 1988).

RVFV was first reported in Kenya in 1931. It spread to Egypt in 1977 and was detected on the Arabian Peninsula in 2000 (Meegan 1979, Fagbo 2002). As the frequency of international travel and trade rises the importation of RVFV infected hosts is likely to increase. It remains to be determined which regions in the U.S. are most at risk for RVFV introduction ultimately hindering the development of appropriate introduction prevention and response strategies (Hartley et al. 2011).

The emergence of arthropod-borne viruses (arboviruses) through geographic expansion is facilitated when amplification hosts include wild or domestic animals, as demonstrated by West Nile virus (WNV), Japanese encephalitis, and epizootic

hemorrhagic disease (Weaver 2005, Weaver and Reisen 2010). Even though RVFV is identified as an emerging infectious disease threat and is classified as a "Category A select agent" by both the Centers for Disease Control and Prevention and the U.S. Department of Agriculture, gaps in data are preventing a proper evaluation of the different roles vectors and vertebrate hosts that potentially may play in RVFV transmission in the U.S. (Hartley et al. 2011, Rolin et al. 2013). Although significant progress is being made with the development of animal vaccines for RVFV, vaccine programs targeting domestic animals might not be sufficient to break the transmission cycle of RVFV in the U.S. if wild animals are responsible for maintaining and amplifying the virus (Kakani et al. 2010, Hartley et al. 2011, Rolin et al. 2013).

In anticipation of continued pathogen emergence, proactive management plans and intervention strategies need detailed information on the regions in the U.S. at risk for RVFV introduction, the key pathways likely to be involved in RVFV introduction, and an understanding of the local vector and host populations in high risk regions. The invasion process is often difficult to foresee, as it is a complex of demographic, evolutionary, and environmental factors. A number of reviews discuss potential vertebrate hosts, disease vectors, and environments potentially conducive to RVFV transmission in the U.S., but none have quantitatively evaluated the relative risk of different introduction pathways into the U.S. or quantitatively evaluated the theoretical importance of different mosquito species and vertebrate hosts to RVFV transmission and amplification in the U.S. (Kasari et al. 2008, Hartley et al. 2011, Barker et al. 2013, Rolin et al. 2013, Golnar et al. 2014).

CHAPTER II

QUANTIFYING PATHWAYS OF RIFT VALLEY FEVER VIRUS INTRODUCTION TO THE UNITED STATES

Introduction

Globalization and the movement of people and goods worldwide is reshaping global ecosystems and facilitating the spread of pathogens. (Tatem and Tatem 2006, Hatcher et al. 2012). The invasion of West Nile virus (WNV) to the United States (U.S.) in 1999, outbreak of monkeypox virus in the Midwest in 2003, and spread of chikungunya to the Caribbean in 2013 underscore the continual threat of pathogen dispersal in even in developed countries such as the U.S. (CDC 2003, Kilpatrick 2011, Powers 2014).

Importation of invasive mosquito species has resulted in dramatic epidemiological consequences. The spread of *Aedes aegypti* to the new world aboard slave trade ships arriving from Africa in the early fifteenth century is an infamous example of mosquito importation. Vector arrival enhanced Yellow Fever virus transmission, which significantly increased mortality in urban areas (Lounibos 2002). The introduction of the more aggressive and anthropophilic malaria vector, *Anopheles gambiae,* to Brazil in 1930 is an equally notorious event which increased malaria transmission up to 25% (Lounibos 2002). More contemporary examples of mosquito import include *Aedes albopictus*, the Asian tiger mosquito, which has spread to the U.S. and 28 other countries through the shipment of car tires (Craven et al. 1988, Benedict et

al. 2007). Similarly, *Aedes japonicus japonicus* arrived to the U.S. through tire imports in 1998 and quickly established throughout the U.S. and Hawaii (Lounibos 2002, Kaufman et al. 2012). Although the shipping network has been implicated as a means for vector dispersal since the 15th century and the unintentional transport of mosquitoes through aerial transport was recognized as early as the 1930s, detailed records describing vector invasions throughout the world remain sparse. Subsequently, rates of mosquito importation and the pathogens they harbor remain undetermined (Griffitts and Griffitts 1931, Lounibos 2002).

The spread of WNV to New York in 1999 and the recent discovery of the Australian mosquito, *Aedes notoscriptus,* in California, August 2014, demonstrates that mosquito invasion in the U.S. is frequent (ProMed-mail 2014). In the last decade, chikungunya virus, a forest dwelling virus maintained among *Aedes* species mosquitoes and non-human primates, spread beyond its historical boundaries in Central, East and South Africa to the Oceana region reiterating the ability for vector-borne diseases to spread through traveling humans (Powers 2014). In 2007 the virus was imported to Italy through an infected human. In December of 2013, local transmission was recorded in the Caribbean, even after preventative measures were taken in Latin America under the guidance of the Center for Disease Control and Prevention (CDC) (Omarjee et al. 2014, Powers 2014). Biological invasions are associated with a variety of adverse affects and are often irreversible once established (Simberloff 2005). For this reason, risk assessments have been adopted internationally to guide human activities by

characterizing the hazard non-native animals pose to ecological systems and evaluating how certain practices modify rates of exposure (Simberloff 2005).

Rift Valley fever virus (RVFV) is an emerging mosquito-borne disease in Africa and the Middle East that adversely affects livestock production and human health. Due to its potential effects to both human and animal health RVFV is listed as a select agent by the U.S. Department of Health and Human Services and considered a foreign arthropod-borne animal disease threat by the U.S. Department of Agriculture (Hartley et al. 2011). Like WNV, RVFV is primarily transmitted through the bite of infected mosquitoes and utilizes wild and domestic animals as amplification hosts. RVFV has already spread from Africa to the Arabian Peninsula and is following a similar global expansion as WNV, which was first described in Uganda and is now the most widely distributed arthropod-borne virus in the world (Bird et al. 2009). RVFV has been isolated from at least 40 mosquito species (Turell et al. 2008b) and can be transmitted by at least six different genera (Turell et al. 2002). Once infected with RVFV, mosquitoes can remain infected for more than 30 days in the laboratory (Turell et al. 1985) demonstrating an innate ability to import RVFV into the U.S. Certain species of floodwater mosquitoes, like *Aedes mcintoshi,* are known to maintain RVFV between RVFV epidemics by infecting their offspring through a process of transovarial transmission (Linthicum et al. 1985). Subsequently, all life-stages of mosquitoes are implicated as potential vehicles of RVFV introduction. As the frequency of international travel and trade rises, the importation of mosquito vectors is likely to increase making the spread of RVFV to the U.S. via an infected mosquito a growing threat.

Mosquito borne viruses (arboviruses) are largely zoonotic because they depend on other animal hosts to maintain the virus in nature while humans are often incidental or dead-end hosts (Gubler 2002). However, the arboviruses that tend to cause the largest public health impact are those that produce a viremia in humans (chikungunya, Dengue, Yellow fever) (Gubler 2002). Dengue virus spread around the world in the nineteenth century through the expanding shipping network and is unique among arboviruses because it does not require an animal reservoir host and is completely adapted to an urban transmission cycle among humans (Gubler 2002, Jones et al. 2008). RVFV is mainly associated with domestic and peri-domestic animals such as goats, sheep, and cattle, therefore activity mainly occurs in rural regions and not urban centers (Rolin et al. 2013). The importation of RVFV infected ruminants to the U.S. is generally assumed to be low because trade bans to prevent the spread of foot-and-mouth disease already restrict trade from many countries with endemic RVFV (Rolin et al. 2013). The role humans may play as amplification hosts largely has been considered low (Chevalier et al. 2010). However, if humans are not dead end hosts and can produce an infectious viremia, their capacity to spread RVFV around the globe is likely high considering the ease and frequency of modern international travel (Hartley et al. 2011).

Although significant progress is being made with the development of animal vaccines for RVFV, vaccine programs targeting domestic animals might not be sufficient to break the transmission cycle of RVFV in the U.S. if wild animals are responsible for maintaining and amplifying the virus (Kakani et al. 2010, Hartley et al. 2011, Rolin et al. 2013). Should RVFV arrive, diagnosing the disease and controlling

the spread of infected mosquitoes and vertebrates will take time, therefore, proactive management plans should be created to minimize the time to react and break transmission of the pathogen. Currently the information on high-risk regions for RVFV introduction is underdeveloped hindering the ability for vector control to properly prepare for an introduction scenario of RVFV as mosquito populations vary geographically (Hartley et al. 2011). The invasion process is often difficult to foresee, as it is a complex of demographic, evolutionary, and environmental factors. Measuring propagule pressure, which is directly related to the frequency of invasion, can provide important information on how to reduce introduction events by identifying high-risk pathways and high-risk regions for introduction (Simberloff 2005, Kilpatrick 2011, Hatcher et al. 2012).

In anticipation of continued pathogen emergence, the development of proactive management plans and intervention strategies will be more efficient and effective than retrospective plans developed after mosquitoes, and the pathogens they harbor, arrive in the U.S. A number of reviews discuss potential vertebrate hosts, disease vectors, and environments potentially conducive to RVFV transmission in the U.S., but none have quantitatively identified high-risk regions and routes of RVFV introduction to the U.S. (Kasari et al. 2008, Hartley et al. 2011, Barker et al. 2013, Rolin et al. 2013, Golnar et al. 2014). Based on the qualitative discussion by Kasari et al. (2008) the most likely pathway of RVFV introduction to the U.S. is proposed to be through an infected mosquito transported on a plane, similar to the putative pathway of WNV introduction to New York in 1999 (Kasari et al. 2008). Other pathways of RVFV introduction include

the importation of RVFV infected animals, entry of RVFV infected people, the transport of larvae via tire trade and the smuggling of live virus (Kilpatrick et al. 2006c, Kasari et al. 2008, Hartley et al. 2011). The overall goal of this analysis is to quantitatively evaluate routes of RVFV introduction into the U.S. to guide prevention efforts and inform control efforts should the virus arrive. Specific objectives are to quantitatively evaluate (a) pathways for RVFV introduction into the U.S., (b) identify high-risk regions for RVFV introduction events and (c) identify RVFV endemic regions at risk for exporting RVFV to the U.S.

Methods

To estimate the relative risk of different pathways of RVFV introduction to the U.S., the number of infectious mosquitoes arriving in the U.S. for each pathway was quantified. Four pathways were considered: infected mosquitoes arriving by airplane, infected mosquitoes arriving by boat, infected humans arriving by flight, and the trade of infected mammals (Kilpatrick et al. 2006b, Kilpatrick et al. 2006c, Kasari et al. 2008). To calculate the number of infectious mosquito days per year resulting from each pathway the (i) number of mosquitoes arriving to the U.S. each year was multiplied by the (ii) fraction likely to transmit virus and the (iii) length of infectiousness (Kilpatrick et al. 2006b, Kilpatrick et al. 2006c). Because no data exists to properly quantify the number of mosquitoes likely to feed on an infected vertebrate imported to the U.S. the rate was estimated as the product of (a) mosquito biting rate, (b) the fraction of bloodmeals likely to be from a mammalian host, the (c) vector host ratio, and the (d)

duration of mammal infection. The (a) biting rate of mosquitoes was estimated to be once every four days (0.25) (Spielman and d'Antonio 2002). The (b) fraction of bloodmeals likely to be from a mammalian host was estimated to be 0.52 based on data aggregated from 39 mosquito-feeding studies across the United States (Golnar et al. 2014). The (c) vector-host ratio was assumed to be constant for humans and mammalian vertebrates and range between 1 and 4 (Johansson et al. 2012). The (d) duration of mammal infection was estimated to be 4 days, even though it can range between 1-7 days (Golnar et al. 2014). Based on these estimates, the number of mosquitoes biting vertebrates in the U.S. is estimated to be 1.32 per day (Low: 0.52; High: 2.08).

Estimates for mosquito infection rate, vertebrate infection rate, human infection rate, rate of mosquito infestation on planes, rate of mosquito infestation on ships, and infectious mosquitoes resulting from feeding on infected vertebrates were estimated based on data obtained from published studies located using Web of Science, NCBI's Pubmed, and the Armed Forces Pest Management Board Literature Retrieval System. Because data obtained from a variety of published studies across the globe were utilized to estimate parameters for this analysis, low and high-risk estimates dictated by the available data were utilized to estimate high and low introduction scenarios.

Model assumptions

Modeling vector-borne pathogen movement is a complex endeavor in comparison to directly transmitted diseases (Tatem 2014). To simplify this analysis key simplifying assumptions were made to explore the frequency of RVFV introduction to

the U.S.: (1) RVFV is considered endemic and circulating year-round in all countries with recorded RVFV activity as indicated by the Center for Disease Control and Prevention (Angola, Botswana, Burkina Faso, Cameroon, Central African Republic, Chad, Democratic Republic of the Congo, Egypt, Ethiopia, Gabon, Gambia, Guinea, Kenya, Madagascar, Mali, Mauritania, Mozambique, Namibia, Niger, Nigeria, Republic of Congo, Saudi Arabia, Senegal, Somalia, South Africa, South Sudan, Sudan, Tanzania, Uganda, Yemen, Zambia, Zimbabwe) (CDC 2013), (2) the infection rate is homogenous among all mosquito species, (3) humans produce an infectious RVFV viremia comparable to other competent mammals, (4) all imported mammals are potentially competent RVFV hosts, (5) vertebrate and mosquito infection rates are the same spatially and temporally in endemic countries, (6) the numbers of mosquitoes imported to the U.S. on ships and airplanes is comparable to studies that quantified infestation rates in other regions other than the U.S. (7) once infected, infectious mosquitoes remain infectious for the duration of their lifetime and (8) estimates of ship traffic, flight traffic, human travel, vertebrate trade, mosquito infestation rate, fraction of bloodmeals likely to be from human hosts and the fraction of bloodmeals likely to be from mammalian hosts were treated as constants. Based on these simplifying assumptions the relative risk different RVFV introduction pathways to the U.S. were quantified.

Host movement data

Predicting pandemic threats remains a difficult task, however the relationship between human movement and disease epidemics is clear. Before the global expansion

of human populations in the last five centuries disease pandemics were relatively confined, but following the increased frequency of international travel pathogen importation is a growing phenomena (Kilpatrick et al. 2006b, Jones et al. 2008). Subsequently, many attempts have been made to quantify local and international movement patterns to understand disease epidemiology. Studies have utilized Census data, border traffic surveys, social media, satellite nightlights, mobile phones, and Air and shipping statistics to capture patterns of movement (Tatem 2014). Although a wealth of data exists to understand movement patterns at a fine-scale, most of this information remains prohibitively expensive. However a variety of sources exist to quantify the movement of hosts to the U.S. from countries with active RVFV.

International flight data: The T-100 International Segment (All Carriers) Database provided by the Research and Innovation Technology Administration of the Bureau of Transportation Statistics contains non-stop segment data that is reported by both U.S. and foreign air carriers. Data for 2012 and 2013 downloaded from the online database on September 3rd, 2014 (United-Nations 2014). Data from 2012 and 2013 on departures, passengers, origin airport, destination airport, and year was combined to estimate the average number of direct flights arriving to the U.S. from countries with RVFV activity. Based on air traffic in 2012 and 2013 obtained from the Transtats database, the number of direct flights to the U.S. from areas with RVFV activity is estimated to be 3,515 flights per year (Bureau of Transportation Statistics-Transtats). Based on passenger data it is estimated that 697,384 humans travel from countries with RVFV activity to the U.S. per year.

International shipping data: The frequency of ships arriving in the U.S. from countries with RVFV activity was estimated based on calculations by Drake and Lodge, 2004. Drake and Lodge explored the role of ballast water as a invasion pathway for freshwater species by creating a gravity model to estimate the total number of ships travelling between each pair of ports yearly. Their analysis utilizes data on 28,748 ship arrivals to the U.S. during the year of 2000 obtained from the National Ballast Water Information Clearing House (Drake and Lodge 2004). Based on the supplemental data (Appendix A) about 474 ships per year arrive in the U.S. from countries with RVFV activity (Drake and Lodge 2004).

Movement of vertebrates: Data from the United Nations Comtrade Database (United-Nations 2014) was obtained to estimate the number of mammals being traded to the U.S. and data from the CITES wildlife trade database (UNEP-WCMC 2014) utilized to estimate the number of wild animals being imported to the U.S. from countries with RVFV activity. In total, 65 live mammals were imported yearly to the U.S. based on commodity codes 0102 (live Animals), 0103 (Live swine), 0104 (live sheep and goats), 010611 (live primates), 010613 (camels and other camelids), 010614 (rabbits and hares) downloaded from the UN Comtrade Database for the years 2010-2013 (United-Nations 2014). On average 120.5 wild animals were imported to the U.S. based on data from the CITES wildlife trade database (UNEP-WCMC 2014).

Mosquito importation

To estimate the average number of mosquitoes transported by airplane, published data that recorded the number of airplanes inspected and the numbers of live mosquitoes found was aggregated to calculate the average number of mosquitoes that are transported alive on each airplane. Multiple studies have recorded the number of mosquitoes found on airplanes since the 1930s with infestation rates ranging from 0.00056 mosquitoes per plane to 5.5 mosquitoes per plane (Highton and van Someren 1970, Le Maitre and Chadee 1983), however to estimate the rate of inadvertent mosquito transport on planes only inspections that utilized pesticides to knock down mosquitoes were considered. Results from three studies 0.057 (Hughes 1961), 0.61 (Mendonca and Cerqueiru 1947), and 2.2 (Russell et al. 1984) mosquitoes per airplane. Therefore the rate of mosquitoes that are transported alive on each airplane was estimated to be 1.13 (Low: 0.057; High: 2.2).

Results from a large-scale study that screened 734 ships arriving from 27 different countries and six different continents demonstrated that mosquito densities varied from 1-346 mosquitoes, however the average number of mosquitoes per ship was estimated to be 15.5 adults (Nie et al. 2004). Mosquito larvae can be transported in a variety of open containers, but used tires have been implicated as the introduction pathway for *Aedes japonicus* and *Aedes albopictus* (Lounibos 2002, Benedict et al. 2007). Therefore, the magnitude of tire trade is utilized to estimate the rates of larvae importation into the U.S. (Benedict et al. 2007). In an effort to quantify the risk of *Aedes albopictus* introduction to the U.S. by tire transport, a study by Craven et al. 1988

inspected 22,051 tires for mosquito eggs and larva in the U.S. and determined that the infestation rate of tires was 0.0006802 (Craven et al. 1988). Based on statistics from the UN Comtrade database (commodity code 401220) the average rate of tires imported into the U.S. from RVFV active regions (South Africa, Tanzania, Kenya) was 4525 per year. Because the average clutch size for Aedes albopictus eggs is about 80, the proportion of pupae that are females is estimated to be 0.5 and the rate of adult emergence is estimated to be 0.83 (based on *Aedes aegypti* life table model) (Armbruster et al. 2002, Focks et al. 1993) the number of larva transported to the U.S. per year is estimated to be 101.6 (0.0006802*4525*80*0.5*0.83).

The fraction of infectious adult mosquitoes capable of transmitting RVFV once in the U.S. was determined based on the product of two estimates: the average RVFV infection rate of mosquitoes in countries with RVFV and the fraction of mosquitoes likely to transmit RVFV after biting a host. Results from six different studies that screened for RVFV infection among mosquitoes in Senegal, Mauritania, Saudi Arabia, Egypt and Kenya (Linthicum et al. 1985, Zeller et al. 1997, Jupp et al. 2002, Diallo et al. 2005, Faye et al. 2007, Hanafi et al. 2011, Ba et al. 2012) indicate infection rates can be as high as 0.00657 in Mauritania (Faye et al. 2007) and as low as 0.000327 in Egypt (Hanafi et al. 2011). Based on this data, the RVFV infection rate in mosquitoes was estimated as the mean 0.00345 (Low: 0.000327; High: 0.00657). The fraction of mosquitoes likely to transmit RVFV by bite was estimated based on the average theoretical transmission competence of 26 mosquito species from six different genera

exposed to a viremia of $10^{7.5}$ Plaque Forming Units (0.14; 95% CI: 0.092-0.183) (Golnar et al. 2014).

To estimate the duration of mosquito infectiousness in the U.S. the logic employed by Kilpatrick et al. (2006) while quantifying the risk of WNV introduction to the Galapagos. Mosquitoes have been shown to live in the lab between 30-60 days and it takes at least 7 days for mosquitoes to develop a disseminated RVFV infection, therefore the duration of mosquito infectiousness was conservatively estimated to be 10-20 days (Turell et al. 1985, Oda et al. 2002, Kilpatrick et al. 2006c).

The fraction of larva arriving in the U.S. likely to transmit RVFV was calculated as the product of three estimates (i) the average RVFV infection rate of mosquitoes in countries with RVFV, (ii) the fraction of Adult mosquitoes likely to transmit RVFV after biting a host, and (iii) the fraction of eggs likely to become infected via transovarial transmission. The first two parameters are the same as described above. The vertical infection rate in mosquitoes was estimated to be the product of mosquito infection rate (0.000327-0.00657) and the rate of transovarial transmission observed in populations of *Aedes mcintoshi* (0.0000299) during the inter-epidemic period in Kenya (Lithicum et al. 1984). The duration of infectiousness for vertically infected adult mosquitoes was estimated to be 10 days longer (20-30 days) than infected mosquitoes transported by plane because they emerge as infectious adults.

Infected vertebrates

Vertebrate hosts need to produce an infectious viremia that can infect mosquitoes in order to import RVFV to the U.S. Experimental infection studies have shown that a number mammals, including rodents, new world monkeys, camels, and bovine animals produce viremia levels sufficient to infect mosquitoes that last between 1-7 days, however no reptiles, amphibians, or birds have ever been implicated as potential amplification hosts (Golnar et al. 2014). The classic RVFV transmission paradigm implicates peri-domestic livestock as important amplification hosts, however serological studies have found RVFV antibodies in a variety of wildlife hosts (Evans et al. 2008). It is more likely that trade in infectious livestock during a RVFV outbreak would result in the importation of RVFV to the U.S., but wildlife mammals cannot be ruled out. Quarantine measures are established in the U.S., however because the extent of these measures is unknown it is assumed that vessel travel time (ship or plane) or any duration of quarantine does not affect the magnitude and duration of vertebrate infectiousness in the U.S. (Rolin et al. 2013).

To estimate the number of infectious mosquito days resulting from mammalian hosts being transported to the U.S. the fraction of individuals likely to be transported to the U.S. (185.5) was multiplied by: (i) the infection rate of mammalian vertebrates (0.00137), (ii) duration and magnitude of mammalian infection (0.17), (iii) the fraction of mosquitoes likely to transmit RVFV by bite (0.14), (iv) the number of mosquitoes feeding on a mammal per day (1.32), (v) and the duration of mosquito infection (15 days). The number of mosquitoes feeding on a mammal per day and the fraction of

mosquitoes likely to transmit RVFV by bite is the same as estimates used to calculate mosquitoes arriving by plane. Similarly, the duration of mosquito infection is expected to be the same as an infectious adult mosquito arriving by plane (10-20 days).

Antibody prevalence was utilized to estimate the mammalian vertebrate infection rate. The prevalence of antibodies against RVFV was estimated based on two studies that selected representative groups of sheep, goats, and cattle and screened for IgG and IgM antibodies after an epidemic and during the enzootic period allowing the calculation of low and high prevalence rates. The rate of RVFV exposure in mammalian populations was estimated by dividing the number of mammals with IgG and IgM antibodies by the total number of mammals tested. IgM has been shown to be detectable in cattle for up to two months post exposure (30 days), and IgG is detectable for up to five months (150 days) (Morvan et al. 1992). To estimate the range of infection observed among mammalian vertebrates the rate of IgM exposure (0.158) (Zeller et al. 1995) and IgG exposure (0.0367) (Zeller et al. 1997) were divided by 60 and 150 respectively. The mean value between these calculations was estimated to be 0.00137 (Low: 0.00024; High: 0.0025).

The duration and magnitude of mammalian host viremia was based on the average mammalian competence value (0.17) estimated by Golnar et al. (2014). The vertebrate competence index estimates the relative number of infectious mosquitoes that may result from feeding on an infected vertebrate host (Komar et al. 2003) and is calculated as the product of susceptibility to infection, mean daily infectiousness to each species of mosquito, and duration of infectiousness (Golnar et al. 2014).

Infected humans

To estimate the average number of infectious mosquito days resulting from infected humans traveling to the U.S. by plane the number of individuals arriving in the U.S. (697,384) was multiplied by (i) the human infection rate (0.00025), (ii) duration and magnitude of mammalian infection (0.17), (iii) the fraction of mosquitoes likely to transmit RVFV by bite (0.14), (iv) the number of mosquitoes feeding on an mammal per day (0.33), (v) and the duration of mosquito infection (10-20 days). The number of mosquitoes feeding on a vertebrate in the U.S. per day (1.32), the fraction of mosquitoes likely to transmit RVFV by bite (0.14), and the duration of mosquito infection (10-20 days) are estimated to be the same as an infectious adult mosquito arriving by plane.

The average RVFV infection rate in humans was estimated based on the prevalence of IgG antibodies in humans and the time IgG remains in the body after infection. Data was obtained from a systematic serosurvey from Senegal during an inter-epidemic period from 1991-1993. Overall, 80 of 3,005 people screened were positive with IgG antibodies against RVFV three different regions (Kedougou, Barkedji, and Dielmo) and prevalence rates ranged from 0.014 to 0.06. (Zeller et al. 1997). It is well known that antibodies wane over time, with IgM being the initial immunoglobulin response, followed by IgG. A study by Morvan et al. (1992) demonstrated that IgG antibodies are detectable 3-5 months after infection in cattle (Morvan et al. 1992). Considering IgG exposure may have taken place anytime during a five-month time frame (150 days), the IgG prevalence rate in humans (Low: 0.014; High: 0.06) was

divided by 150 to calculate the average infection rate: 0.00025 (Low: 0.00009; High: 0.0004).

It remains undetermined whether humans contribute to the amplification of RVFV during enzootic and epidemic outbreaks. After the accidental infection of a laboratory worker in the 1940s with RVFV blood with a viremia equated to $10^{6.2}$ LD_{50} was isolated, however it was never quantified over time (Smithburn et al. 1949). The presence of a viremia in humans was also demonstrated during the 1977 Egyptian RVFV outbreak where humans produced a viremia between $10^{4.1}$-$10^{8.6}$ LD_{50} (Meegan 1979). Although it appears humans may produce an infectious viremia insufficient data exists to characterize the magnitude and duration of human infectiousness. Therefore, the average mammalian competence value (0.17) calculated by Golner et al. 2014 was applied to estimate the duration and magnitude of human infectiousness.

Frequency of invasion in U.S. cities

To identify high-risk areas for RVFV introduction in the U.S. the number of infectious mosquito days was estimated for each city in the U.S. resulting from flight and ship traffic multiplied by parameters described above. To estimate the number of infectious mosquito days arriving at each port the number of flights arriving in each region was multiplied by 0.00819, which was calculated as the product of mosquito infestation rate, mosquito infection rate, fraction of mosquitoes estimated to transmit RVFV by bite, and the number of infectious days. The number of infectious mosquito days resulting from human travel to U.S. cities was estimated by multiplying the number

of humans arriving by 0.0000295, which was calculated as a product of human infection rate, mammal host infectiousness, number of mosquitoes biting a human per day, fraction of mosquitoes estimated to transmit RVFV by bite, and the duration of mosquito infection. To estimate the number infectious mosquito days resulting from ship traffic the number of ships arriving was multiplied by 0.0374, which was the sum the risk displayed by adult mosquitoes. Because data from the UN Comtrade Database (Vertebrate and tire importation) and CITES Database (Wildlife trade) did not provide any resolution regarding the port of arrival or final destination, these pathways were not utilized to calculate regional propagule pressure or identify which countries abroad are most likely to import RVFV to the U.S.

Results

Pathways of introduction: Parameters from published literature are listed in Table 2.1. The relative risk calculated for each pathway is outlined in Table 2.2. Humans travelling to the U.S. from regions with RVFV activity represent the highest risk of RVFV introduction to the U.S. (Table 2.2). It is estimated that human travel will result in 82.16 (5.28-276) infectious mosquito days per year. Adult mosquitoes arriving by plane and by ship will result in 28.8 (0.06-186) and 25.7 (0.32-128) infectious mosquito days per year, respectively. Imported mammals will result in 0.029 (0.0009-0.14) infectious mosquito days per year. Vertically infected larvae arriving by ship will result in 0.0003 (0.000016-0.0009) infectious mosquito days per year.

Table 2.1. Range of parameter estimates based on published literature

Parameter	Low	High	Mean
Number mosquitoes biting vertebrates per day	0.52	2.08	1.32
Mosquitoes infestation rate of planes	0.057	2.2	1.13
Mosquito RVFV infection rate	0.000327	0.00657	0.00345
Transovarial RVFV infection rate	9.78E-09	1.96E-07	0.0000299
Mammal RVFV infection rate	0.00024	0.0024	0.00137
Human RVFV infection rate	9.33E-05	0.0004	0.00025
Mosquito RVFV transmission rate	0.092	0.183	0.14
Mosquito by airplane duration of infection	10	20	15
Mosquito by ship duration of infection	1	10	5
Larvae by tire trade duration of infection	20	30	25
Mosquito infected by vertebrate duration of infection	10	20	15

See text for references

Table 2.2 Estimated risk of Rift Valley fever virus introduction to the United States

Pathway	Number arriving to U.S. per year	Fraction likely to transmit by bite	Infection duration*	Infectious mosquito days per year
Mosquito by plane	(3515)(1.13)[a]	(0.00345)[a](0.14)[a]	15	28.78 (0.06-186)
Mosquito by ship	(474)(15.5)	(0.00345)[a](0.14)[a]	5	25.72 (0.32-128)
Larvae by tire	(4525)(0.0006802)(80)(0.5)(0.83)	(0.00345)[a](0.0000299)[a](0.14)[a]	25	$3.7x10^{-5}$
Human travel	697384	(0.00025)[a](1.32)(0.17)(0.14)[a]	15	82.16 (5.28-276)
Mammal Import	185.5	(0.00137)[a](1.32)(0.17)(0.14)[a]	15	0.12 (0-0.56)

[a] *Parameter estimates are the middle of the identified range listed in Table 2.1*

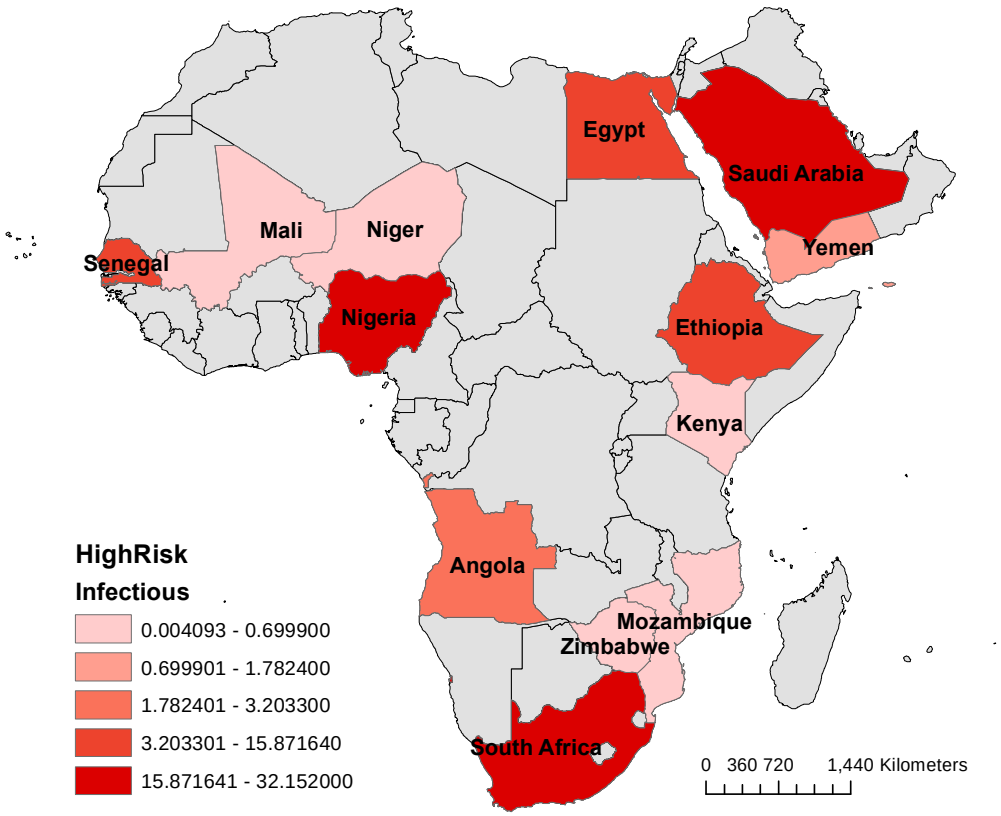

Figure 2.1. Countries in Africa and the Arabian Peninsula implicated to have high-risk connectivity with the U.S. in the context of RVFV importation. A gradient highlights the risk displayed by each country for importing infectious mosquitoes into the U.S. through humans, shipping and air traffic.

High-risk ports: Based on human travel, flight traffic, and shipping, 15 countries are implicated as potential importers of RVFV to the U.S. displayed in Figure 2.1 (Saudi Arabia, South Africa, Nigeria, Morocco, Egypt, Senegal, Ethiopia, Yemen, Angola, Kenya, Mozambique, Zimbabwe, Mali, Niger, and The Gambia). Movement from Saudi Arabia to the U.S. is estimated to result in 24% of all infectious mosquitoes arriving in the U.S., followed by South Africa (22%), Nigeria (18%), Senegal (12%), Egypt (11%), Ethiopia (9%), Angola (2.0%), Yemen (1%) and Kenya, Mozambique, Zimbabwe, Mali,

Niger, and The Gambia each are estimated to be responsible for less than 1%. Travel from Botswana, Burkina Faso, Cameroon, Central African Republic, Chad, Democratic Republic of the Congo, Gabon, Guinea, Madagascar, Mauritania, Namibia, Republic of Congo, Somalia, South Sudan, Sudan, Tanzania, Uganda, and Zambia are estimated to pose no threat for RVFV introduction. The import of vertebrates and import of car tires are not accounted for in this estimate, but considering the low risk of these pathways (Table 2.1), vertebrate imports from the Congo, Botswana, Tanzania and used tire imports from Tanzania represent a relatively minimal threat.

The frequency of RVFV invasion was estimated to be highest in the North East region of the U.S. Specifically in New York/New Jersey there was an estimated 26.5 infectious mosquito days per year (Figure 2.2). Based on this assessment, Washington D.C. (14.5), Houston (11.2), Atlanta (10), and Philadelphia (4) receive the next highest frequency of arriving infectious mosquitoes.

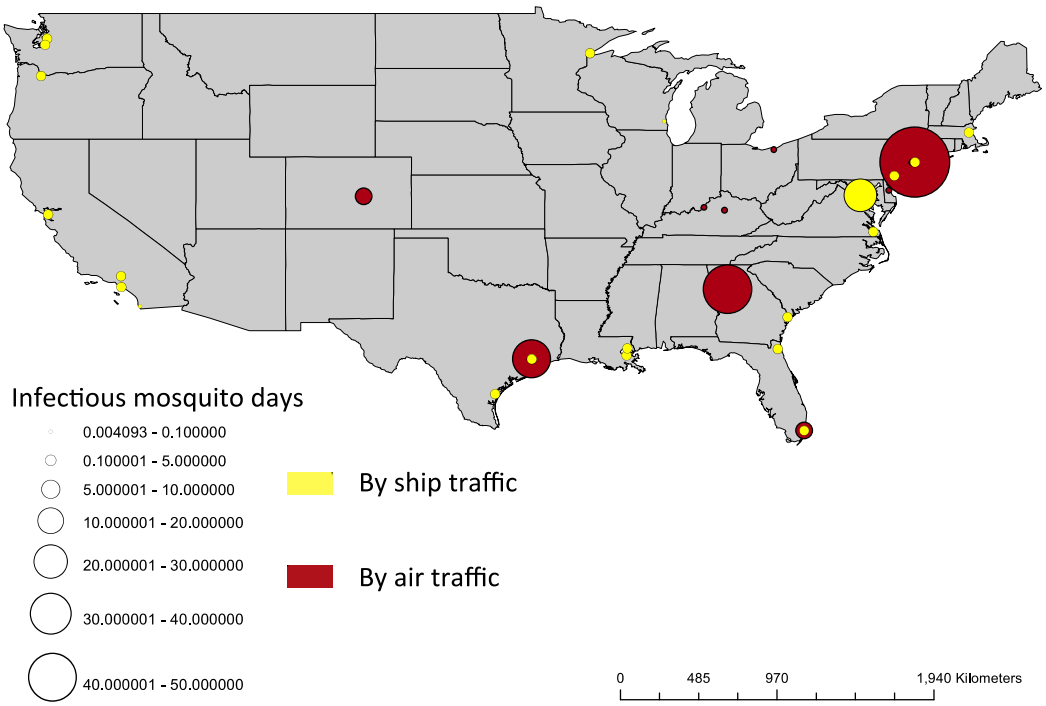

Figure 2.2. The number of RVFV infectious mosquitoes days in the U.S. per year. This value was estimated as a product of (i) the number of mosquitoes arriving to the U.S. per year, (ii) the fraction likely to transmit RVFV by bite, and (iii) the duration of infection. Data from three pathways were combined: mosquitoes arriving by plane, mosquitoes arriving by ship, and the travel of infected humans. Red indicates infectious mosquito days per year resulting from flight traffic and yellow indicates the risk infectious mosquito days per year resulting from ship traffic.

Discussion

The increased emergence of vector-borne diseases over the past 30 years is a result of increased vector range and land-use change, but largely due to human movement (Jones et al. 2008, Tatem et al. 2012). The spread of RVFV to the U.S. is generally considered low, but concerns remain high due to the significant economic and public health impacts associated with the virus (Tatem et al. 2012, Rolin et al. 2013). Overall, results from this analysis suggest that human travel is the most important route

of RVFV introduction to the U.S. followed by mosquito transport by airplane and mosquito transport by ship (Table 2.2). Results also suggest the importation of mammals and the trade of tires are a relatively low risk for RVFV entry into the U.S.

The role of humans in RVFV amplification and pathogen dispersal remains unknown (Smithburn et al. 1949, Meegan 1979, Kasari et al. 2008, Chevalier et al. 2010, Hartley et al. 2011, Rolin et al. 2013). Although RVFV is associated with rural livestock communities, international tourists have indeed acquired RVFV, including a French Canadian woman and members of the French military (Durand et al. 2001, Rolin et al. 2013). As of 2003 arboviruses were one of the most common causes of viral fevers in returning tourists, often presenting with non-specific symptoms such as fever and myalgia (Spira 2003). If humans produce a RVFV viremia comparable to other mammals this analysis indicates human travel would be a significant route of RVFV dispersal to the U.S. (Table 2.2). The unintentional importation of mosquitoes by airplane and ship are also important pathways of RVFV introduction to the U.S. and should not be neglected considering the spread of vectors worldwide has spearheaded some of the most important epidemics throughout history, including yellow fever, typhus, plague, and malaria (Lounibos 2002).

The small numbers of vertebrates being imported into the U.S. (120.5 per year) is responsible for the low risk highlighted by this analysis. RVFV has been spread through infected animals, but the time to travel across the Atlantic Ocean combined with quarantine measures would likely be longer than the 1-7 day viremic period of most mammals (Rolin et al. 2013, Golnar et al. 2014). Utilizing the magnitude of tire trade between the U.S. and RVFV endemic regions as a surrogate to estimate mosquito eggs

or larvae present on freighter ships likely results in an underestimate of mosquito infestation rates. However, vertical infection of mosquito eggs in the RVFV system has only been demonstrated in one species of mosquito, therefore, the role vertically infected larva play in importing RVFV to the U.S. is expected to be negligible in most scenarios.

The frequency of RVFV introduction to the U.S. is largely concentrated in the North East, Central East Coast and Houston, Texas (Figure 1). Reminiscent of the 1999 WNV invasion, New York is estimated to receive the highest introduction pressure. However should an infectious mosquito arrive in New York, the potential for local transmission and establishment remains unknown and further evaluation of local hosts and ecological conditions would be important for gauging invasion success. The use of climate matching and remote sensing techniques can be utilized to identify environmental conditions supportive of RVFV transmission and the harsh winters of New York would likely prevent over-wintering of the virus (Barker et al. 2013). Therefore, regions further south with warmer climates year-round may be more appropriate for local transmission and establishment. By calculating the number of infectious mosquito days per year that occur regionally in the U.S., Houston, Texas receives the fourth most introduction pressure. This region should be monitored closely considering the warm climactic conditions, abundance of cattle, and abundance of the highly competent salt marsh mosquito, *Aedes sollicitans*, which is known to reach populations so large that cows die of exsanguination (Abbitt and Abbitt 1981, Gargan et al. 1988, Golnar et al. 2014).

Considering 32 countries have been identified with RVFV activity, only 14 countries have been identified as likely origins of a RVFV importation into the U.S. The

importation of RVFV to the U.S. is likely to be the highest during an outbreak and understanding which countries are most likely to import RVFV to the U.S. is informative to surveillance programs and the creation of preventative strategies. The U.S. is highly connected with Saudi Arabia and preventative efforts should be fully activated when outbreaks occur in this region. Overall, authorities responsible for limiting the spread of RVFV or other organisms to the U.S. should be particularly concerned with outbreaks in Saudi Arabia, South Africa, Nigeria, Egypt, Senegal, Ethiopia, Yemen and Angola (Figure 2.1).

Limitations: The evaluation of vector-borne pathogen invasion is a complex process; therefore this model utilized a number of simplifying assumptions. Infection rates, infestation rates, host/vector abundance levels, competence levels, and movement data could be estimated from available studies, however, the addition of local data for parameter estimates would improve the resolution of results and help explore introduction probabilities at the local scale.

Conclusion

To quantitatively evaluate routes of RVFV introduction to the U.S. the number of infectious mosquitoes arriving in the U.S. per year was estimated for five pathways: infected mosquitoes arriving by plane, infected mosquitoes arriving by ship, infected mosquito larvae/eggs arriving through tire transport, infected humans travelling to the U.S., and the trade of infected mammals. The movement of infectious humans by flight is estimated to be the most significant route of RVFV introduction to the U.S., followed by the movement of adult mosquitoes on ships and airplanes. High-risk regions for

RVFV introduction were identified to New York, Washington D.C., Atlanta, and Houston. Saudi Arabia, South Africa, Nigeria, Egypt, Senegal, Ethiopia, Yemen and Angola were identified as countries that pose a risk for importing RVFV to the U.S. through movement connectivity.

Although introduction events are often stochastic and unpredictable, unlikely scenarios of disease spread happen, as demonstrated by the spread of WNV to the U.S. With the growing frequency of international travel the threat of RVFV introduction will only increase. Key pathways of introduction and high-risk regions within the U.S. quantified in this analysis will function as important parameters for comprehensive risk models combining environmental data with epidemiological data to evaluate RVFV invasion in the U.S.

In the event that RVFV emerges in the U.S. it will be state and county public health departments and the associated vector control agencies that will be critical members of the response task force. Results from this analysis help judge the relative risk of RVFV introduction regionally in the U.S. important to mosquito control and vaccination strategy development. Should RVFV reach the U.S., clear case definition for clinicians and veterinarians will be essential for effective diagnosis and timely response efforts.

CHAPTER III

PREDICTING THE MOSQUITO SPECIES AND VERTEBRATE SPECIES INVOLVED IN THE THEORETICAL TRANSMISSION OF RIFT VALLEY FEVER VIRUS IN THE UNITED STATES*

Introduction

Rift Valley fever virus (RVFV) is an emerging infectious disease in Africa and the Middle East. If introduced to North America, RVFV is capable of serious health and socioeconomic consequences potentially incapacitating large numbers of humans, decimating susceptible farm animals, and instigating heavy restrictions on livestock trade (Weaver and Reisen 2010, Hartley et al. 2011). Although transmission of the virus can occur through aerosol inhalation or direct tissue-tissue contact by handling of infected organisms, an enzootic cycle between mosquito vectors and domestic or wild animals has been repeatedly proposed as a main mechanism of transmission (Meegan and Bailey 1988). Clinical signs vary by vertebrate species and age, but infected pregnant ruminants generally suffer spontaneous abortions and juvenile ruminants suffer high mortality while occasional spillover into human populations results in a self-limiting, febrile illness that may progress to encephalitis, retinitis, blindness, hemorrhagic fever or death (Meegan and Bailey 1988, Mandell and Flick 2010, Weaver and Reisen 2010, Ikegami and Makino 2011). In 1931, RVFV was first reported in

*Adapted and reprinted with permission from "Predicting the Mosquito Species and Vertebrate Species Involved in the Theoretical Transmission of Rift Valley Fever Virus in the United States" by Golnar, A. J., M. J. Turell, A. D. LaBeaud, R. C. Kading, and G. L. Hamer. 2014. *PLoS Neglected Tropical Diseases* 8: e3163. Copyright [2014] by Andrew John Golnar. URL: http://www.plosntds.org/article/info%3Adoi%2F10.1371%2Fjournal.pntd.0003163

Kenya. It spread to Egypt in 1977 and was detected on the Arabian Peninsula in 2000 (Meegan 1979, Fagbo 2002). Since advancing beyond African borders in 2000, total human cases of RVFV include 768 confirmed fatalities, 4,248 confirmed infections and over 75,000 suggested unconfirmed cases (CDC 2000a, b, c, WHO 2007a, b, Bouloy and Flick 2009, WHO 2010, Hassan et al. 2011).

The emergence of arthropod-borne viruses (arboviruses) through geographic expansion is facilitated when amplification hosts include wild or domestic animals, as demonstrated by West Nile virus (WNV), Japanese encephalitis, and epizootic hemorrhagic disease (Weaver 2005, Weaver and Reisen 2010). *Aedes* and *Culex* spp. mosquitoes are proposed to be the main vectors of RVFV, where *Aedes* spp. act as the reservoir and maintenance vectors that emerge after flood events and feed heavily on livestock (Pepin et al. 2010). *Culex* spp. mosquitoes then become involved as amplifying hosts of RVFV leading to epizootics and the eventual spillover to human populations (Bird et al. 2009, Pepin et al. 2010, Ikegami and Makino 2011, Bird and Nichol 2012). However, the understanding of RVFV transmission biology in Africa and the Arabian Peninsula remains underdeveloped. Additionally, unresolved questions surround endemic persistence of the virus, such as transovarial transmission (Pepin et al. 2010).

Should RVFV arrive, diagnosing the disease and controlling the spread of infected vertebrates will take time, and proactive management plans should be created to minimize the time to react and break transmission of the pathogen. Even though RVFV is identified as an emerging infectious disease threat and is classified as a "Category A

select agent" by both the Centers for Disease Control and Prevention and the US Department of Agriculture, gaps in data are preventing a proper evaluation of the different roles vectors and vertebrate hosts potentially may play in RVFV transmission in the U.S. beyond qualitative conjecture (Hartley et al. 2011, Rolin et al. 2013). To prepare for an arbovirus introduction, it is essential to understand which vectors and vertebrate hosts may be responsible for viral amplification and transmission, as disease control methods vary depending on the target species (Turell et al. 2008b, Kakani et al. 2010). For example, mosquito species using small container habitats for larval development are often controlled using larvicides and source reduction of aquatic habitat, whereas mosquito species with synchronous emergence following flooding events are controlled by adulticides or granular larvicides applied prior to flooding (Rose 2001, Medlock et al. 2012).

To assess the role of mosquitoes and hosts in the transmission of a virus, it is important to quantify the ability for a mosquito species to transmit a pathogen (vector competence), the infectiousness of vertebrate host species (host competence), and contact rates between mosquitoes and vertebrate hosts. In the WNV system, Kilpatrick et al. (2005) combined data on vector competence, abundance, and mosquito feeding patterns to identify the species of mosquitoes responsible for bridge transmission of WNV to humans. Several studies have then implicated important avian hosts disproportionately responsible for WNV amplification based on mosquito host feeding patterns, mosquito vector competence data, and vertebrate host competence data (Hamer et al. 2009, Hamer et al. 2011). By applying models utilized in the WNV system, we can implicate potentially important vectors and vertebrate hosts in RVFV transmission

should the virus arrive. A number of reviews discuss potential vertebrate hosts, disease vectors, and environments that may support RVFV transmission in the U.S., through environmental receptivity models (Barker et al. 2013) and spatial overlap of important host populations (Kakani et al. 2010). However, to our knowledge, no study has quantitatively evaluated the theoretical importance of different mosquito species and vertebrate hosts to RVFV transmission and amplification in the U.S. (Barker et al. 2013).

This study utilized published and unpublished vector and host competence data and mosquito feeding patterns to model the theoretical roles of different mosquito and vertebrate species in the amplification and transmission of RVFV in the U.S. Although predictions from this analysis are strictly theoretical, and limited by available data, these results highlight critical gaps in knowledge necessary to properly evaluate the potential transmission activity of RVFV in the U.S. and provide hypotheses that can support proactive arbovirus surveillance and control programs.

Methods

Vector competence: Mosquito vector competence studies evaluate the ability of mosquitoes to develop an infection and ultimately transmit the pathogen during feeding. Data generated from vector competence studies include viral dissemination and transmission rates. Viral dissemination rates are defined as the percentage of orally exposed mosquitoes with virus detected in their legs seven or more days after RVFV infection. Transmission rates are defined as the percentage of orally exposed mosquitoes (regardless of infection status) that transmitted virus by bite upon refeeding (Turell et al. 2008b). Selected studies evaluated mosquito species that occur in the U.S. and

monitored dissemination and transmission rates after feeding on a RVFV infected animal at the incubation temperature of 26ºC. RVFV vector competence studies were located using Web of Science, NCBI's Pubmed, and the Armed Forces Pest Management Board Literature Retrieval Systems (Gargan et al. 1988, Turell et al. 1988, Turell et al. 1996, Turell et al. 2008a, Turell et al. 2008b, Turell et al. 2010, Turell et al. 2013, Turell et al. 2013).

Analyzing viral dissemination and transmission data drawn from multiple studies is problematic because these data are dependent on the viremic titer of exposure (Turell et al. 1988) and the compiled transmission data for this analysis reflects mosquitoes exposed to viremia that ranged from $10^{4.3}$ to $10^{10.2}$ plaque-forming units/ml (PFU/ml). To address this issue, a regression analysis of log viremia versus experimental transmission data from 17 mosquito species (Figure S1, A and B) was utilized to estimate the dependence of dissemination and transmission rates on viremic dose. Slopes from these regressions were combined with experimental data from each mosquito species to interpolate what the dissemination and transmission rates would be at the exposure viremia of $10^{7.5}$ PFU/ml (equations shown in Table S1, Appendix A). Mosquito species that demonstrated low overall vector competence in experimental transmission studies due to midgut escape barriers or salivary gland barriers (i.e. *Anopheles crucians* (Wiedemann), *Cx. nigripalpus* (Theobald) and *Ae. infirmatus* (Dyar & Knob)) or had a limited sample size (N<2 mosquitoes) were not used in the regression analyses (Turell et al. 2013c).

The viremia-dissemination equation was equal to $0.098*(\text{Log}_{10}\text{ viremia}) - 0.268$ and the viremia-transmission rate of a mosquito with a disseminated infection equation

was equal to 0.056*(Log_{10} viremia)-0.0155 (Figure S1, A and B; Table S1, Appendix A). Both equations show a positive relationship for dissemination (N= 27; R^2= 0.28; p = 0.0049) and transmission (N= 27; R^2= 0.13; p = 0.07) as viremic dose increases. For each mosquito species we generated a linear equation and the y-intercept was adjusted based on the difference between the experimentally observed rate and what the standardized equations described above (Figure S1, A and B) would predict at a specific viremic dose. This adjusted y-intercept and the standardized slopes from Figure S1, A and B (Dissemination m = 0.098, Transmission m = 0.056) were utilized to create two unique linear equations for each mosquito species: one to calculate dissemination rate and one to calculate transmission rate with respect to viremic dose for each vector species. By solving for y when x= $\log_{10}$ 7.5 PFU/ml we were able to estimate dissemination and transmission rates at an exposure viremia of $10^{7.5}$ PFU/ml for each mosquito species (Table S1-Appendix A). When there were multiple data points for a mosquito species the averages of exposure viremia and the observed experimental transmission data were used to calculate the two linear equations for vector competence standardization.

Additional data points were estimated that describe transmission rates for *Ae. dorsalis* (Meigen), *Cx. erythrothorax* (Dyar), *Cx. tarsalis*, and *Cx. erraticus* (Dyar-Knab) mosquitoes that developed a disseminated infection based on the estimated transmission rates of Turell et al. (Turell et al. 2010). These data were standardized with the same methodology described above. Vector competence (C_v) was calculated by multiplying the fraction of mosquitoes that develop a disseminated infection after feeding on a viremic host by the transmission rate of mosquitoes with disseminated

infection based on estimated values for an exposure viremia of $10^{7.5}$ PFU/ml (Turell et al. 2007).

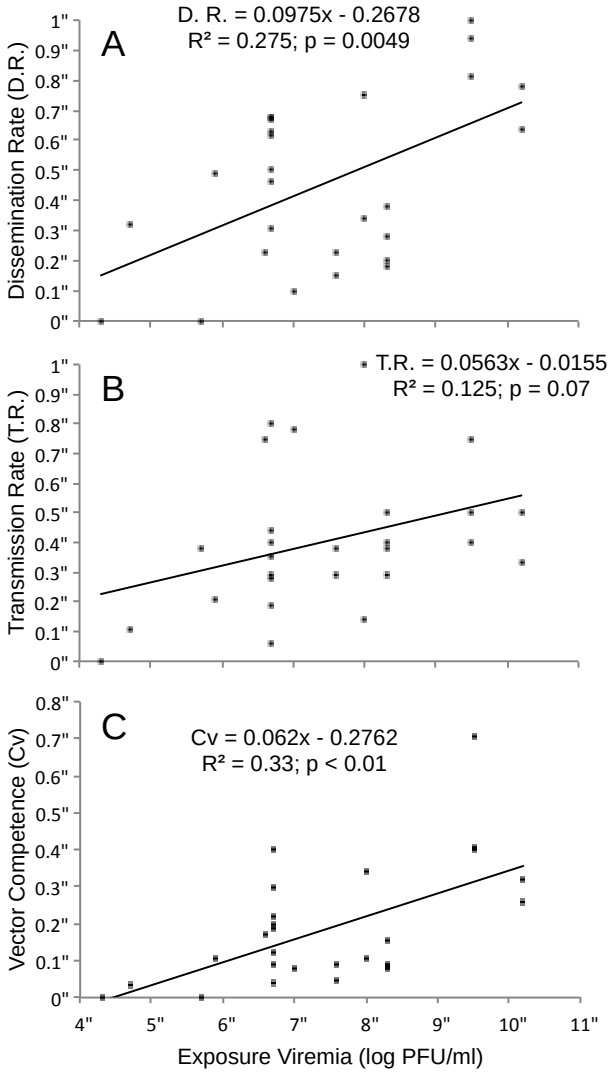

Figure 3.1. Dose-dependent relationship between exposure viremia and vector competence. The dose-dependent relationship between exposure viremia and dissemination rate (A), transmission rate (B), and vector competence (C) displayed by 17 mosquito species in seven experimental transmission experiments: *Ae. aegypti, Ae. albopictus, Ae. atlanticus, Ae. canadensis, Ae. cantator, Ae. sollicitans, Ae. taeniorhynchus, Ae. triseriatus, Ae. vexans, Cq. perturbans, Cx. erraticus, Cx pipiens, Cx. salinarius, Cx. tarsalis, Cx. territans, Ma. dyari*, and *Ps. ferox*.

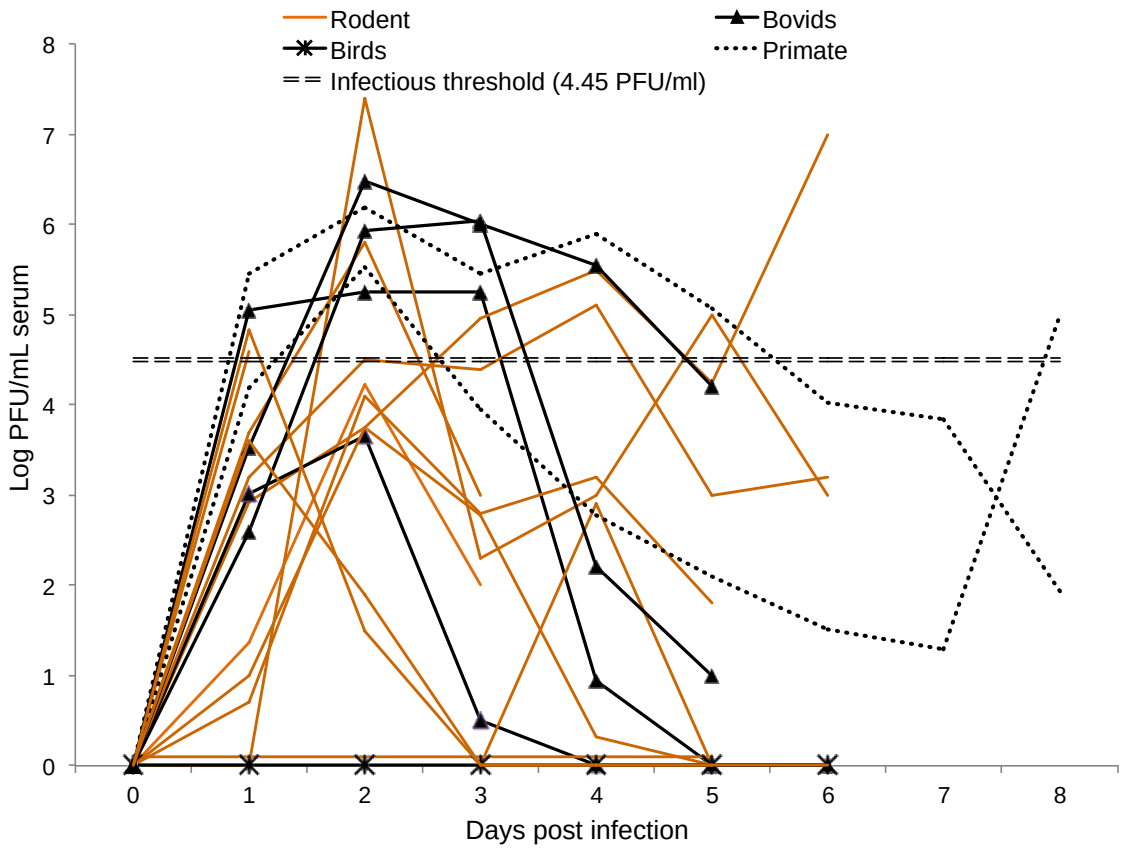

Figure 3.2. A graphical representation of the mean viremia profiles demonstrated by 20 different vertebrates after exposure to virulent strains of Rift Valley fever virus. Data was compiled from 17 published experimental infection studies and unpublished data from Dr. John Morrill and Dr. Michael Turell. Viral titers were quantified each day after infection by Plaque Assay or Tissue Culture Infectious Dose 50, which was converted to PFU/ml by the following equation: PFU/ml = $TCID^{50}$/ml x 0.69 (O'Reilly et al. 1994, Mena et al. 2003). When a vertebrate host's viremia was calculated to be negative the daily infectiousness was set to zero as discussed in the methodology. References: Bovids: (Davies and Karstad 1981, Morrill et al. 1991, Rippy et al. 1992, Nfon et al. 2012); Birds: (Findlay and Daubney 1931) (Turell unpublished data); Primate: (Peters et al. 1988, Morrill et al. 1989, Smith et al. 2012) (Morrill unpublished data); Rodent: (Swanepoel et al. 1978, Anderson et al. 1987, 1988, Rossi and Turell 1988, Anderson et al. 1991b, Anderson et al. 1991a, Pretorius et al. 1997, Gora et al. 2000, Geffers et al. 2010, Smith et al. 2010).

Vertebrate host competence: When mosquitoes feed on an infected vertebrate a fraction of those mosquitoes will become infectious depending on the intensity of the vertebrate host's viremia and the mosquito's susceptibility to the virus (Kilpatrick et al. 2007). Experimental infection studies that exposed vertebrate species to RVFV and monitored post-infection viremias were used to create a host competence index (C_i). The vertebrate reservoir competence index represents the relative number of infectious mosquitoes that may result from feeding on infected vertebrate hosts and is calculated as the product of susceptibility to infection, mean daily infectiousness to each species of mosquito, and duration of infectiousness (Komar et al. 2003). Published studies were located using Web of Science, NCBI's Pubmed, and the Armed Forces Pest Management Board Literature Retrieval Systems. Studies utilizing PFU/ml and Tissue Culture Infectious Dose 50% ($TCID_{50}$) techniques to quantify viral titers after experimental infection with virulent strains of RVFV (ZH501,T1,T46, AN1830, Kabete, 80612A, AnD100286, AnD100287, Z8548, FRhL2) were the only inclusion criteria for host competence data as no universal conversion between Lethal Dose 50% (LD_{50}) and Mouse Lethal Dose 50% (MLD_{50}) was found. Conversion from $TCID_{50}$ to PFU/ml was obtained by the equation: PFU/ml = $TCID_{50}$/ml x 0.69 (O'Reilly et al. 1994, Mena et al. 2003).

To calculate the vertebrate host competence index for RVFV, an equation describing vector competence was calculated utilizing available mosquito transmission experiments performed at 26ºC as a linear function of log (host viremia). This viremia-vector competence equation (Figure S1, C) describes the fraction of mosquitoes that would become infected after feeding on a single viremic host indicating the

infectiousness of a vertebrate (Komar et al. 2003, Kilpatrick et al. 2007). Because of limited species-specific experimental transmission data, the viremia-vector competence equation is based on the combined experimental transmission data of 17 mosquito species (See Figure S1). Mosquito species that demonstrated low overall vector competence in experimental transmission studies due to midgut escape barriers or salivary gland barriers or had a limited sample size as described above were not used to calculate the viremia-vector competence relationship (Turell et al. 2013). The viremia-vector competence equation (vector competence = 0.062 (Log_{10} viremia) -0.276; R^2=0.27; N=27; P= <0.001) was used to calculate the daily infectiousness of vertebrate hosts by inserting daily vertebrate host viremia titers into the equation. When the equation calculated a vertebrate host's infectiousness to be negative the vertebrate host's daily infectiousness was set to zero (Kilpatrick et al. 2007). These daily values were summed over the host's viremic period and used as the vertebrate species' competence index (C_i). When multiple experimental studies existed for a particular vertebrate species or taxonomic group a mean C_i was calculated (Komar et al. 2003, Kilpatrick et al. 2007, Perez-Ramirez et al. 2014).

Vector amplification fraction: To determine the theoretical importance of a mosquito to RVFV transmission it is important to consider contact rates between vectors and vertebrate hosts. The amplification fraction estimates the number of infectious mosquitoes resulting from feeding on a particular host and can be utilized as an index to compare the relative role of various vectors in transmission. In the WNV system, the relative number of infectious (transmitting) mosquito vectors resulting from feeding on a vertebrate host was estimated by Kent et al. (Kent et al. 2009) utilizing the following

equation: $F_i = B_i^2 * C_i$ where F_i = the relative number of infectious mosquitoes resulting from feeding on each vertebrate species *i*, where B_i = the proportion of blood meals from species *i* and C_i = reservoir competence. This equation was modified from Kilpatrick et al. (Kilpatrick et al. 2006a) which estimated the fraction of WNV-infectious mosquitoes, F_i, resulting from feeding on each avian species, *i,* as the product of the relative abundance, the vertebrate reservoir competence index, C_i, and the mosquito forage ratio. Kent et al. (Kent et al. 2009) found that the relative abundance of each avian species cancelled out when multiplied by the forage ratio, of which the denominator is relative abundance. F_i as defined by Kilpatrick et al. (Kilpatrick et al. 2006a) was therefore reduced to the product of C_i and the proportion of blood meals from species *i*. Because the viremia-vector competence relationship used in this analysis is based on data from multiple mosquito species, Kent et al's (Kent et al. 2009) F_i equation was modified to multiply by the mosquito's vector competence value (C_v) to account for the differences observed in mosquito vector transmission competence across species. The modified equation is referred to as the vector amplification fraction (F_{vi}) and provides a theoretical means to compare the role of various vector species in the transmission of RVFV.

$$F_{vi} = B_i^2 * C_i * C_v$$

In the F_{vi} equation, the number of infectious mosquitoes resulting from feeding on a vertebrate host, F_{vi}, is equal to vertebrate host competence (C_i), multiplied by the vector competence (C_v), multiplied by the fraction of the total blood meals from host *i* squared (B_i^2) (Kent et al. 2009, Hamer et al. 2011). B_i represents the number of blood meals taken from a vertebrate host species divided by the total blood meals taken. B_i is unique to each mosquito species and is used as an indicator of exposure to RVFV and as

an indicator of potential RVFV-infectious bites received by a host species, or taxonomic group (Muñoz et al. 2012). Mosquito host feeding data from 39 studies were combined to generate a robust estimate of mosquito feeding patterns at the taxonomic resolution of Class and Order compiled into Table S2 (See Appendix A)**.** Vertebrate hosts fed on by mosquitoes lacking a competence index (C_i) were assigned the closest taxonomic mean (Perez-Ramirez et al. 2014). Only mosquito species with over 40 recorded blood meals to calculate vertebrate host feeding proportions (B_i) were included in this analysis. When vector competence data were missing for a given mosquito species, vector competence values were substituted based on the taxonomic subgenus average (*Aedes- Ochlerotatus*: 0.15; *Culex- Melanoconion*: 0.04, *Culex*: 0.11), genus average (*Anopheles*: <0.01; *Psorophora*: 0.18, *Mansonia*: 0.07) or family average (*Culicidae*: 0.15). To include *Ae. aegypti* in this analysis host-feeding patterns were estimated based on mosquito feeding patterns in Puerto Rico (Barrera et al. 2012).

F_{vi} is unique to each mosquito vector-vertebrate host pair and assumes initial seroprevalence, susceptibility and competence values are equal among all adult and juvenile vertebrate hosts (Dye and Hasibeder 1986, Woolhouse et al. 1997, Hamer et al. 2011). In an attempt to control any effect of the exposure dose of RVFV on the outcome of mosquito transmission competency, the F_{vi} calculation only utilized mosquito competence values standardized to an exposure dose of $10^{7.5}$ PFU/ml as described above. To calculate a mosquito species' vector amplification fraction resulting from feeding on all vertebrate hosts, all F_{vi} values reflecting a vector-vertebrate pair were summed for each mosquito species (equations shown in Table S3, Appendix A). This overall risk for

a mosquito species to contribute to RVFV transmission in the U.S. was calculated based on a weighted percentage relative to the total F_{vi} displayed by all mosquitoes.

Vertebrate host amplification fraction: To explore the theoretical contribution of vertebrates to RVFV amplification and transmission in the U.S., F_{vi} values unique to each vector-vertebrate pair described above were summed across each vertebrate host instead of by mosquito vector. The resulting index expresses the relative number of infectious mosquitoes generated by each vertebrate host. Since species-specific competence data was lacking for all vector-vertebrate host contacts, the role of vertebrate hosts was explored at the taxonomic resolution of class, order, and family. By summing F_{vi} values with respect to vertebrate host at different taxonomic levels we were able to quantify the theoretical amplification fraction displayed by each vertebrate host taxonomic group. This index was expressed as a weighted average by dividing the summed F_{vi} values for a vertebrate group by the total F_{vi} value calculated for the mammalian order (Table S3, Appendix A).

Results

Vector competence: Eight experimental studies were identified that fit the inclusion criteria for this analysis (Gargan et al. 1988, Turell et al. 1988, Turell et al. 1996, Turell et al. 2008b, Turell et al. 2008a, Turell et al. 2010, Turell et al. 2013c). Data for 26 mosquito species were adjusted utilizing the viremic dose-dependent relationship of dissemination and transmission rates based on 17 species of mosquitoes (Figure S1, A and B). Standardized dissemination and transmission values were multiplied together to calculate vector competence (Table 3.1 and S1). The most

competent transmission vectors of RVFV when exposed to $10^{7.5}$ PFU/ml of viremia are estimated to be *Coquillettidia perturbans* (Walker) (0.38), *Ae. japonicus japonicus* (Theobald) (0.37), *Cx. tarsalis* (0.33), and *Ae. excrucians* (0.28). Some mosquito species were estimated to be incompetent for RVFV, such as *An. crucians* (<0.01), *Ae. infirmatus* (<0.01), and *Cx. quinquefasciatus* (Say) (<0.01) (Table 3.1).

Table 3.1. Estimated dissemination rate, transmission rate, and vector competence for mosquitoes exposed to 7.5 log PFU/ml Rift Valley fever virus.

Species[citation]	Dissemination rate[a]	Transmission rate[b]	Vector Competence (Cv)[c]
Coquillettidia perturbans [29]	0.53	0.72	0.38
Aedes j. japonicus [30]	0.74	0.51	0.37
Culex tarsalis [31, 32]	0.38	0.87	0.33
Aedes excrucians [31]	0.28	1	0.28
Aedes canadensis [31]	0.7	0.4	0.28
Aedes sollicitans [31]	0.76	0.34	0.25
Aedes triseriatus [31]	0.75	0.32	0.24
Psorophora ferox [29]	0.55	0.32	0.18
Culex territans [31]	0.39	0.45	0.17
Aedes atlanticus [29]	0.36	0.42	0.15
Aedes taeniorhynchus [21, 31]	0.49	0.27	0.13
Aedes albopictus [33]	0.52	0.25	0.13
Culex salinarius [31]	0.54	0.24	0.13
Culex pipiens [32, 34, 35]	0.13	0.9	0.12
Aedes vexans [21, 29]	0.26	0.41	0.11
Aedes aegypti [34]	0.7	0.11	0.08

Table 3.1. Continued

Species[citation]	Dissemination rate[a]	Transmission rate[b]	Vector Competence (Cv)[c]
Aedes cantator [31]	0.71	0.11	0.07
Mansonia dyari [29]	0.17	0.4	0.07
Culex erythrothorax [32]	0.17	0.26	0.04
Culex erraticus [32]	0.15	0.28	0.04
Culex nigripalpus [21, 29, 32]	0.06	0.24	0.01
Anopheles bradleyi-crucians [31]	0.17	0.05	0.01
Aedes infirmatus [29]	0.29	0	<0.01
Anopheles crucians [29]	<0.01	<0.01	<0.01
Culex quinquefasciatus [32, 34]	<0.01	0.14	<0.01
Aedes dorsalis [30]	0.32	<0.01	<0.01

[a]Average rate of mosquitoes, regardless of infection status, containing virus in their legs

[b] Average rate of refeeding mosquitoes with a disseminated infection that transmitted virus

[c] Average rate of disseminated infection after ingesting RVFV multiplied by percentage of mosquitoes with disseminated infection that transmitted virus by bite

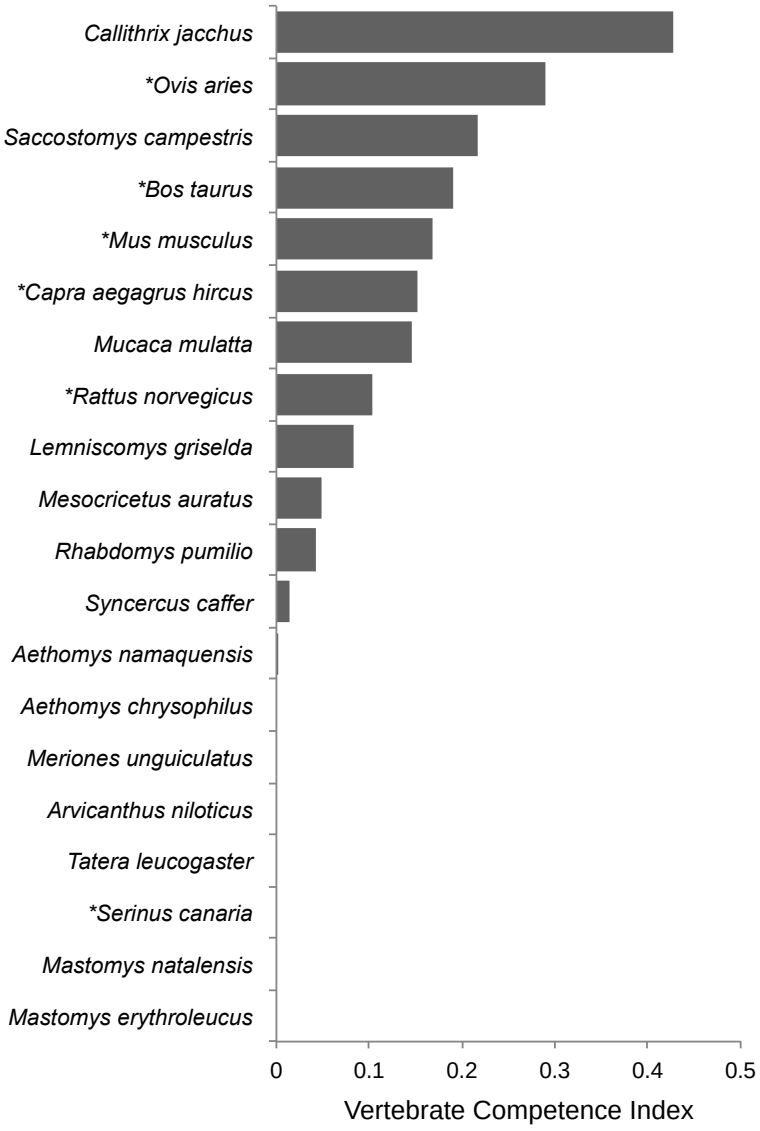

Figure 3.3. Rift Valley fever virus host competence index values for 20 vertebrate hosts based on experimental infection studies characterizing viremia profiles in PFU/ml or TCID^{50}. The vertebrate host competence index value depends on the viral titer circulating in the blood and the duration of the infectious viremia (Komar et al. 2003). Each value represents the sum of daily probabilities that an infected vertebrate host will transmit RVFV to a biting mosquito. This value was obtained by inserting the recorded daily viremia of experimentally infected hosts into the viremia-vector competence equation [% infectious = 0.062 (Log_{10} viremia) - 0.276 (R^2 = 0.27; p <0.001; N = 27)] (Figure S1, C). When a vertebrate host's viremia was calculated to be negative the daily infectiousness was set to zero. Conversion from TCID^{50} to PFU/ml was obtained by the equation: PFU/ml = TCID^{50}/ml x 0.69 (O'Reilly et al. 1994, Mena et al. 2003). *Denotes a vertebrate species found in the U.S.

Host competence: To estimate vertebrate host competence, published data and unpublished data provided by Dr. John Morrill from RVFV experimental infections (Figure 1) (Findlay and Daubney 1931, Swanepoel et al. 1978, Davies and Karstad 1981, Anderson et al. 1987, 1988, Peters et al. 1988, Rossi and Turell 1988, Morrill et al. 1989, Anderson et al. 1991b, Anderson et al. 1991a, Morrill et al. 1991, Rippy et al. 1992, O'Reilly et al. 1994, Pretorius et al. 1997, Gora et al. 2000, Mena et al. 2003, Geffers et al. 2010, Nfon et al. 2012, Smith et al. 2012) were inserted into a viremia-vector competence equation that describes the relative number of infectious mosquitoes resulting from feeding on a vertebrate host (Figure S1, C). Exposure viremia dosages ranged from $10^{4.3\text{-}10.2}$ PFU/ml at an incubation temperature of 26ºC. With this approach, 12 vertebrate species demonstrated reservoir competence by producing sufficient viremia titers to infect mosquitoes after exposure to RVFV, all of which were mammals (Figure 2) (O'Reilly et al. 1994, Komar et al. 2003, Mena et al. 2003). Vertebrate host species demonstrating competence for viral amplification were the following: sheep (*Ovis aries*, Class Artiodactyla), domestic cow (*Bos taurus*, Artiodactyla), domestic goat (*Capra aegagrus hircus*, Artiodactyla), mouse (*Mus musculus*, Rodentia); brown rat (*Rattus norvegicus*, Rodentia), the common marmoset (*Callithrix jacchus*, Primates); four-striped grass mouse (*Rhabdomys pumilio,* Rodentia); South African pouched mouse (*Saccostomus campestris,* Rodentia); Rhesus macaque (*Macaca mulatta*, Primates); Griselda's striped grass mouse (*Lemniscomys griselda*, Rodentia); African buffalo (*Syncerus caffer*, Artiodactyla); and namaqua rock rat (*Aethomys namaquensis*, Rodentia). Many species were considered incompetent because they did not develop a sufficient viremia profile to infect mosquito vectors ($\leq 10^{4.7}$ PFU/ml), such as the red

rock rat (*Aethomys chrysophilus*, Rodentia), african grass rat (*Arvicanthis niloticus*, Rodentia), guniea multimammate mouse (*Mastomys erythroleucus*, Rodentia), natal multimammate mouse (*Mastomys natalensis*, Rodentia), Mongolian gerbil (*Meriones unguiculatus*, Rodentia), Atlantic canary (*Serinus canaria*, Passeriformes), domestic chickens (*Gallus gallus*, Galliformes) and the Bushveld gerbil (*Taera leucogaster*, Rodentia).

The vertebrate host competence index averages based on taxonomy were the following: Class: Mammalian (0.17), Aves (0.00); Order: Primates (0.25), Artiodactyla (0.21), Rodentia (0.05); Family: Bovidae (0.21), Muridae (0.05), Cricitidae (0.05); Genus: *Ovis* (0.29), *Bos* (0.19), *Capra* (0.15), *Rattus* (0.04).

Table 3.2. Relative risk of mosquitoes contributing to Rift Valley fever enzootic transmission in the U.S.

Mosquito Species	Vector Competence (C_v) [a]	($\sum F_{vi}$) [b]	% Risk [c]
Aedes japonicus japonicus	0.37	3.10E-02	11.42%
Aedes thibaulti	0.15 ‡	2.30E-02	8.80%
Aedes canadensis	0.28	2.00E-02	7.42%
Culiseta inornata	0.15 *ƒ*	1.80E-02	6.75%
Wyeomyia mitchellii	0.15 *ƒ*	1.80E-02	6.63%
Aedes sollicitans	0.25	1.50E-02	5.37%
Coquillettidia perturbans	0.38	1.50E-02	5.36%
Aedes sticticus	0.15 ‡	1.40E-02	5.40%
Aedes aegypti	*0.08*	1.30E-02	5.04%
Aedes nigromaculis	0.15 ‡	1.20E-02	4.46%
Aedes cantator	0.07	9.60E-03	3.34%
Psorophora columbiae	0.18†	8.70E-03	3.25%
Aedes trivittatus	0.15 ‡	8.30E-03	3.12%
Aedes fulvus pallens	0.15 ‡	8.10E-03	3.04%
Aedes taeniorhynchus	*0.13*	7.80E-03	2.92%
Psorophora discolor	*0.18†*	7.00E-03	2.64%
Psorophora ferox	0.18	6.60E-03	2.49%

Table 3.2. Continued

Mosquito Species	Vector Competence (C_v) [a]	($\sum F_{vi}$) [b]	% Risk [c]
Aedes albopictus	0.13	5.90E-03	2.22%
Aedes atlanticus	*0.15*	5.70E-03	2.10%
Mansonia titillans	0.07 †	4.70E-03	1.78%
Aedes triseriatus	0.24	4.30E-03	1.57%
Aedes vexans	0.11	3.30E-03	1.26%
Culex erythrothorax	0.04	3.10E-03	1.02%
Culex salinarius	0.13	1.90E-03	0.71%
Culex cedecei	0.04 ‡	1.00E-03	0.37%
Deinocerites cancer	0.15 *ƒ*	9.90E-04	0.37%
Culex tarsalis	0.33	5.90E-04	0.22%
Culex erraticus	0.04	5.30E-04	0.19%
Culex stigmatosoma	0.11 ‡	3.70E-04	0.14%
Culex nigripalpus	0.01	3.30E-04	0.09%
Culex restuans	0.11 ‡	2.30E-04	0.09%
Anopheles crucians	<0.01	2.30E-04	0.08%
Anopheles quadrimaculatus	<0.01 †	2.10E-04	0.08%
Anopheles punctipennis	<0.01 †	2.10E-04	0.08%
Culex pipiens	0.12	1.70E-04	0.07%
Culex pilosus	0.04 ‡	1.20E-04	0.05%
Culiseta moristans	0.15 *ƒ*	1.10E-04	0.04%
Aedes infirmatus	0	8.28E-05	0.03%
Culex territans	0.17	4.80E-06	0.00%
Culiseta melanura	0.15 *ƒ*	3.40E-06	0.00%
Culex peccator	0.04 ‡	2.10E-07	0.00%
Aedes dorsalis	0	0.00E+00	0.00%
Culex quinquefasciatus	0	0.00E+00	0.00%

[a] Estimated Transmission Rate (C_v) (Values from Table 1)

[b] $(\sum F_{vi})$ for each mosquito species where $F_i = B_i^2 * C_i * C_v$

[c] $\sum F_{vi} \div$ total F_{vi} demonstrated by all mosquitoes

† Genus average (Anopheles: <0.01; Psorophora: 0.18; Mansonia: 0.07)

‡ Subgenus average (Aedes- Ochlerotatus: 0.15; Culex: Melanoconion: 0.04, Culex: 0.11)

ƒ Family average substituted (Culicidae: 0.15)

Vector amplification fraction: Among mosquito species evaluated, the vector amplification fraction ($\sum F_{vi}$) ranged from 0 to 0.018 (Table 3.2). The resulting index

was expressed as a weighted percentage relative to the total amplification fraction demonstrated by the 40 mosquito species included in this analysis, which ranged from 0% to 11.7% (Table 3.2; See Table S3 for calculations, Appendix A). This index estimates the relative probability that a mosquito will feed on an infectious vertebrate host, develop a disseminated infection into the salivary glands, and ultimately transmit RVFV to a vertebrate host during a subsequent blood-feeding event. Mosquito species with the highest amplification fractions were: *Ae. japonicus japonicus* (Theobald) (11.4%), *Ae. thibaulti (*Dyar and Knab*) (*8.8%), *Ae. canadensis* (Theobald) (7.4%), *Culiseta inornata* (Williston) (6.7%), *Wyeomyia mitchellii* (Theobald) (6.6%), *Ae. sollicitans* (Walker) (5.4%), *Cq. perturbans* (5.4%), *Ae. sticticus* (Meigen) (5.4%), *Ae. aegypti* (5.0%) and *Ae. nigromaculis* (Ludlow) (4.4%) (Table 3.2).

Vertebrate host amplification fraction: Overall four classes (Mammalia, Aves, Amphibia, and Reptilia), eight mammalian orders (Artiodactyla, Carnivora, Chiroptera, Didelphimorpha, Lagomorpha, Perissodactyla, Primates, Rodentia), six families (Bovidae, Cervidae, Cricitidae, Muridae, Sciuridae, Suidae) and seven genera (*Bos, Capra, Dama, Homo, Odocoilius, Ovis, Rattus*) of vertebrates were evaluated with this model. As indicated by vertebrate competence studies, only mammals are competent hosts and are estimated to contribute 100% of theoretical RVFV amplification in the U.S. The order Artiodactyla is estimated to contribute 64.3% of all theoretical mammalian RVFV amplification followed by the orders Lagomorpha (16.8%), Primates (6.8%), Carnivora (4.4%), Rodentia (0.8%), Perissodactyla (0.4%), Didelphimorpha (0.1%), and Chiroptera (0.0%) (Table S3, Appendix A). Because some blood meal data was only specific to the taxonomic resolution of Class there were undefined mammalian

hosts that represent 6.3% of the risk, which means all % risk estimates are potentially underestimated (Table S3, Appendix A). Similarly, within the Artiodactyla order 10.5% risk is undefined, therefore, the family Cervidae accounts for at least 56% of the theoretical RVFV amplification contributed to Artiodactyla, while Bovidae contributes 34%, and Suidae contributes <1% (Table S3, Appendix A).

Discussion

Vector competence: Rift Valley fever virus has been isolated from at least 40 African mosquito species and currently 19 North American species have been shown to be competent laboratory vectors of RVFV, several of which are known vectors of enzootic viruses of large mammals (e.g., *Cx. tarsalis* and western equine encephalitis virus or *Ae. taeniorhynchus* (Wiedemann) and Venezuelan equine encephalitis). These data suggest that a suite of mosquito vectors could potentially transmit RVFV should the virus reach North America (Turell et al. 2008b).

Overall, results from previous studies have indicated that vector competence for RVFV is variable between mosquito species and among different populations of the same mosquito species. These variations in vector competence within mosquito species could be due to differences in development temperatures, phenotype, or parasite interactions that facilitate or block viral transmission (Turell 1993, Vaughan and Turell 1996, Kilpatrick et al. 2005, Turell et al. 2010, Iranpour et al. 2011). Viral infection, dissemination rates, and transmission rates are also dependent on the titer of the viremic exposure (Turell et al. 1988). Because mosquito control methods vary for different

mosquito species, future RVFV transmission experiments are necessary to better understand variations in vector competence (Turell et al. 2010, Iranpour et al. 2011).

Vertebrate host competence: The vertebrate host competence index value depends on the viral titer circulating in the blood and the duration of this infectious viremia (Komar et al. 2003). As the classic RVFV transmission paradigm would hypothesize, which implicates peri-domestic livestock as important amplification hosts, the calculated vertebrate host competence index shows sheep, domestic cow, domestic goat, and African buffalo may potentially contribute to RVFV amplification (Figure 3.2) (Pepin et al. 2010). Primates from the new world also demonstrate a high competence suggesting humans may play a role in RVFV transmission. In the 1977 Egyptian outbreak of RVFV, Meegan et al. (Meegan 1979) demonstrated that humans produce a viremia of $10^{4.1}$-$10^{8.6}$ LD_{50}, but how this relates to vertebrate competence values of new world monkeys remains unclear. The vertebrate competence index indicates rodents can be competent amplification hosts, but their role in viral amplification may be limited as mosquitoes rarely use them as blood meal hosts. The lack of RVFV competence for parakeets, canaries, and pigeons has been described, however our analysis of the class Aves was limited to a study evaluating the Atlantic canary (*S. canaria*) (Findlay and Daubney 1931) and an unpublished study by Turell et al. evaluating domestic chickens (*G. gallus*), both of which have a competence index of zero.

It is apparent that RVFV viremia profiles vary between vertebrate hosts (Figure 3.1 and Figure 3.2). These variations emphasize the importance of characterizing RVFV viremia profiles of domestic and wild animals present in the U.S., especially since their immune systems may be more susceptible to a foreign virus. Experimental infection

studies evaluating vertebrate species from the U.S. with larger sample sizes will manifest in more accurate competence values and provide a finer set of data to better implicate important vertebrate hosts for RVFV amplification should the pathogen emerge in the U.S.

Vector amplification fraction: Previous experimental transmission studies conclude that *Cx. tarsalis* and *Ae. j. japonicus* are the most competent vectors with the highest risk to transmit RVFV should it arrive in the U.S.; however, vector competence does not directly imply a significant role in disease transmission (Gargan et al. 1988, Turell et al. 1988, Turell et al. 2007, Turell et al. 2008b, Turell et al. 2010, Iranpour et al. 2011, Turell et al. 2013). The vector amplification fraction provides a means to quantitatively compare theoretical risk of various mosquito species based on their potential to contribute to RVFV transmission in the U.S. Vector-host contact rates, as dictated by mosquito feeding patterns, is a key component to consider when evaluating the risk of a mosquito vector, as illustrated by the *Cx. tarsalis* mosquioto. *Cx. tarsalis* is one of the most competent vectors of RVFV in the U.S. (Table 3.1), which feeds mainly on avian hosts (Table S2, Appendix A), and therefore, is predicted to have a low amplification fraction in comparison to other vectors as seen in Table 2 (0.2% of total risk). Recent transmission experiments by Turell et al. (Turell et al. 2013) suggest that *Ae. j. japonicus* mosquitoes are the most competent vector of RVFV in the U.S. (previously *Cx. tarsalis*). The vector amplification fraction calculated in this study further implicates *Ae. j. japonicus* as a high risk vector with the potential to contribute to RVFV transmission in the U.S. (11.4%, Table 2). This invasive mosquito has a high vector competence (0.37, Table 1), feeds heavily on competent hosts (Artiodactyla 80%

and Primates 16%, Table S1), and is found in all U.S. states east of the Mississippi river except for Florida and Louisiana (Kaufman and Fonseca 2014). Should RVFV spread to the U.S., *Ae. j. japonicus* populations should be carefully monitored for infection and potentially targeted for mosquito control (Turell et al. 2013).

Ae. sticticus and *Cs. inornata* both demonstrate varying degrees of transmission competency, but vector competence for these two species remains undetermined. In the study by Iranpour (Iranpour et al. 2011), RVFV was detected in the saliva of *Ae. sticticus* after experimental infection and *Cs. inornata* demonstrated both a high infection rate (100%; N=5) and high dissemination rate after exposure to RVFV viremia between $10^{7.9}$ to $10^{9.4}$ PFU/ml (60%; N=3). Considering both these species feed heavily on the order Artiodactyla (*Ae. sticticus* 94% and *Cs. inornata* 80%, Table S2) their role in RVFV transmission in the U.S. is uncertain and should be evaluated. *Ae. trivittatus* is another mammal-biting mosquito estimated to have a moderate role in transmission that occurs in large populations in the Eastern U.S. and is lacking experimental data.

Among the top 10 mosquito species theoretically contributing to RVFV transmission in the U.S., only five species (*Ae. j. japonicus, Ae. sollicitans, Ae. canadensis, Cq. perturbans* and *Ae. aegypti*) have data comprehensive enough for this analysis. This underscores the lack in data necessary to estimate the theoretical role of different mosquito vectors in RVFV transmission in the U.S. Of those ranking as high-risk for contributing to RVFV enzootic transmission, some are limited in geographic range within the U.S. (e.g. *Wy. mitchellii*) underscoring the importance for including spatial and temporal mosquito abundance data while evaluating local regions for RVFV transmission potential. These results indicate a gap in experimental transmission data

and requisite further vector competence evaluations to properly evaluate the potential risk of mosquitoes contributing to RVFV transmission in the U.S. Future studies should pay particular emphasis on assessing and re-evaluating the regional transmission competence and population dynamics of *Ae. j. japonicus, Cs. inornata, Ae. sollicitans, Ae. sticticus* (only 13 individuals have been evaluated) (Kaufman and Fonseca 2014), *Ae. nigromaculis* (all data from one study in 1988) (Gargan et al. 1988), and *Ae. trivittatus* because of their estimated risk and abundance in the Eastern U.S.

Vertebrate host amplification fraction: Artiodactyla, Lagomorpha, Primates, and Carnivora are estimated to be theoretically involved in RVFV amplification in the U.S., while the Mammalian orders Perissodactyla, Didelphimorpha and Chiroptera are not (Table S3). The order Chiroptera may deserve further investigation as a potential reservoir host as RVFV has been isolated from several bat genera (Calisher et al. 2006) and even though antibodies against RVFV have been detected in horses, the family Equidae has demonstrated low viremic titers (Yedloutschnig et al. 1981, Olive et al. 2012).

Our results suggest that Artiodactyla contributes 64.3% of the theoretical risk for RVFV transmission in the U.S., which supports the currently held paradigm that Artiodactyla are the most important vertebrate host for RVFV amplification and transmission. Research and control efforts should place a particular emphasis on the families Cervidae and Bovidae as they account for at least 56% and 34% of the total risk contributed by the order Artiodactyla, respectively (Table S3). Based on the 2012 Census of Agriculture (USDA National Agriculture Statistics Service) there are about 90 million cattle, 5 million sheep, 3 million goats, and 300,000 captive cervids. There are

an estimated 25 million white-tailed deer (*Odocoileus virginianus*) in the U.S. (Miller et al. 2003). Throughout the U.S. captive and wild ruminants are widely available and heavily utilized by mosquitoes (Table S2) emphasizing their potential role in RVFV transmission.

It is important to note that the role of the order Lagomorpha (17%) may be inflated by the vector amplification fraction because their estimated vertebrate competence was based on a mammalian average (0.17). No studies provide evidence supporting that Lagomorphs are capable of producing an infectious viremia, but little research has evaluated their role in RVFV ecology (Findlay and Daubney 1931). Similarly, vertebrate competence of the order Carnivora is lacking. Studies demonstrate susceptibility in cats, dogs, ferrets and serological studies demonstrate antibodies against RVFV in lions (*Panthera leo*) and the polecat (*Ictonyx striatus*) (Gear et al. 1955, Darsie and Ward 2005, Olive et al. 2012, CDC 2014a)[72,75-77]. Experimental evaluation within the Order Carnivora should focus on the competence of dogs, cats, and raccoons because mosquito host-feeding is mainly associated with these species (Table S2).

Arbovirus amplification in domestic and peridomestic animals and eventual spillover to humans is a well-documented phenomenon. However the permanent establishment of dengue and chikungunya viruses in urban, tropical environments demonstrates the ability for arboviruses to subsist through human reservoirs (Weaver and Reisen 2010), especially important given the recent emergence of chikungunya in the Caribbean in 2013 (CDC 2014a). The vertebrate amplification fraction estimates Primates will contribute about 7% of the theoretical RVFV amplification in the U.S. (Table S3). This estimate is based on the assumption that the human viremia profile is

comparable to Rhesus macaques and common marmosets. Viremia data from new-world monkeys as a surrogate for human viremia may overstate the role of humans in RVFV transmission. In the 1977 Egyptian outbreak of RVFV, Meegan et al. (Meegan 1979) demonstrated that indeed humans produce a viremia of $10^{4.1}$-$10^{8.6}$ LD_{50}, however socio-economic factors in the U.S. may limit mosquito-human contact rates, and dampen any role in amplification of RVFV. As such, the role of humans as vertebrate hosts for RVFV amplification remains unknown.

Hypotheses implicating rodents as important hosts for RVFV amplification started when high death rates of *Arvicanthis abyssinicus* and *Rattus rattus* coincided with sheep deaths caused by RVFV in 1932 (Olive et al. 2012). Experimental studies demonstrate rodents can be competent amplification hosts for RVFV (Figure 1 & 2) depending on the viremic dose, age, and species (Olive et al. 2012). However, results from the vertebrate amplification fraction suggest members of the order Rodentia are at low risk for contributing to RVFV transmission because of infrequent contact with mosquitoes (Table S2).

Limitations: Given the gaps in data preventing a complete analysis of the amplification fraction potentially produced by all mosquito and vertebrate hosts, we made several assumptions that limit the accuracy of these results. This analysis does not account for spatial or temporal variation in mosquito abundance or competence, both of which are known to be spatially heterogeneous and influence pathogen transmission dynamics (Darsie and Ward 2005, Turell et al. 2010). Many of the mosquito species and vertebrate hosts included in the analysis have no competence data and for these species we assigned taxonomic averages. It is important to note that taxonomic averages are not

always appropriate and extrapolations based on taxonomic averages for both vectors and vertebrate hosts can lead to spurious results (e.g. disparate RVFV vector competence exists for several *Culex* spp.) (Perez-Ramirez et al. 2014). By combining data on 39 studies reporting mosquito host-feeding patterns in different regions and landscapes across the U.S, we aim to incorporate a robust measure of vertebrate host utilization. However, the mosquito host-feeding patterns for several species are based on a single study, and given the importance of host availability (Chaves et al. 2010), a single study might not be broadly representative of host feeding patterns. Despite these limitations, the results from this study highlight potentially important mosquito vectors and vertebrate hosts of RVFV that should be monitored in the event RVFV emerges in the U.S. Additionally, this study identifies knowledge gaps that can be filled by future experimental work on both vectors and vertebrate species.

Conclusion: World-wide zoonotic disease emergence is an increasing phenomenon due to environmental changes, ecological disturbances, and globalization(Patz et al. 2000). The U.S. has already been affected by the emergence of WNV, recently identified a new zoonotic disease (Heartland virus) (McMullan et al. 2012, Savage et al. 2013)[80,81], and is threatened by the spread of chikungunya virus to the Caribbean (CDC 2014a). During the initial epidemics of WNV in the U.S. in 2002 and 2003, many mosquito control programs did not have a strong focus on *Culex* spp. mosquitoes. As knowledge of the WNV transmission system increased, vector control has improved by targeting *Culex* species to reduce human exposure events. The delay of *Culex* spp. vector control might have allowed more human WNV disease and

may have contributed to the rapid spread of the virus across the U.S. highlighting the importance of *a priori* response strategies for potential viral threats.

RVFV is of particular concern in the U.S. because it causes disease in humans and economically important animals alike. Even more, its emergence throughout Africa and the Arabian Peninsula make it a conceivable threat for future geographic expansion. We combined published data to provide an estimate of each vector and vertebrate taxon's contribution to RVFV amplification in the U.S. However, major gaps in knowledge exist preventing a comprehensive evaluation of potentially important vectors and vertebrate hosts to RVFV transmission in the U.S. Results, combined with information on abundance of vectors and vertebrate hosts, can provide guidance for proactive management programs and aid parameterization for further modeling efforts evaluating environmental receptivity of RVFV in the U.S. (Kakani et al. 2010, Barker et al. 2013). Additionally, the framework of this analysis can also be applied to regions in Africa and the Arabian Peninsula with endemic RVFV transmission to help identify important vectors and vertebrate hosts for vector control and vaccination programs.

Future research efforts should focus on: 1) further evaluating the dose-dependent nature of RVFV vector competence in geographically widespread mosquitoes quantified as high risk: *Ae. j. japonicus, Ae. canadensis, Cs. inornata, Ae. sollicitans, Cq. perturbans, Ae. sticticus, Ae. nigromaculis, Ae. cantator and Ae. trivitattus* 2) characterizing local vector competence in high risk areas for RVFV introduction, and 3) evaluating the RVFV viremia profiles of vertebrates in the U.S. with particular emphasis on the orders Artiodactyla (Cervidae, Bovidae, Suidae), Lagomorpha, and Carnivora (domestic dog, domestic cat, raccoon), respectively.

CHAPTER IV

CONCLUSION

World-wide zoonotic disease emergence is an increasing phenomenon due to environmental changes, ecological disturbances, landscape domestication, and globalization (Patz et al. 2000). Although introduction events are often stochastic and sometimes unpredictable, scenarios of introduction, no matter how unlikely, will occur with more frequency as international connectivity increases. The U.S. is not immune to vector-borne disease import and has already been affected by the emergence of WNV and is threatened by the spread of chikungunya virus to the Caribbean (CDC 2014a). RVFV is of particular concern in the U.S. because it causes disease in humans and economically important animals alike. Even more, its emergence throughout Africa and the Arabian Peninsula make it a conceivable threat for future geographic expansion.

This thesis aims to improve the prediction and mitigation of RVFV importation and transmission in the U.S. by identifying key pathways of RVFV introduction and evaluating potentially important vectors and vertebrate hosts to RVFV transmission in the U.S.. Results, combined with information on abundance of vectors and vertebrate hosts, can provide guidance for local management programs to reduce the risk of RVFV introduction. New York, Washington D.C., Atlanta, and Houston are implicated as high-risk regions for introduction, and future research efforts should evaluate locally abundant vectors for RVFV transmission competence and evaluate the host competence of locally abundant vertebrate hosts, especially Artiodactyla, Lagomorpha, and Carnivora. With fine-scale abundance data and host competence data the intensity of RVFV transmission

can be predicted at a local scale. In the short-term control programs should work with port authorities to monitor international traffic for infectious mosquitoes or humans arriving from Saudi Arabia, South Africa, Nigeria, Egypt, Senegal, Ethiopia, Yemen and Angola, especially during RVFV epidemic periods.

Should RVFV reach the U.S. via an infected traveler or mosquito, clear case definition for clinicians will be essential for the effective diagnosis of RVFV. It will be state and county public health departments and the associated vector control agencies that will be critical members of the response task force. Although introduction risk and important transmission hosts are only pieces of the larger invasion complex, results from this thesis offers regional guidance for control efforts, vaccination strategy, and provides parameter estimates for more comprehensive modeling efforts combining environmental receptivity and epidemiological factors to evaluate the introduction, establishment, and spread of RVFV in the U.S.

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APPENDIX A

Table S1. To standardize Rift Valley fever virus experimental transmission data two equations referenced in row 60 that estimate the viremia dose dependence of dissemination rate and transmission rate (see Figure S1-A and Figure S1-B) were utilized to interpolate what the dissemination and transmission rates would be at the exposure viremia of $10^{7.5}$ PFU/ml. A species average was calculated (Columns H and K) and multiplied together to calculate the vector competence at the same exposure viremia (Column L). This table is freely available online through the *PLoS NTD website.*
doi: 10.1371/journal.pntd.0003163.s002
(XLS)

Table S2. Number and percentage of mosquito blood meals grouped by vertebrate host class and selected orders. Data is based on 39 combined mosquito feeding studies across the United States. This table is freely available online through the *PLoS NTD website.*
doi:10.1371/journal.pntd.0003163.s003
(DOCX)

Table S3. Vector competence data, vertebrate competence data, and mosquito feeding patterns were combined to estimate the Rift Valley fever virus amplification fraction displayed by the vectors and vertebrates in the United States. In the F_{vi} equation ($F_{vi} = B_i^2 * C_i * C_v$), the number of infectious mosquitoes resulting from feeding on a vertebrate host, Fvi, is equal to vertebrate host competence (C_i: located in row 5), multiplied by the vector competence (C_v: located in column C), multiplied by the fraction of the total blood meals from host i squared (B_i^2: indicated in each cell as a number divided by total blood meals in column B). All F_{vi} values reflecting a vector-vertebrate pair were summed for each mosquito species (Column AC) and summed for each vertebrate species (Row 49). To present these values as a % risk (Column AD) the values of the vector amplification fraction were weighted over the total amplification demonstrated by all vectors, then multiplied by 100. To express the vertebrate contribution to RVFV amplification as a % risk (Row 50), the amplification values at the taxonomic resolution of Family and Order were weighted over the total amplification estimated by all mammals (Cell: Y49), then multiplied by 100. Because some blood meal data was only specific to the Mammalian class, 6.3% of the estimated amplification fraction is undetermined at the resolution of Order. Therefore, all order % risk estimates are minimum estimates. This table is freely available online through the *PLoS NTD website.*
doi:10.1371/journal.pntd.0003163.s004
(XLS)