DISSERTATION

ILLUSTRATING THE POST-INTRODUCTION ECOLOGY OF RIFT VALLEY FEVER VIRUS IN THE UNITED STATES OF AMERICA

Submitted by

Daniel A. Hartman

Department of Microbiology, Immunology, and Pathology

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2021

Doctoral Committee:

Advisor: Rebekah C. Kading

Gregory Ebel
Brian Foy
Colleen T. Webb

ABSTRACT

ILLUSTRATING THE POST-INTRODUCTION ECOLOGY OF RIFT VALLEY FEVER VIRUS IN THE UNITED STATES OF AMERICA

Rapid urbanization, increasing international travel, and our changing climate are modifying the existing interspecies interactions at the interfaces between human, wildlife, and livestock interfaces, increasing the potential for outbreaks and transboundary disease introductions. It is more important than ever to maintain proactive research programs that integrate data across disciplines to maintain a working knowledge the potential transmission cycles of high-threat pathogens in novel environments. For vector-borne pathogens, entomological parameters, as well as interactions with the pathogen and host are highly informative, while representing avenues for control prior to an introduction.

The work of this dissertation seeks to inform the potential transmission cycles of Rift Valley fever virus (RVFV) in the United States. Currently restricted to Africa and the Arabian Peninsula, RVFV infects domestic ruminants and humans with substantial degrees of morbidity and mortality. Throughout its current range, transmission involves a diversity of vectors, which are capable of transmitting the virus horizontally between vertebrates and vertically to mosquito progeny. The ecology of RVFV presents a great deal of complexity, with many unknown factors such as the roles of wildlife hosts, and relative contributions of vectors to transmission.

To gain some insight into the potential ecology of RVFV in the United States, we first performed extensive sampling of mosquitoes at feedlots in northern Colorado to explore the potential for these operations to act as amplification foci after an introduction. We discovered

that the most competent mosquito in Colorado that has been tested to date is highly abundant, and feeds readily on cattle, making these operations high risk for an epizootic.

In this previous study we also identified blood-feeding on deer for some mosquitoes (*Ae. vexans*, *Ae. melanimon*, *Ae. dorsalis*) as well as domestic ruminants. We then set out to determine whether *Ae. melanimon* is capable of transmitting RVFV biologically, as there were no data to date for this species. We conducted infection experiments with these three *Aedes* species and others to determine the efficiencies with which they can transmit RVFV horizontally and vertically. We found substantial evidence for horizontal transmission and susceptibility of ovaries to infection, a prerequisite for vertical transmission, in all species but for *Aedes increpitus*. For these data we also developed a model to estimate the infection susceptibilities and barriers in mosquito organs in a functional manner.

Finally, we sought to investigate the potential for transmission of RVFV in white-tailed deer by describing the community of mosquitoes in a riparian woodland habitat. We revealed some interesting patterns in the abundances of some mosquito species which stood in contrast to those observed at the feedlots. Several mosquito species exhibited the capacity to feed on white-tailed deer, including *Ae. increpitus* and *Ae. vexans*, both previously shown the ability to transmit RVFV by bite. By scoring the digestive stage of the blood meals in mosquitoes that were later identified to vertebrate source species, we uncovered an interesting pattern suggestive of interrupted feeding on eastern cottontail rabbits, in contrast to blood meals taken from white-tailed deer, from which mosquitoes fed to repletion. The implications of interrupted feeding for transmission by mosquitoes is unclear, but highlights the important factor of behavioral interactions between mosquito vectors and hosts which is often overlooked.

ACKNOWLEDGEMENTS

First and foremost, I have an enormous amount of gratitude for my advisor, Rebekah Kading, for her support and mentorship. Under her leadership I have become a proper medical entomologist and have gained a new multi-level perspective on disease transmission. As my mentor, she has provided an excellent example of how to be a great scientist as well as a great human being. Her seemingly limitless compassion, and collaborative, empathetic spirit has been massively inspirational.

The members of my dissertation committee have also been crucial to the success of the work detailed here, as well as my professional development. The evaluation of this work and my progress as a scientist from experts across fields with a diversity of perspectives has been extremely valuable. Thanks so much to Greg Ebel, Brian Foy and Colleen Webb for your time and support throughout this process.

This research was made possible and fun by many undergraduate trainees in the laboratory. With them I have become adept at training students, while they have added a level of joy to the scientific process and changed the way that I measure my success. Many thanks to Therese Kondash, Lucy Robb, and Aya Safira.

I owe the technical virology skills that I've gained over the last five years almost exclusively to Erin Borland. Starting with basic cell culture techniques, through virus handling and assays, her thoroughness and patience throughout my formal training in the wet laboratory brought me into the field of virology with confidence. The Colorado State University biosafety

staff, especially Robert Ellis and Heather Blair also provided invaluable training on containment procedures. I fully appreciate their dedication to the safety of students.

Thanks also to Will Schlattmann. Aside from sharing our resources, working with a local vector control agency puts the feet of this work on the ground. It's also a great reminder of why I'm doing what I'm doing.

DEDICATION

This work is dedicated to my grandmother, Marian Hartman, who instilled in me the importance of humility, compassion, and baseball.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: EXPLORING THE ECOLOGICAL COMPLEXITY AND VARIATION THROUGHOUT THE EXTANT RANGE OF RIFT VALLEY FEVER VIRUS

Rapid urbanization, increasing international travel, and our changing climate are modifying the existing interspecies interactions at the interfaces between human, wildlife, and livestock interfaces, increasing the potential for outbreaks and transboundary disease introductions (Patz et al. 1996, Gould et al. 2017, Hassell et al. 2017, Mangili et al. 2021). Vector-borne diseases, and arthropod-borne viruses in particular represent a large part of transboundary disease expansions (Gould et al. 2017, Ryan et al. 2019). Due to the current increase in the expansion of arboviruses, it is more important than ever to maintain proactive research agendas with the objective of informing introduction risks and potential transmission cycles.

The introduction of West Nile Virus teaches us that reactive measures to control novel pathogens is inadequate to prevent their establishment. Introduced in 1999 into New York City, it established quickly in avian populations, while causing human cases including encephalitis and febrile illness (Nash et al. 2001). Only after the isolation of virus as part of avian necropsies was the causative agent recognized to be WNV (Steele et al. 2000). Over the next decade, WNV spread across North America to reach the west coast, though the route of introduction for the introduction remains elusive (Roehrig 2013).

Much of the disease control that has been implanted in the wake of the WNV introduction has involved vector control; this, in turn has been informed predominantly by research on the ecology of mosquito vectors of WNV. Perhaps the most important discoveries have been the incrimination of North American vectors, as well as investigations into their feeding behaviors,

which include a higher proportion of humans late in the season to cause human cases (Reeves et al. 1965, Kent et al. 2009, Campbell et al. 2013). It is impossible to calculate the number of human cases that have been mitigated by vector control programs that have been able to focus resources with this knowledge.

Predicting the next pandemic arbovirus may be difficult; however, we may be able to make some prioritization based on past pandemic arboviruses. The introduction of WNV to the United States came on the heels of several other outbreaks around the world, including the introduction to Romania in 1996 (Campbell et al. 2006), and a major outbreak in Israel which was the likely source for the U.S. introduction (Lanciotti et al. 1999). Likewise, the Zika virus pandemic was causing outbreaks in the South Pacific with significant human disease (Duffy et al. 2009, Cao-Lormeau et al. 2014), prior to introduction to Brazil and spread throughout the Americas (Petersen et al. 2016).

If recent and current range expansions bear the mark of high introduction risk for North America, Rift Valley fever virus (RVFV) has earned its place as a Category A High Priority Pathogen ("NIAID Emerging Infectious Diseases/ Pathogens | NIH" 2021). Recently RVFV has been expanding its range from continental Africa to include Egypt, the Arabian Peninsula, and Madagascar in recent decades (discussed in detail below). There have been a number of confirmed cases of RVFV in Mali who subsequently travelled to France, Ukraine, and Crete, as well as suspected cases involving travel elsewhere (Tong et al. 2019). China recently had its first imported case of RVF in a laborer returning from Angola (Liu et al. 2017); this patient maintained a measurable blood viremia for 30 days under the care of medical staff. Risk of RVFV introduction has long been assumed for the United States, and this dissertation represents

primary research to investigate the entomological and ecological factors that will be informative to risk and control.

This objective of this first chapter is to explore the variability of RVFV epidemiology and ecology throughout its current range, while highlighting historic outbreaks and recent introductions. The epidemiology of RVFV transmission is determined by a number of interacting factors, including environmental and entomological factors, as well as agricultural practices. By understanding the contributions of these factors to the regional ecologies of RVFV, we hope to gain some insight into the potential ecology of RVFV in the United States.

1.1 Rift Valley Fever Virus Molecular Epidemiology

Rift Valley fever virus RVFV is a mosquito-borne virus in the family Buyavirales, and the order Phlebovirus. It is an enveloped virus with a single-stranded RNA genome that is comprised of three negative or ambisense segments: S, M, and L (Pepin et al. 2010). The most recent phylogenetic analyses support the existence of thirteen clades (A-M); these show a high degree of geographic mixing throughout the endemic distribution of RVFV (Jansen van Vuren et al. 2019). Genomic characterization of virus isolates during an outbreak in Kenya has demonstrated the contribution of multiple lineages within an outbreak, as well as reassortment of M segments between lineages, producing viruses with new combinations of segment genotypes (Bird et al. 2008). Reassortment has also occurred during an outbreak in Tanzania, producing a novel combination of a Tanzanian M segment with Kenyan S and L segments (Samy et al. 2017). Genetic analysis of the recent RVFV case in China represented a novel reassortment, with L and M segments resembling segments from Zimbabwe, Kenya, and South Africa. The S segment clustered with strains from Egypt, Madagascar, Zimbabwe, Central Africa, Namibia and South Africa (Liu et al. 2017). Still, the contributions of reassortment to the epidemiology of disease, or

differences in replicative fitness between lineages in vertebrates and vectors are unclear (Pepin et al. 2010).

1.2 Rift Valley Fever Virus Ecology – A Historical Overview of Regional Patterns

In order to understand what the potential ecology of a pathogen may look like outside of its current range, it is crucial to understand the ecology within its current distribution. For RVFV, the consensus ecological model is based on the understanding of the ecology in east-Africa (Bird et al. 2009) (**Figure 1.1**). Briefly, high rainfall events trigger the hatching of *Aedes* mosquito eggs in large numbers, some of which are infected by vertical transmission. Amplification begins in ruminants (cattle, sheep, goats) when infected by these mosquito vectors. Humans can be infected by vector-borne transmission, primarily by *Culex* species, but also *Aedes*. Human infections can also result from the handling of infected ruminant tissues, putting veterinary care providers and abattoir workers are at especially high risk (Mohamed et al. 2014, Msimang et al. 2019). Infectious *Aedes* species mosquitoes then lay eggs near the edges of the floodwater pools, or 'dambos'. Due to vertical transmission in *Aedes spp.* mosquitoes, these egg populations can serve as a reservoir for the virus, allowing the virus to persist until the next flooding event. Low levels of enzootic transmission also occur, although there is evidence for strong involvement by specific vertebrate species.

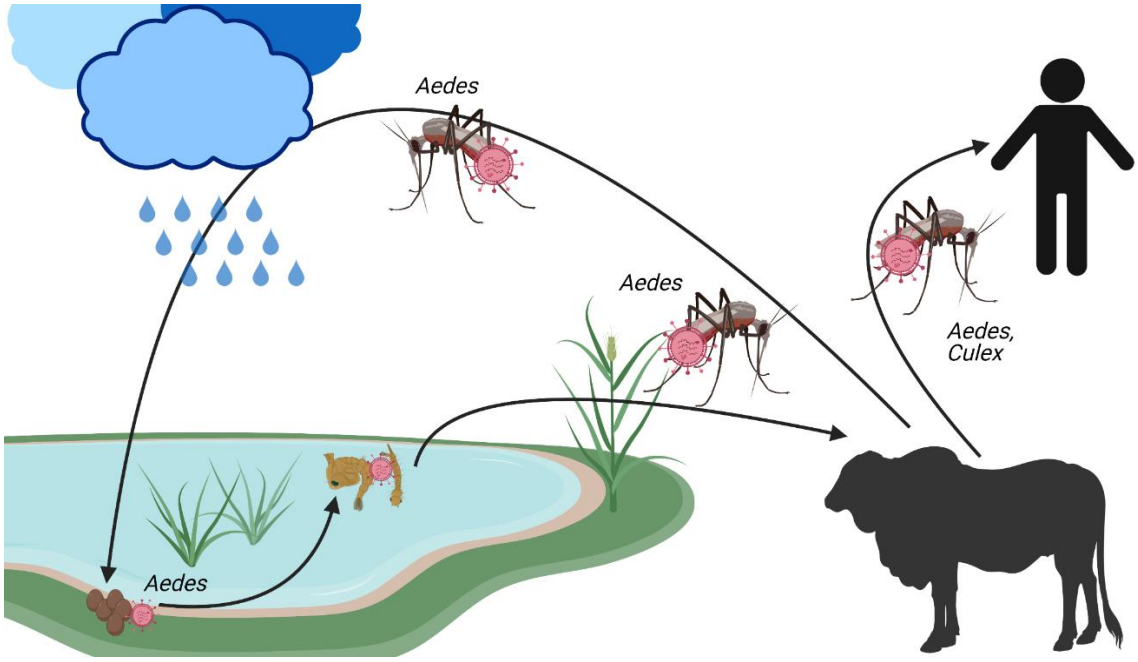


Figure 1.1. Schematic of the Rift Valley fever virus transmission cycle.

As RVFV has spread from its focus of eastern Africa over the last 90 years, it has taken on a more complexity in these new environments (Bron et al. 2021). While this transmission cycle may be somewhat generalizable, understanding this complexity is crucial to forming an illustration of the potential transmission of RVFV in the United States, similar to **Figure 1.1**. The current range of RVFV includes a significant amount of variation in the mosquito species, vertebrate species, environmental conditions, agricultural practices, and the complex interactions between all of these factors. RVFV ecology in the United States post-introduction is unlikely to closely resemble the generalized African transmission cycle; it is informative then to review the geographic variation in RVFV ecology throughout its extant range, so that the known vector ecology can be applied to the United States, and research gaps filled.

1.2.1 Eastern Africa

By far the most research has been conducted on RVFV transmission in its original foci of eastern Africa, informing the description of the transmission cycle above. The original description of RVFV resulted from a 1930 outbreak on a sheep ranch near Lake Naivasha in Kenya (Daubney et al. 1931). This epizootic exhibited the common clinical signs in these animals, with high incidences of abortions, hepatic necrosis, and high mortality in ewes (Pepin et al. 2010). The link between exceptionally high rainfall, as well as the involvement of mosquito vectors in this epizootic was noted within this report, and healthy animals were relocated to higher altitudes (Daubney et al. 1931).

The first formal investigations into the link between high rainfall and the onset of epizootic/epidemic events focused on the temporal patterns of disease in eastern Africa, wherein outbreaks typically occur in 10-15 year cycles (Davies et al. 1985). High sea surface temperature anomalies in the Indian and Pacific Ocean preceding very high rainfall in this region lend strong predictive power for major outbreaks (Linthicum et al. 1999).

This observation, along with the apparent lack of livestock transmission during interepidemic period, led to the speculation of transovarial transmission among *Aedes spp.* mosquitoes as a mechanism of viral persistence between epidemics/epizootics. Floodwater breeding *Aedes spp.* oviposit desiccation resistant eggs into moist soil, and these must dry and be re-inundated for hatching to occur. Strong evidence for this phenomenon was provided by subsequent mosquito collections, for which immature stages were collected in naturally and artificially flooded dambos (Linthicum et al. 1985). This resulted in the isolation of RVFV from reared eight pools consisting of both male and female *Ae. lineatopennis* mosquitoes, assumed now to be *Ae. mcintoshi* (Huang 1985). This collection, together with the lack of isolations

during interepidemic periods (Linthicum et al. 1985), represent the strongest base of evidence for the contribution of vertical transmission among *Aedes* mosquitoes to viral maintenance.

While these collections are a seminal part of the body of research on RVFV, the importance of vertical transmission of RVFV to viral maintenance between epidemics is not without controversy. Seroconversions of sheep and goats born during an interepidemic periods in Kenya have been reported, indicating low rates of transmission during this period implicating cryptic sylvatic transmission (Rostal et al. 2010). This is supported by evidence from virus isolation of mosquito pools representing *Culex*, *Mansonia*, *Anopheles*, *Coquilletidia*, and *Aedes*, including *Aedes mcintoshi* (Rostal et al. 2010). More recent mathematical modeling also supports that desiccation resistant *Aedes* eggs alone likely does not account for all interepidemic maintenance (Manore and Beechler 2015). The observation of seroconversions concentrated near forest edges in Kenya during interepidemic periods also supports the involvement of wildlife (Davies 1975, Murithi et al. 2011). Still, interepidemic transmission among livestock is considered to be quite low in comparison with other regions with RVFV circulation.

A number of wildlife species have been speculated to act as wildlife reservoirs of RVFV. Perhaps the most evidence exists for the involvement of the African Buffalo (*Syncerus caffer*). Experimental infections have supported the susceptibility and production of blood viremias (Davies and Karstad 1981). Isolations of virus from wild buffalo, however have been elusive. Detections of viral RNA by RT-qPCR may be the strongest evidence for natural infections in these animals (Bird et al. 2008). Other speculated reservoir species include giraffes, impala, white rhinoceroses, bushbuck, and waterbuck (Davies 1975, Anderson and Rowe 1998, Fischer-Tenhagen et al. 2000, Paweska et al. 2005, 2008, Bird et al. 2008, Evans et al. 2008).

1.2.2 Madagascar

The isolation of Madagascar from the eastern coast of Africa, as well as its unique biotypes and mosquito fauna makes for a potentially unique natural laboratory for the study of arbovirus ecology. Rift Valley fever virus has been recognized in Madagascar since 1979, when it was isolated from pools of *Mansonia uniformis*, as well as multispecific pools in the absence of documented human or livestock cases (Fontenille 1989). In 1990-1991, a significant outbreak of RVFV occurred. Observations included the abortions of Zebu cattle and several human cases on the east coast and central highlands, including one human mortality (Morvan, Fontenille, et al. 1991, Morvan, Saluzzo, et al. 1991). Unfortunately, entomological investigations were performed but there were no virus isolations.

Subsequent outbreaks occurred during the rainy seasons of 2008 and 2009, also exhibiting classical transmission including cattle abortions and human disease, coinciding closely with a major outbreak throughout the horn of Africa (Andriamandimby et al. 2010). Virus was isolated from mosquitoes from the Central Highland during this outbreak: *Anopheles coustani*, *An. squamosus*, and *Culex antennatus.* Phylogeographic analyses of these isolates as well as clinical samples have provided evidence for multiple introductions of the east-African lineage as the causes of these outbreaks, rather than circulation of the original 1979 isolate, between these two events (Carroll et al. 2011, Ratovonjato et al. 2011). The likely route of introduction for both was the importation of livestock from continental Africa (Morvan, Saluzzo, et al. 1991, Carroll et al. 2011).

In Madagascar, there exists a very wide range of mosquito taxa with known associations with RVFV elsewhere, spanning 23 species and 5 genera (Tantely et al. 2015). Only of these, *Cx. antennatus* and *An. coustani*, has been incriminated as a local RVFV vector based virus

isolations, vector competence experiments, and demonstration of feeding on susceptible hosts (Reeves 1958, Tantely et al. 2015, Nepomichene et al. 2018). Still, the diversity of incriminated and potential vectors across the island may inform some of the ecological complexity, as these represent a diversity of life history strategies as permanent water breeders (*Culex*, *Anopheles*, *Mansonia*), floodwater species (Aedes spp.) as well as container breeders (*Ae. aegypti*, *Ae. albopictus*).

Madagascar represents an outlier for which classical RVFV transmission model may not apply. The 1990-1991 outbreak did not follow abnormally high rainfall, but actually occurred prior to the 1994 Geralda cyclone (Lancelot et al. 2017). Risk models developed for the horn of Africa also failed predict the 2008-2009 outbreak due to inadequate rainfall (Sang et al. 2010). Instead, the major drivers behind RVFV in transmission appear to be more closely related to other factors: the illegal importation of cattle from east African countries (Lancelot et al. 2017), and the widespread distribution of *Cx. antennatus* which can reproduce effectively outside of heavy flooding conditions (Sang et al. 2010). For these reasons, Madagascar is a fascinating example of how the interactions between environmental conditions, the ecology of local vectors, and agricultural practices preclude a one-size-fits-all model for RVFV ecology.

1.2.3 Western Africa

Rift Valley fever virus was studied in western Africa for nearly a decade before its identity was known. Zinga virus was isolated in the Central African Republic, Senegal, and Madagascar from mosquitoes and from humans in the Central African Republic and Senegal (Digoutte, Cordellier, et al. 1974, Digoutte, Jacobi, et al. 1974, Digoutte 1981). In 1983, Zinga virus was determined to be a strain of RVFV by plaque reduction neutralization tests (Meegan et

al. 1983). Isolations from these mosquitoes represented *Aedes (Aedimorphus) dalzieli* and *Aedes (Neomelaniconion) palpalis s.l.* in Senegal (Meegan et al. 1983).

The first identified focus of RVFV in western Africa was in southern Mauritania, where seroprevalences among shepherds (13%), as well as a diversity of livestock (16% in goats, 14% in sheep, 13% in cattle, and 33% in camels) were identified to be higher than surrounding areas (Saluzzo et al. 1987). Seroprevalences in younger animals indicated enzootic transmission during drought years (1982-1985), contrary to the patterns observed in eastern Africa. In 1987 an outbreak ensued in southern Mauritania in the Senegal River basin, and was characterized by abortions in goat and sheep, and many human cases. Human mortality and morbidity was significant, with 6% of cases presenting as encephalitis, and a case-fatality rate of 10% (Jouan et al. 1988). Anecdotal reports with this investigation included abnormally high rainfall in the Senegal River Basin, the recent construction 2 major dams on the river, and abnormally high mosquito abundances.

Serological evidence suggested that this Mauritanian outbreak extended to Senegal (Ksiazek et al. 1989), prompting a large effort of mosquito surveillance, as well as serosurveys of humans and animals. These serosurveys concluded that transmission was occurring at low levels following the Mauritania/Senegal outbreak, however without the levels of clinical human disease observed in Mauritania (Zeller et al. 1997). Virus isolations from pools of *Ae. vexans* and *Ae. ochraceus,* implicating these as the most likely enzootic vectors (Zeller et al. 1997).

Considering the total effort throughout the region, isolations of RVFV from mosquitoes in West African countries show a somewhat different, and perhaps more diverse set of potential vectors, including *Aedes spp.* (*Neomelaniconia and Aedimorphus* subgenera), *Cx. poicilipes*, *Mansonia spp., An. pharoensis,* and Culicoides biting midges (Fontenille et al. 1998). While

virus isolates are not vector incriminations, the arthropod fauna represented here presents a substantial taxonomic breadth. The most attention has been granted to *Ae.* (*Aedimorphus*) *vexans* and *Cx. poicilipes* in Senegal, and abundances of both of these vectors exhibit annual peaks coinciding with the rainy season (Fontenille et al. 1998). This may present a somewhat different set of environmental conditions in comparison to eastern Africa, where abnormally high rainfall every ~10 years drives large epizootics.

The involvement of wildlife may be even less studied in western African countries than elsewhere throughout the range of RVFV. There has been one notable exception, represented by the isolation of two strains of RVFV: one from the Aba roundleaf bat (*Hipposideros abae*), and one from Peters's dwarf epauletted fruit bat (*Micropteropus pusillus*) (Boiro et al. 1987). The role of rodents has been explored; reports include two positive *Mastomys sp*. by immunofluorescence assay out of 268 animals (Zeller et al. 1997), so these do not likely represent important reservoirs. Spatially explicit modelling has provided evidence for endemic transmission of RVFV under Sahel conditions (periodic rainfall, Culex and Aedes spp. mosquitoes) without a wildlife reservoir, given some heterogeneity among rainfall and adequate human migration (Favier et al. 2006).

The factor of human migration is mentioned frequently throughout the literature on RVFV in western Africa (Saluzzo et al. 1987, Jouan et al. 1988, Zeller et al. 1997), though it has not been studies specifically with respect to RVFV or other zoonoses. Mali, Senegal and Mauritania lie in the Sahelian region, a unique ecotype at the interface of the Sahara and sub-Saharan Africa. Rainfall is highly seasonal north of the Senegal River Valley, which runs east to west. During the rainy/flood season (June to November), nomadic pastoralists travel both north and south of the valley, and move back to the valley during the dry season (December to May)

(Bicout and Sabatier 2004). This “transhumanance” allows for the optimal use of quality pasture (Bicout and Sabatier 2004). The importance of animal movement is highlighted by a more recent outbreak in 2010, during which unusually high rainfall attracted herdsman and their animals to northern Mauritania for grazing, causing a massive epizootic/epidemic event (El Mamy et al. 2011).

1.2.4 South Africa

South Africa represents a significant focus of RVFV transmission, with outbreak/quiescent slightly different from eastern Africa. Historic outbreaks have occurred in 1950-1951, 1973-1976, and 2008-2011, and 2008-2011 (Jansen van Vuren et al. 2019), putting South Africa in a 20-30 year cycle which is longer than 10-15 year cycles observed in areas of eastern Africa (Ngoshe et al. 2020). Outbreak cycles are interspersed with higher levels of interepidemic transmission than seen in eastern Africa, in the form of smaller outbreaks in the wake of larger epidemics (Pienaar and Thompson 2013). Transmission is more prevalent in the central, more temperate areas of the country (Pienaar and Thompson 2013). The lack of coincidence with the east African outbreaks, as well as the phylogenetic clustering of the South African genetic lineages (Jansen van Vuren et al. 2019) suggest local circulation following potentially unique drivers.

The longer periodicity in South Africa is probably determined by climate. Rainfall patterns are continuous, and high rainfall events can cause dambo flooding when soils are saturated (Williams et al. 2016). While risk models developed for the eastern African climate do not perform for South Africa (Linthicum et al. 1999), risk or previous outbreaks have been assessed based on soil saturation, precipitation and irrigation (Williams et al. 2016). Inland South

Africa also experiences a cool, dry winter that may significantly limit transmission (Ngoshe et al. 2020).

The primary vector of RVFV in the South African inland plateau in early outbreaks was determined to be *Culex theileri*, with other *Culex* species acting as secondary vectors (McIntosh et al. 1980). These mosquitoes have been shown to overwinter as larval and pupal stages (*Culex pipiens*, *Culex fatigans, Culex theileri*) with emergence limited to sparse windows of warmer weather during the winter (Jupp 2009a, 2009b). *Culex zombaensis* and *Aedes circumluteolus* were incriminated as the primary vector in the 1973-1976 outbreak, which occurred primarily on the eastern Natal coast where *Cx. theileri* is scarce (McIntosh et al. 1983).

In South Africa there seems to be more evidence for interepidemic circulation of RVFV among wild ungulates as well as livestock. Seroconversion rates in the central region among cattle goats and sheep remained quite high four years after the 2011 outbreak, with seroprevalences of 43%, 9.3%, and 28% respectively (Ngoshe et al. 2020). Similar rates of interepidemic seroconversion in cattle and goats were also recorded in eastern South Africa where RVFV has never caused a large outbreak (van den Bergh et al. 2019). It is possible that sustained transmission limits the density of susceptible animals; a low (R0 between 0 and 1) may determine the time until herd immunity is low enough for another epizootic. Vaccines are available in South Africa, but vaccine sales are negligible during the long interepidemic periods, so this is likely not a factor in the outbreak periodicity (Williams et al. 2016).

In light of the sustained interepidemic periods with seemingly higher rates of seroconversion, the potential roles of wildlife species in viral maintenance have been explored. Kruger National Park in the eastern region of South Africa is home to a large population of African buffalo. Seroconversions outside of large outbreaks have been recorded in these animals,

with higher rates in the southern part of the park, potentially indicated differences in mosquito ecologies between these areas (Beechler et al. 2013). Temporal trends in seroprevelance have been explored following a period of high rainfall in Kruger, showing seroconversions in buffalos including young animals, but with an overall decline in seroprevalences through time (Getz et al. 2011). Given that interepidemic transmission occurs in these buffalo, and they act as significant amplifying hosts during large outbreaks.

South Africa, with its more temperate climate and abundance of both *Culex* and *Aedes* mosquitoes, and susceptible wildlife populations bordering agricultural areas, presents many parallels that may inform the post-introduction RVFV ecology in the United States. Overall, entomological investigations into transmission of RVFV in South Africa are relatively few, especially lacking with respect to vertical transmission. Vegetation density, as a proxy for rainfall, was a significant risk factor for animal exposures in the 2008-2011 outbreak (Métras et al. 2015), further highlighting the need for entomological investigations. Further work on the population biology of these species across different regions in South Africa, and their responses to meteorological variables would be insightful into the drivers of RVFV transmission.

1.2.5 Egypt

A significant epizootic event occurred in 1977-1978 in the Nile delta and valley, representing one of the first major outbreaks of RVFV outside of sub-Saharan Africa (Hoogstraal et al. 1979). This epizootic coincided with a major epizootic during the same year in Kenya (Davies et al. 1985). Subsequent large outbreaks occurred in 1993 and 2003, putting Egypt into an outbreak periodicity more similar to South Africa than eastern African countries. Introduction of RVFV through the importation of infected camels or sheep from Somalia was proposed for the 1977-1978 outbreak (Hoogstraal et al. 1979). Genomic analyses of isolates from this outbreak

indicated the involvement of a single genotype (Bird, Khristova, et al. 2007), suggesting a single introduction event for this initial outbreak.

In addition to the classical signs of RVFV outbreaks (abortions and mortality among sheep), an unusually high amount of human morbidity and mortality was involved in the 1977 outbreak in Egypt (Laughlin et al. 1979), with an estimated 200,000 cases and 600 fatalaties (Kenawy et al. 2018). This was attributed to the practice of slaughtering sick animals for food prior to death (Hoogstraal et al. 1979). Seven Naval Medical Research Unit Employees stationed in Cairo documented the traditional Islamic slaughter by exsanguination of a sick sheep; only one researcher who exited the room did not succumb to infection (Hoogstraal et al. 1979). This resulted in the first documentation of RVFV infection by the respiratory route with infectious blood aerosols.

Two additional large outbreaks have been recorded since this initial introduction; the 1993 and 2003 outbreaks have been attributed to the recurring importation of infected animals rather than local, interepidemic persistence (Abd El-Rahim et al. 1999, Ahmed Kamal 2011). There is little evidence overall of interepidemic transmission of RVFV in Egypt, except for few suspected cases reported in cattle (Sharaf El-Deen 1987). A human serosurvey conducted 13 years after the 1978 outbreak (just prior to the 1993 outbreak) showed seropositivity among older age classes, with little evidence of exposure in younger age classes, indicating lack of interepidemic exposure (Corwin et al. 1993).

In contrast to the geographic areas discussed so far, Egypt has relatively little ecological complexity, which is reflected in the relatively lower levels of mosquito diversity (Hoogstraal et al. 1979). The main mosquito breeding sites in include irrigation canals which are ubiquitous throughout the Nile delta and valley (Hoogstraal et al. 1979). *Culex pipiens* mosquitoes are the

dominant mosquito in this area, and were implicated as the primary vector since the first in this outbreak by virus isolation and subsequent vector competence experiments (Hoogstraal et al. 1979, Meegan et al. 1980).

Like the outbreaks in South Africa, these outbreaks do not conform to the east African dynamics that permit prediction via meteorological anomalies in the horn of Africa. Hydrological indicators are still useful for outbreak prediction, however. Rainfall at Gambiela (upstream of the Nile from Egypt), and monthly discharge at the Aswan Dam are strong predictors of transmission, reflecting the importance of *Culex* mosquito habitat (Drake et al. 2013). The annual festival of Eid al-Adha brings animal importation from Sudan, and is celebrated with the sacrificing of sheep, which may also inform outbreak risk (Drake et al. 2013). Rather than having a single strong predictor, animal movement and hydrological conditions both represent strong factors in the risk for RVFV epizootics in Egypt. Social and cultural factors underly the risk with respect to animal movement as well as the human-animal interface, similar to the Sahel of western Africa. While the Egyptian ecology may be simpler than sub-Saharan Africa, the interplay between these factors represents extra layers of complexity in the prediction of outbreaks.

1.2.6 Arabian Peninsula

The first large outbreaks outside the continent of Africa occurred in the transboundary outbreaks in Saudi Arabia and Yemen, 2000-2001 (CDC 2000a, 2000b). These outbreaks happened simultaneously, but as multiple independent foci (CDC 2000c). This spatiotemporal pattern, as well as the genomic similarity to the 1997-1998 east African outbreak strain suggest an earlier introduction to the peninsula (Shoemaker et al. 2002). Similar to the initial Egyptian

outbreak, the Saudi Arabia outbreak showed a human high case-fatality rate of 17%, the highest observed across RVFV outbreaks.

Entomological investigations reported high abundances of *Culex tritaeniorhynchus* and *Aedes caspius* throughout flood irrigated areas in Saudi Arabia initially (CDC 2000c). Virus isolations were performed from pools of *Culex tritaeniorrhnchus* and *Aedes vexans arabiensis* (Jupp et al. 2002). These species transmitted between hamsters efficiently, and fed readily on humans and sheep, fulfilling the criteria for incrimination as RVFV vectors in Saudi Arabia (Jupp et al. 2002). Flood irrigated agricultural fields were common in areas throughout the epidemic in Saudi Arabia, as well as seasonally flowing streams or "wadis" with small dams (Jupp et al. 2002).

Rift Valley fever virus seems to have established a cryptic, local transmission cycle in Saudi Arabia, but limited to the Jazan region of the original outbreak (Al-Afaleq and Hussein 2011). Low rates of recent exposure were observed in 2004 among herds of sheep and goats in which abortions had been observed (Elfadil et al. n.d.). In 2010, one cow and five sheep were confirmed infected in the Jazan region, as well as one confirmed human case, and a single *Aedes* mosquito (Al-Afaleq and Hussein 2011). Still, no large outbreaks have occurred since year 2000. Abnormally high rainfall similar the conditions spurring during the 2000-2001 outbreak were observed during 2007, 2013, and 2016-2018, but no cases were observed during these times (Tucker et al. 2020). Seven years following the 2000-2001 outbreak, the seroprevalence of antibodies to RVFV was determined to be zero in children born after the 2000-2001 outbreak, though these children may not interact with cattle as much as adults (Al-Azraqi et al. 2012). While cryptic circulation is occurring at low levels in animals, risk to humans is either low, or unreported.

The contribution of sleeping outdoors during hot temperatures likely put humans at higher risk for vector-borne transmission during the 2000 outbreak, as opposed to direct contact (Al-Hazmi et al. 2003). Exposure to mosquitoes presented a much higher risk of infection to humans than exposure to animals alone (Madani et al. 2003). This also may contribute to a lower risk of interseasonal transmission among livestock given Saudi Arabia's highly seasonal mosquito populations. However, the risk of animal importation still presents major risk of reintroduction. This should be especially high at the haj in Mecca. During Ramadan, 10-15 million small ruminants are slaughtered during the festivals, and most of this are imported across the Red Sea from eastern African countries (Davies 2006). Efforts are made to transport these animals very quickly, so that weight loss during transport does not decrease their value. Infected animals are unlikely to recover from RVFV infections during the journey (Davies 2006).

1.2.7 Conclusions

These regional summaries all represent variations on the theme of the classical Rift Valley Fever transmission cycle (**Figure 1.1**). The drivers of transmission in these areas represent a complex set of interactions between environment, the mosquito vector community, the susceptible vertebrate community, and agricultural practices. By taking a historical perspective, the objective of this chapter is to build a context of the qualitative features of these epidemics and epizootics throughout the current range of RVFV.

In all of these regions, Rift Valley fever virus transmission is highly driven by precipitation and resulting hydrological conditions. The classical transmission cycle modeled after eastern Africa is described by the flooding of dambos by high precipitation events. Dambo flooding is also described for South Africa. Irrigation is also a significant factor in the maintenance of mosquito breeding habitats. The damming of the wadis in Saudi Arabia, as well

as the Senegal river near the Senegal/Mauritania border were both described in relation to very high mosquito densities during outbreaks. This significance of flooding to outbreaks has been attributed to the hatching of infectious floodwater *Aedes spp.* mosquitoes capable of vertical transmission; however, this is based on a single study with a small set of samples and has yet to be demonstrated for these species under controlled conditions.

Madagascar may be a slight outlier with respect to this pattern, with transmission occurring in the highlands by *Culex spp.* which do not require flooding for egg hatching. In fact, the classical transmission cycle may overrepresent the contributions of *Aedes spp.* mosquitoes when applying this model to regions outside of eastern Africa, most of which report significant transmission contributions by *Culex spp.* mosquitoes. This may be due to irrigation in the Nile River Valley of Egypt, the dammed wadis and irrigated agriculture in Saudi Arabia, as well as damming along the Senegal River, providing organic-rich semi-permanent water habitat for *Culex spp.*. Drawing these connections between mosquito species and larval habitats could provide valuable insight into the transmission cycles and control of RVFV.

The more temperate climates of Egypt and South Africa may represent the opportunity to explore overwintering of RVFV in mosquito populations. While the classical transmission model presents *Aedes spp.* as a reservoir for RVFV due to interepidemic maintenance of the virus in egg populations, the ability of *Culex* mosquitoes to overwinter as adults may present an additional interseasonal maintenance mechanism if these mosquitoes emerge infectious from diapause when conditions are favorable.

Interepidemic transmission represents a major gap in the knowledge of RVFV transmission throughout the African continent and the Arabian Peninsula. Serological surveys in most areas are able to confirm exposures of animals to RVFV between epidemics, but the extent

to which domestic ungulates act as reservoirs relative to wildlife remains to be determined. Interepidemic surveillance of mosquitoes may fill this gap, in conjunction with analysis of feeding behaviors; however, due to the low levels of circulation during interepidemic periods this may require major resources while yielding little data.

Rift Valley fever virus as it effects humans is largely an agricultural disease, effecting domestic ungulates, pastoralist peoples, veterinarians, and abattoir workers. Agricultural practices also vary regionally in their contribution to transmission. Much of this is due to the movement of both humans and animals. The introductions of RVFV to Saudi Arabia and Egypt, as well as recurring outbreaks in Madagascar have all been attributed to importation of cattle from the Horn of Africa. The nomadic Sahel tribes of western Africa interact dynamically with their herds and the changing conditions north of the Senegal River, which likely increases transmission significantly. The slaughtering of sick animals before death for human consumption also puts pastoralist families at additional risk, as observed in both Egypt and Mauritania.

The history of RVFV outbreaks, and the contributions of the environment, local mosquito vectors, and agricultural practices are the first step toward building a working illustration of RVFV ecology in the United States. The proceeding chapters (2-4) represent entomological work to fill the gaps in our understanding Colorado mosquito ecology in agricultural and sylvatic settings. This information will be synthesized into a working illustration of RVFV in the United States in Chapter 5, aided by the relationships and patterns described above.

CHAPTER 2: ENTOMOLOGICAL RISK FACTORS FOR POTENTIAL TRANSMISSION OF RIFT VALLEY FEVER VIRUS AROUND CONCENTRATIONS OF LIVESTOCK IN COLORADO[1]

2.1: Introduction

Rift Valley fever virus (RVFV) is an emerging arbovirus that infects ruminants and humans. Epizootics may result in up to 100% mortality in neonatal animals, along with sweeping 'abortion storms', during which spontaneous abortions occur in ruminants (Bird et al. 2009). Zoonotic transmission during outbreaks occurs through mosquito-borne transmission as well as through the handling of infected ruminant carcasses (Terasaki and Makino 2015). Infection in humans causes febrile illness in most cases, although 1%–2% of cases may develop more severe symptoms such as blindness, retinitis, encephalitis and haemorrhagic fever (Meegan and Bailey 1989).

Rift Valley fever virus was first described in 1931 during an outbreak in sheep on a ranch in Kenya (Daubney et al. 1931). Outbreaks have occurred periodically across the African continent since this initial description, expanding from the Rift Valley region to western Africa, Egypt and South Africa (Rolin et al. 2013). Outbreaks in Egypt in 1977–1978, (Hoogstraal et al. 1979), Madagascar in 1991 (Morvan, Saluzzo, et al. 1991) and the Arabian Peninsula in 2000 (CDC 2000b, 2000a, 2000b) have demonstrated the ability of this virus to cross major geographic boundaries and invade new areas. With increasing global trade and travel, research on the ecology of RVFV is necessary to inform disease control strategies. Multiple routes of

[1] This chapter includes the complete manuscript cited as "**Hartman, D. A., L. M. Rice, J. DeMaria, E. M. Borland, N. A. Bergren, A. C. Fagre, L. L. Robb, C. T. Webb, R. C. Kading. 2019**. Entomological risk factors for the potential transmission of Rift Valley fever virus around concentrations of livestock in Colorado. Transbound Emerg Dis. 66(4): 1709-1717". This article is reproduced with permission and only minimal modifications were made to meet formatting requirements.

entry into the United States exist for RVFV, with the most likely pathway of introduction being an infected traveller from Africa (Golnar et al. 2017). The environmental receptivity of southern Central Valley of California to the virus has also been modelled (Barker et al. 2013), indicating the potential for epizootics from May to September based on environmental suitability and abundances of domestic ungulate hosts and vectors. The high susceptibility of white-tailed deer is also a major concern, due to the potential for RVFV to establish a sylvatic transmission cycle (Wilson et al. 2018). Intensive livestock farming practices common in the United States create huge populations of susceptible amplification hosts for RVFV, and many North American mosquito species are expected to be ecologically significant vectors (Golnar et al. 2014).

Much laboratory work has been conducted to assess the competence of North American mosquitoes to transmit RVFV (Turell et al. 1988, 2001, 2013, 2015). *Culex* (*Cx*.) *tarsalis* Coquillett is among the most competent vectors tested to date, with mosquito populations from Colorado and California exhibiting a laboratory transmission rate of 52% (Turell et al. 2010). *Cx. tarsalis* exhibits an overall feeding preference for birds; however, this host selection is also highly opportunistic based on the availability of alternative available hosts (Reeves et al. 1965, Kent et al. 2009, Thiemann et al. 2011, 2017, Campbell et al. 2013). *Culex tarsalis* mosquitoes are also highly abundant in riparian and flood-irrigated areas in the Colorado plains between 1,215 and 1,487 m of elevation (Barker et al. 2009), corresponding with many feedlots in Northern Colorado, where they also serve as the primary bridge vector of West Nile virus (WNV) (Hayes et al. 2005). This predominance of avian host utilization predicted *Cx. tarsalis* to have minimal involvement in the theoretical transmission of RVFV (Golnar et al. 2014). However, due to the opportunistic blood selection of *Cx. tarsalis* in Colorado (Kent et al. 2009),

we hypothesized that *Cx. tarsalis* could be a locally important vector of RVFV, particularly in areas with abundant susceptible amplifying hosts.

To this end, we investigated the potential role of cattle housed in feedlots in Colorado as theoretical amplification foci for RVFV, assuming a successful introduction and sylvatic establishment of RVFV in the United States. Feedlots represent a habitat disturbance, with sparse vegetation and a high concentration of domestic ungulates to serve as a blood source for *Cx. tarsalis* mosquitoes. Our specific aims were to (a) investigate differences between mosquito community assemblages at feedlots and nearby sites where sylvatic transmission of RVFV may occur; and (b) to investigate mosquito blood-host choices at feedlots and nearby sites. We hypothesized that community assemblages would differ between feedlots and surrounding areas, exhibiting lower abundances for some mosquito species at feedlots, as well as lower overall diversity. We also hypothesized that the blood meal composition of *Cx. tarsalis* mosquitoes would differ between these habitat types, to include more cattle at feedlot sites.

This study provides the first ecological assessment of *Cx. tarsalis* as a potential vector of RVFV in the United States. Describing the community of mosquitoes present at feedlots, as well as their feeding behaviors, is crucial to informing vector control strategies as well as contextualizing existing vector competence data.

2.2: Materials and Methods

2.2.1 Study area and mosquito collections

This study was conducted in Larimer and Weld Counties, Colorado, near the towns of Fort Collins, Ault, Greeley, and Wellington. Elevation in the study area ranges from 1,340 to 1,530 m, and the primary land uses in this area are irrigated row crops (sod, hay, alfalfa and corn), residential, and intensive livestock production. Fort Collins and Greeley have relatively

larger human populations (152,000 and 97,000 people, respectively) while Ault and Wellington are smaller farm communities (1,519 and 6,289 people, respectively).

We used a paired-site design (one livestock operation site, and one site without livestock approximately 1–2 km away), with a total of four pairs. A map of sampling sites is shown in **Figure 2.1. Map of the Study Area.** Map shows the study area in northern Colorado, just east of the Rocky Mountain Range. Numbers indicate the site pair number as referenced throughout the text. Control sites were chosen to be representative of the habitat in areas surrounding the feedlot sites. Site pair 1 included a medium-sized cattle feeder located in Wellington, CO (N 40.834,400°, W 105.083,310°, 1,714 m elevation), with its paired control site 1.11 km away, near irrigated crop circles on Boxelder Creek; site pair 2 included a large cattle feedlot located in Greeley, CO (N 40.321,000°, W104.785,959°, 1,448 m elevation) near the South Platte River, with its paired control site near a horse pasture 1.28 km away; site pair 3 included a small family-owned cattle feeder located in Fort Collins (N 40.632,000°, W 105.018,491°, 1,710 m elevation), with its paired control site near a private residence 1.67 km away and site pair 4 included a medium sized lamb feeder located in Ault, CO (N 40.580,000 W 104.799,686, 1,528 m elevation), with the Collins Lateral irrigation ditch running through its center. Its paired control site was a private residence surrounded by irrigated cropland, 2.12 km away. Mosquitoes were collected during epidemiological weeks 23, 24, 25, 28, 30, 32 and 34 (6 June–23 August), 2016. During each of these sampling weeks, all site pairs were sampled on consecutive nights, except for week 34, during which only site pairs 1 and 2 were sampled.

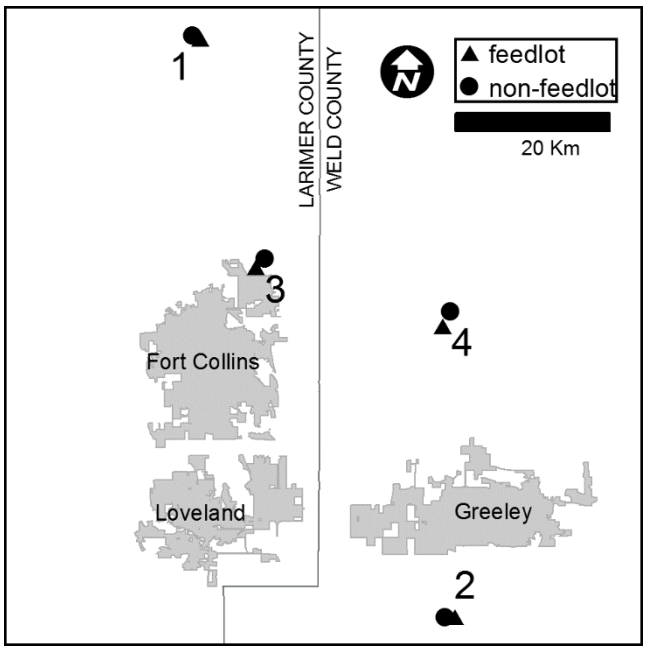


Figure 2.1. Map of the Study Area. Map shows the study area in northern Colorado, just east of the Rocky Mountain Range. Numbers indicate the site pair number as referenced throughout the text.

We used CDC light traps (Bioquip, Rancho Dominguez, CA; or John W. Hock, Gainesville, FL) to collect mosquitoes from each site pair during a single night. Traps were operated from approximately 15:00 hr until 08:00 hr the following morning, using light and dry ice as attractants. We deployed 10–15 traps at each site during each night of trapping. Mosquitoes were killed by placing in a −20°C freezer for at least 20 min and stored at −80°C until morphologically identified (Darsie and Ward 2005). Mosquitoes were sorted on a chill table, and female mosquitoes were preserved at −80°C in pools ranging in size from 1 to 27. Blood-fed females were pooled individually. Male mosquitoes were discarded.

2.2.2 Blood Meal Identification

Blood meal analysis was performed to explore blood host choice of mosquitoes in the study area. DNA was extracted from bloodfed mosquito abdomens using the Qiagen DNA

Investigator kit (Qiagen, Valencia, CA) and amplified by PCR using GoTaq Hot Start Green Master Mix (Promega, Fitchburg, WI). PCR was performed using primers targeting approximately 700 nucleotides (nt) of the vertebrate Cytochrome c Oxidase I (COI) gene (Ivanova et al. 2007). Primers contained M13 sequence tails for sequencing using M13F (−12) and M13R (−27) primers (Messing 1983). Cycling conditions were programmed according to (Crabtree et al. 2013). Reactions were prepared in 25 μL volumes, using either 2 or 10.5 μL of template. PCR amplicons were visualized on agarose gels, and purified using the QIAquick PCR Purification kit (Qiagen). Amplicons were submitted to Quintara Biosciences (San Francisco, CA) for Sanger sequencing with the M13F primer. Sequence quality was checked visually using Geneious 10.0.7 software (https://geneious.com) and searched against the Barcode of Life Database (BOLD) (Ratnasingham and Hebert 2007) as well as GenBank using Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990). Sequences with a 98%–100% match were identified to the species level; those with a 91%–97% match were identified to the genus level and those with a 88%–90% match were identified to the family level (Kent et al. 2009). Eight of our sequences amplified well and appeared to have been sourced from deer, but had poor homology with existing COI reference sequences. To resolve this, we performed PCR using the primers L15926 and H16501A, which target a 685 nucleotide section of the hypervariable region of the mitochondrial control region (Purdue et al. 2000) as recommended by (Hopken et al. 2015) . A BLAST search of the sequences returned 99%–100% homology to the expected local species, the white-tailed deer (*Odocoileus virginianus*) and the mule deer (*Odocoileus hemionus*).

2.2.3 Statistical Analysis

To explore associations between mosquito abundances and site type (feedlot vs. non-feedlot sites), we performed an ordination based on abundances (n/trap*night) at each site,

omitting week 34 data in favor of a balanced dataset. Unconstrained ordination was created by fitting a Bayesian latent variable model using the 'boral' package (Hui 2016) for R (R Core Team 2017), and plotting the median posterior values for the two latent variables. Mosquito abundances were assumed to fit a negative binomial distribution. Latent variables were estimated by Markov Chain Monte Carlo (MCMC) methods implemented by boral in Just Another Gibbs Sampler (JAGS), using the default, weakly informative normal priors. Markov Chain Monte Carlo was run as a single chain with 40,000 iterations, with the first 10,000 discarded as burn-in iterations.

We tested the effect of feedlots on mosquito community assemblages using a multivariate generalized linear model framework using the 'mvabund' (Wang et al. 2012) package for R. This model assumed negative binomial distributions of mosquito abundances. Using this analysis we only included species that occurred at more than a single site; this excluded *Ae.* (*Och.*) *epactius* Dyar & Knab, *Ae*. (*Och.*) *flavescens* (Müller), *Ae*. (*Och.*) *hendersoni* Cockerell, *Ae*. (*Och*.) *intrudens* Dyar and *An. fransicanus* McCracken. We included abundance as the dependent variable and the additive effects of site type (feedlot vs. non-feedlot) and site pair as independent variables. We included the log of trapping effort as an offset to account for differences in the number of traps that were successfully deployed at each site through the course of the study. Effects were evaluated based on 1,000 residual permutations and likelihood ratio (LR) tests. Univariate (single species) models were assessed for significant effects by species, with p values adjusted using Holm's stepdown procedure. Data from week 34 were omitted from this analysis due to the lack of data from all sampling sites, as with the ordination.

The diversity of each site was characterized using Hill numbers, or the 'effective number of species' (Hill 1973) using the 'iNEXT' package (Hsieh et al. 2016) for R (R Core Team 2017),

to investigate differences at feedlots versus surrounding areas. These indices differ only by the exponent q (here, q = 0, 1, 2), and give different weights to the rare species in the sample. When q = 0 the index represents species richness; q = 1 simplifies to exponential Shannon index; q = 2 simplifies to inverse Simpson's index. We compared point estimates of Hill numbers between paired sites by rarefying to a common coverage level and comparing 95% confidence intervals. For site pair 3, we compared the observed diversity indices without rarefying as the sample coverage was estimated to be one for both sites.

To assess the shifting of *Cx. tarsalis* blood host choice from non-feedlot to feedlot sites, analysis of mosquito blood meal data was performed using a Bayesian softmax regression model (Kruschke 2014) with runJags (Denwood 2016) in R (R Core Team 2017). We first binned vertebrate blood meal identifications to the family level; this permitted adequate sample sizes for each vertebrate group in the analysis while preserving the relevant life history information. For this analysis, a reference category must be defined. We chose *Bos taurus* as the reference outcome for two reasons: adequate sample size and intuitive interpretation of results. Two MCMC chains were run for 4,000 burn-in iterations followed by 200,000 additional iterations.

2.3 Results

2.3.1 Field Sampling

CDC light traps were operated for a total of 603 trap-nights: 108 trap-nights during week 23, 114 trap-nights during week 25, 99 trap-nights during week 28, 113 trap-nights during week 30, 115 trap-nights during week 32 and 54 trap-nights during week 34. In total, 29,561 female mosquitoes from 19 species were collected throughout the study period. A total of 15,269 (52%) of these were morphologically identified as *Cx. tarsalis*, 5,129 (18%) were *Ae.* (*Och.*) *vexans*

(Meigen), 4,094 (14%) were *Ae*. (*Och*.) *dorsalis* (Meigen), 2,714 (9%) were *Cx.* (*Cx.*) *pipiens* (Linnaeus) and 1,128 (4%) were *Ae*. (*Och.*) *melanimon* (Dyer). The remaining 4% were comprised of *Ae*. (*Och*.) *epactius* (Coquillett), *Ae*. (*Och*.) *fitchii* (Felt & Young), *Ae*. (*Och*.) *flavescens* (Müller), *Ae*. (*Och.*) *hendersoni* Cockerell, *Ae*. (*Och*.) *increpitus* (Dyar), *Ae*. (*Och*.) *intrudens* Dyar, *Ae*. (*Och*.) *nicromaculis* (Ludlow), *Ae*. (*Och*.) *spencerii idahoensis* (Theobald), *Ae*. (*Och*.) *trivittatus* (Coquillett) and *Cs*. *inornata* (Williston). We collected a single individual from each of the following species: *Ae. epactius*, *Ae. flavescens*, *Ae. intrudens* and *An. franciscanus* McCracken. West Nile virus infection rates in *Cx. tarsalis* from site pair 2 were as high as 29 per 1,000 mosquitoes during this sampling period (Robb et al. 2019). Abundances, defined here as the number of mosquitoes divided by trapping effort (trap*night), are shown in **Figure 2.2**. Voucher specimens are available at the C.P. Gillette Museum of Arthropod Diversity at Colorado State University, Fort Collins, Colorado.

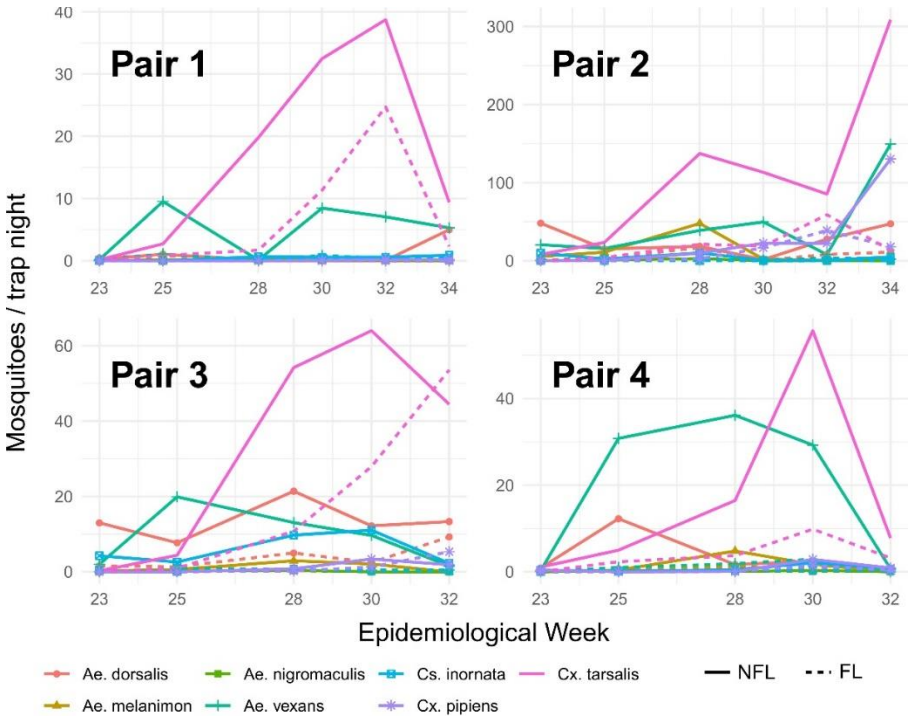


Figure 2.2. Abundances throughout the sampling period are shown for mosquito species by site type. Species for which fewer than 45 specimens were collected are omitted.

2.3.2Diversity

Using Hill numbers for characterization of mosquito diversity at each field site, an overall trend towards higher diversities on control sites was evident for site pairs 1–3 (**Figure 2.3**). Site pair 4 did not show this trend. While species richness (q = 0) was higher at non-feedlot site 4, exponential Shannon's entropy (q = 1) was higher at the feedlot site 4, and inverse Simpson's index (q = 2) showed no difference. These results are indicative of rare species driving the difference in species richness at site pair 4. Indeed, *Ae. fitchii*, *Ae. increpitus* and *Ae. trivitattus* occur in very low abundances at non-feedlot site 4, and were absent at the paired feedlot.

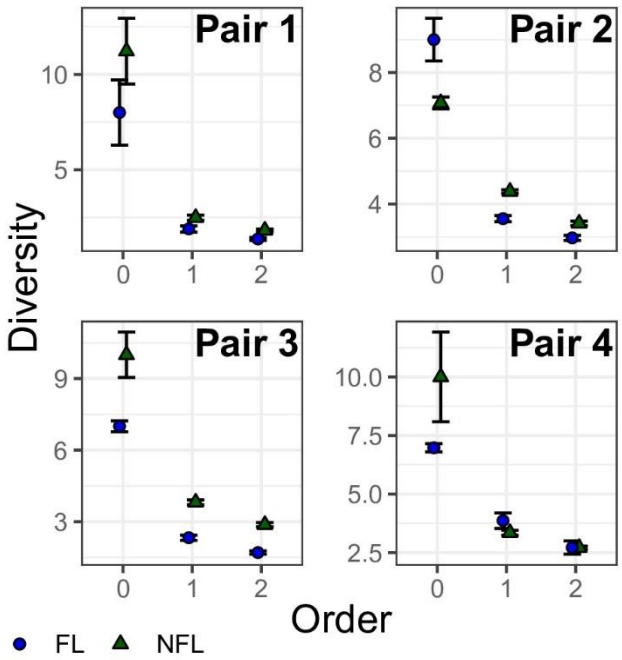


Figure 2.3. Estimated Hill numbers are plotted as point estimates with 95% confidence intervals, for comparisons between paired sites. Orders 1, 2, and 3 represent Hill numbers for q=0 (species richness), q=1 (exponential Shannon's index) and q=2 (inverse Simpson's index). Increasing the order of q allots less weight to relatively rare species for each estimate. Circles show diversitites for feedlot sites, while triangles show non-feedlot sites.

2.3.3Feedlot Associations

Ordinations were performed to visualize differences between plots based on the presence of livestock, as well as the associations between mosquito species and these two groups of sites. The resulting ordination biplot is shown in **Figure 2.4**. Feedlot and non-feedlot sites showed separation, indicating differences between the community assemblages. *Aedes vexans* and *Ae. fitchii* appeared highly associated with non-feedlot sites. The remainder of the species, including *Cx. tarsalis*, did not show strong associations with either site type.

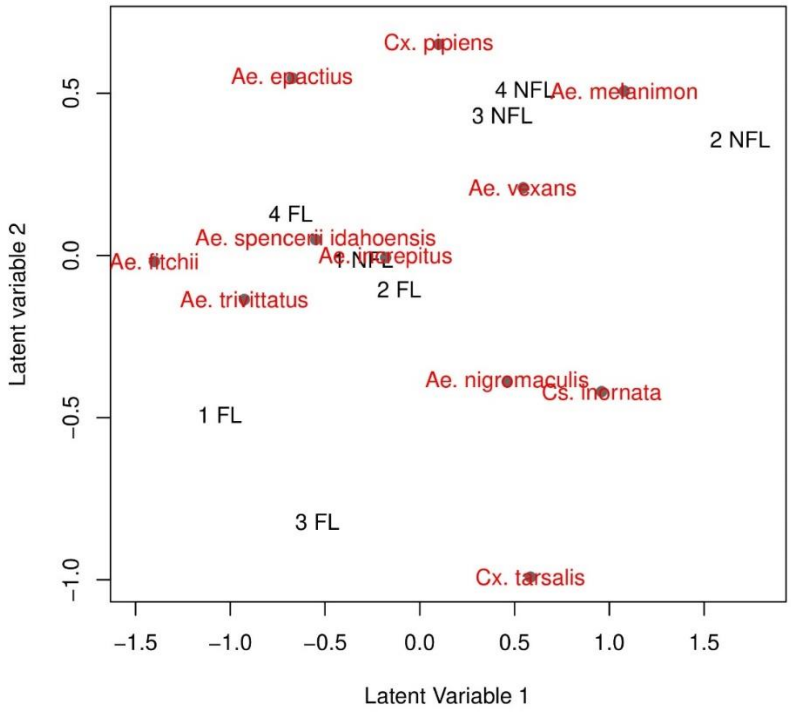


Figure 2.4. Ordination biplot based on a Bayesian latent variable model. Sites are plotted in black as the site pair number, followed by "FL" for feedlot, or "NFL" for non-feedlot. Species are plotted in red according to estimated latent variable coefficients, with their exact coordinate represented by a grey point.

Results of the multivariate generalized linear model showed evidence for both feedlot effect (sum of LRs = 135.34, p = 0.003), and site-pair effects (pair 2: LR = 150.69, p = 0.003; pair 3: LR = 90.91, p = 0.018; pair 4: LR = 87.63, p = 0.006) on the overall mosquito community assemblages. When assessing the univariate models for the drivers of their combined, community-level effect, feedlot/non-feedlot site status was a statistically significant predictor for *Ae. vexans* abundances (LR = 45.417, padj = 0.038), but not other mosquito species.

2.3.4 Blood Meal Analysis

We collected a total of 273 (0.9% of total collections) mosquitoes containing blood in the abdomen. We identified 229 (84%) of these blood meals to the species level; six were identified

to genus, and one sample was identified at the family level. Blood meal sequence identification results are summarized in **Figure 2.5**, and included in **Supplemental Table A1**. At non-feedlot sites, 31/118 (26%) vertebrate blood meals originated from cattle (*Bos taurus*), while at feedlots cattle comprised 97/116 (84%) vertebrate blood identifications. Considering only *Cx. tarsalis* blood meal identifications, cattle represented 5/31 (16%) blood meal identifications at non-feedlot sites and 67/72 (93%) at feedlots.

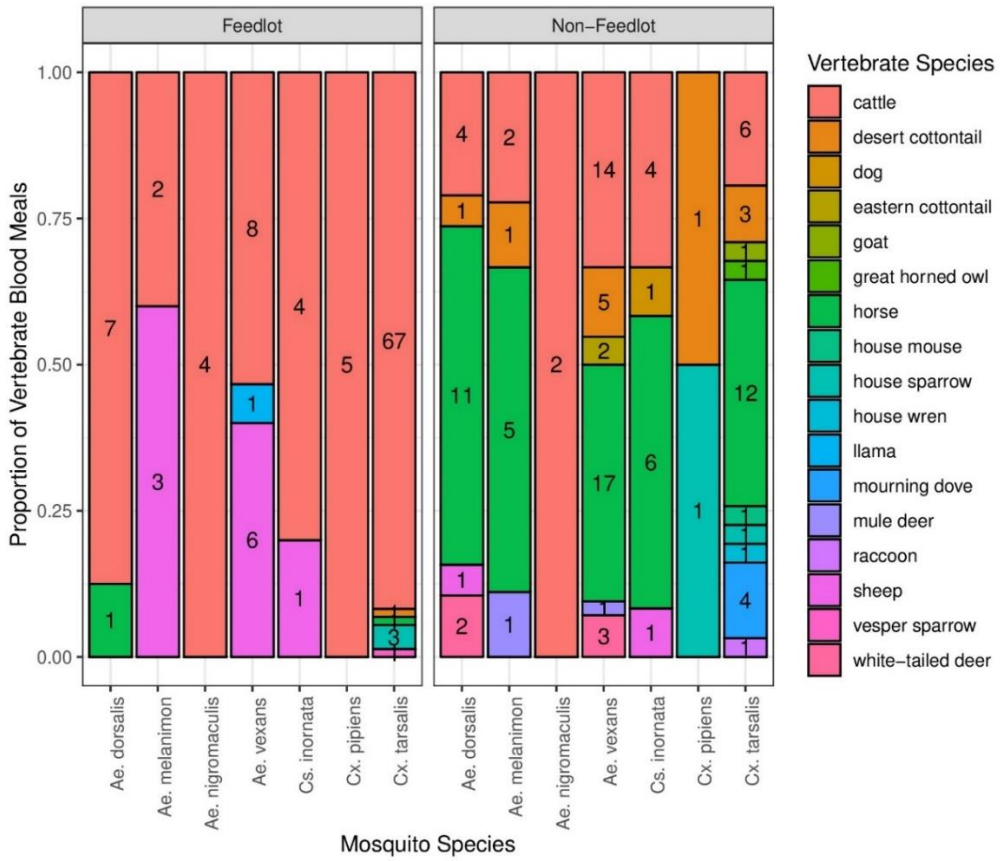


Figure 2.5. Vertebrate blood host composition is shown by mosquito species and site type (feedlot/non-feedlot). *Cx. tarsalis* showed a major shift from a diversity of hosts at non-feedlot sites to cattle on feedlots.

The regression coefficients estimated using the softmax regression model are interpreted biologically as the strength of association between vertebrates and feedlots, compared to Bovidae (comprised of cattle [*Bos taurus*] and sheep [*Ovis aries*]). Highest density posterior intervals (HDPIs) show the range of highest credibility for these estimates (**Figure 2.6**). Results of this analysis suggest that while shifting towards Bovidae occurred on feedlots, *Cx. tarsalis* feeding on the families Leporidae (rabbits), Equidae (horses), Strigidae (owls), Muridae (mice), Procyonidae (raccoons), Troglodytidae (wrens) and Columbidae (doves) was less associated with feedlot sites.

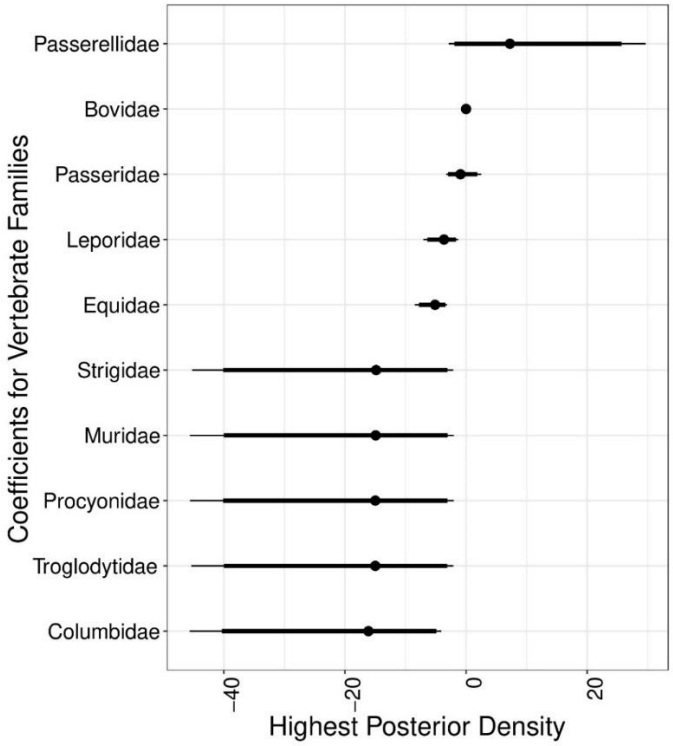


Figure 2.6. Regression coefficients for the effect of "feedlot" on the probability of *Cx. tarsalis* feeding on each vertebrate family, compared to family Bovidae. Points show the median estimate, and thick lines indicate intervals of the highest posterior density (HPD). Asterisks indicate families that differ significantly from Bovidae.

2.4 Discussion

This study characterizes the entomological risk factors at the interface between intensive livestock production facilities and the agricultural and residential community in northern Colorado in the context of a hypothetical RVFV introduction. Currently there is no commercially available vaccine for RVFV in the United States, and vector control is likely to be a major component of outbreak response efforts. In order to direct vector control efforts in an evidence-based manner, predicting which competent mosquito vectors are likely to have epidemiologically significant interactions with vertebrate amplifying hosts including cattle and sheep is paramount for strategic response plan development. We performed this study to characterize mosquito community assemblages at feedlots, specifically in terms of overall mosquito diversity and mosquito abundances. We also determined the specific mosquito species that associate with feedlots, using abundance as a measure of habitat suitability. Finally, we investigated the degree to which mosquito species may vector RVFV in a hypothetical post-introduction scenario by investigating blood-feeding behaviors.

We were interested in mosquito diversities at feedlots in comparison with surrounding areas as a measure of habitat and environmental suitability for mosquito community assemblages as a whole. Comparisons between paired sites show a general trend of higher mosquito diversities on control sites, showing evidence for feedlots as resource poor or oppressive environments for some mosquito species. Site pair 4 provided an exception, as differences in species richness were determined by rare species at the non-feedlot site. Feedlot site 4 was unique among our sites as the only site housing sheep and llamas rather than cattle, which may provide some explanation.

Exactly what overall mosquito diversity means for the risk of mosquito-borne diseases such as RVFV is not entirely clear. Loaiza et al. (2017) found that while mosquito diversities

peaked in relatively less disturbed forests, mosquito species incriminated as disease vectors were more highly represented in the fraction of disturbance tolerant, 'colonist' species. As *Cx. tarsalis* maintains an increasing presence on feedlots throughout the spring and summer, we may consider this species as a colonist species. Larval sampling will provide valuable insight into the actual degree of resource utilization by *Cx. tarsalis* and other mosquito species on feedlots, and the relative importance of local mosquito production on feedlots compared to dispersal to feedlots from nearby areas. These data will be critical to informing vector control in response to the introduction of RVFV.

The ordination biplot (**Figure 2.4**) revealed some patterns associating mosquito species and treatment (feedlot vs. non-feedlot). First, *Ae. vexans* appeared associated with non-feedlot sites. While this species was relatively abundant on feedlots, its high abundance at nearby sampling sites may reflect plentiful floodwater larval habitat present at those control sites. Second, *Cx. tarsalis* appeared between the clusters of feedlot and non-feedlot sites, indicating a lack of strong association with either feedlot sites or non-feedlot sites. In addition, *Cx. tarsalis* was the most abundant species at these feedlot sites overall. Periodic enrichment of larval habitat is a requirement for stable production of *Cx. tarsalis* (Beehler and Mulla 1995), and abundances increased at feedlots later than non-feedlots (**Figure 2.2**). Investigation of possible larval habitat on feedlots, as well as patterns of enrichment may inform this trend. These data collectively support our hypothesis of *Cx. tarsalis* as a potential bridge vector capable of transferring virus between sylvatic vertebrate reservoirs and feedlot ungulates in the post-RVFV introduction ecology.

Analysis of mosquito abundances by the multivariate generalized linear model framework showed an overall negative effect of feedlot land use on mosquito abundances at the community

level. In concordance with the ordination biplot, *Ae. vexans* showed significantly lower abundances at feedlots, and this association was the largest driver of the observed community-level differences. (Turell et al. 2010) showed an exceptionally low rate of transmission of RVFV by Colorado and California populations of *Ae. vexans* in the laboratory. In addition, low abundances at our feedlot sites (=<2.2 mosquitoes/trap night season-long abundance) gives additional evidence that *Ae. vexans* may not be an important vector for domestic ungulates compared to *Cx. tarsalis* in Colorado. This is likely to vary geographically, as *Ae. vexans* populations in Florida are competent for transmitting RVFV under laboratory conditions (Turell et al. 2013).

The blood feeding behaviors exhibited by mosquitoes are crucial to understanding their potential to vector RVFV among domestic ungulates, while shifts in feeding behaviors between feedlots and nearby areas informs their potential to bridge-vector viruses between potential sylvatic hosts and domestic ungulates. *Culex tarsalis* exhibited a strong shift in feeding behavior from a variety of vertebrates in more natural areas surrounding feedlots to feeding predominantly on cattle while on feedlots (**Figure 2.5**). This observation is consistent with previous work describing the opportunistic blood host choice by this species in Colorado (Kent et al. 2009). Two of the vertebrate blood meals detected in *Cx. tarsalis* on non-feedlot sites were identified as vertebrate taxa suspected to play significant roles in RVFV transmission in the United States: one from *Mus musculus* (house mouse) and the other from *Capra hircus* (domestic goat) (Golnar et al. 2014). While host selection shifted toward cattle on feedlots, *Cx. tarsalis* host selection waned from the several vertebrate families (Leporidae, Equidae, Strigidae, Muridae, Procyonidae, Columbidae) at feedlots (**Figure 2.6**). Unbiased collections of blood-fed mosquitoes are difficult to perform, with collections from light traps collecting more partially fed individuals (Thiemann

and Reisen 2012), so this representation of vertebrate hosts chosen by *Cx. tarsalis* is likely not exhaustive.

It is worth noting that this analysis assumed that each blood feeding event, represented by a blood-fed mosquito collected in a light trap, occurred at the site where the trap was deployed. We detected several cattle blood meals from non-feedlot sites, where cattle were not kept, which most likely violates this assumption. These events likely dilute our ability to detect statistically significant shifts in blood host utilization as a function of site type (feedlot/non-feedlot). Furthermore, the collection of *Cx. tarsalis* mosquitoes with cattle blood meals at sites where cattle are not present is highly suggestive of dispersal from feedlots to surrounding areas. As *Cx. tarsalis* is a highly competent vector in the laboratory (Turell et al. 2010) and exhibits promiscuous blood feeding behavior in the northern Colorado Front Range (Kent et al. 2009), these results suggest that the amplification of RVFV at feedlots may also pose major risk to susceptible wildlife in nearby areas.

While floodwater *Aedes* species (*Ae. dorsalis*, *Ae. melanimon*, Ae. vexans) were not as abundant as *Cx. tarsalis* on feedlots, the blood feeding data suggest that low frequency feeding events on feedlots may be more consequential, as blood meals from deer were detected in these species. Even if *Ae. vexans* and *Ae. dorsalis* exhibit very low vector competence in the laboratory (Turell et al. 2010), this is troublesome, as white-tailed deer are highly susceptible to RVFV (Wilson et al. 2018). High vector abundance of these species combined with frequent contact with competent vertebrate amplifying hosts may offset the low vector competence to increase the overall vectorial capacity and contribution of these species to RVFV transmission. This phenomenon was hypothesized for *Psorophora* species; while a salivary gland barrier to RVFV reduced transmission efficiency in the laboratory, high population densities and intensive feeding

on large mammals by *Psorophora spp.* could result in an effective contribution to RVFV circulation in the field (Turell et al. 2015). (Golnar et al. 2014) predicted a considerable number of mosquito species to play secondary roles in RVFV transmission in the United States. The vector competence of *Ae. melanimon* is unknown, and should be a high priority for future laboratory studies considering the utilization of cattle, sheep and deer as blood sources by this species (**Figure 2.5**, **Supplemental Table A1**). These floodwater mosquitoes appeared most abundant from epidemiological weeks 25–30, or from mid-June to mid-July. Targeted vector control for these species during mid-summer may prevent spillover to feedlots from wildlife if sylvatic transmission becomes established.

Taken together, these results support the hypothesis of feedlots as amplification foci of RVFV in the United States. Feedlots represent a habitat disturbance that negatively impacts overall mosquito abundance and diversity; however, the abundance of the highly competent vector *Cx. tarsalis* as well as its feeding behavior at these sites is worrisome. The detection of cattle blood meals from *Cx. tarsalis* mosquitoes collected ~1–2 km away from feedlots suggests that *Cx. tarsalis* disperse readily from feedlots to surrounding areas. While we show here that this species associates with domestic ungulates on feedlots, determining resource use at these operations (i.e. larval habitat, resting habitat) is the crucial next step towards informing control upon the arrival of RVFV.

In the absence of an Food and Drug Administration-approved vaccine, vector control will be crucial to the control of RVFV (Britch et al. 2007). Knowing which mosquito species are associated with feedlots and capable of bridging virus between susceptible human and livestock communities will be critical to directing targeted vector surveillance and control efforts. Mosquito control should include not only livestock feeding operations, but surrounding areas

where these mosquitoes are highly abundant to prevent their dispersal to feedlots. Investigations into mosquito dispersal in agricultural areas near feedlots, particularly for *Cx. tarsalis*, are needed to determine a radius for mosquito control that is manageable and effective for preventing RVFV spillover into these operations from surrounding zoonotic transmission cycles.

CHAPTER 3: VECTOR COMPETENCE AND BARRIERS TO INFECTION OF COLORADO MOSQUITOES WITH RIFT VALLEY FEVER VIRUS

3.1 Introduction

Rift Valley fever (RVFV) is a mosquito-borne virus (Order: *Bunyavirales*, Family: *Phenuiviridae,* Genus: *Phlebovirus*) endemic to sub-Saharan Africa that affects both humans and domestic ungulates (Pepin et al. 2010). Clinical signs in animals include spontaneous abortion, and near total mortality of neonatal ungulates, while human illness manifests as acute febrile illness, with low rates of encephalitis, hemorrhagic fever and blindness (Bird et al. 2009, Pepin et al. 2010).

While the epidemiology of RVFV is nuanced across its range, the importance of mosquito-borne transmission seems to be universal. While direct transmission of RVFV occurs between infected animals and humans, vector-borne transmission is critical to epizootics as well as interepidemic transmission (Bird et al. 2009). In addition to horizontal transmission by mosquitoes, there is strong evidence for vertical transmission by some floodwater *Aedes spp.* mosquitoes (Linthicum et al. 1985, Romoser et al. 2011). This persistence in the mosquito population is thought to be a mechanism of viral maintenance, allowing the virus to survive long inter-epidemic periods in mosquito egg populations, which can hatch following periods of high rainfall (Davies et al. 1985, Linthicum et al. 1985). Vertical transmission of viruses in the vector is well-documented throughout the order *Bunyavirales* (Bergren and Kading 2018).

The first described RVFV epizootic event occurred on a sheep ranch on Lake Naivasha, Kenya, where abortion storms were observed among ewes, along with high mortality in lambs (Daubney et al. 1931). Subsequent epizootics and epidemics have been observed throughout the

African continent, with notable expansions into Egypt (Hoogstraal et al. 1979), Madagascar (Morvan, Saluzzo, et al. 1991), and Saudi Arabia (CDC 2000a, Al-Hazmi et al. 2003) making RVFV an increasing emerging disease risk for other continents such as Europe and North America. RVFV is listed as an overlap select agent pathogen in the United States ("Select Agents and Toxins List | Federal Select Agent Program" 2020), and as such represents a biosecurity and bioterrorism threat.

The main potential introduction pathway to the United States is suspected to be human travel via airline (Golnar et al. 2017). Establishment of RVFV, however requires the presence of competent vectors and amplification hosts. The United States has both competent vectors and amplifying hosts for RVFV (Golnar et al. 2014). White-tailed deer exhibit high RVFV titers upon infection (Wilson et al. 2018), and some theoretical evidence exists regarding the competency of animals in the orders Artiodactyla, Lagomorpha, and Carnivora to serve as amplification hosts (Golnar et al. 2017). Overall, however, the data on vertebrate competence are lacking.

A wealth of work has been produced on vector competence of North American mosquitoes in the laboratory (Turell et al. 1988, 2010, 2013, Turell, M.J., Byrd, B.D., Harrison 2013); however important gaps exist in light of the transmission efficiencies of mosquito vectors that feed on potential amplifying hosts for RVFV in the United States, such as white-tailed deer. This ecological context is imperative to assessing the potential role different mosquito vectors might play in the event this virus is introduced, and informing risk models. Further, a myriad of mosquito species are predicted to contribute to RVFV transmission based on laboratory competency and blood feeding patterns (Golnar et al. 2014), which will necessitate a complex vector surveillance and intervention strategy post-invasion. Therefore, filling in data gaps for

species with epidemiologically significant host selection patterns, but for which vector competence data are lacking is paramount.

Aedes melanimon (Dyar), *Ae. vexans* (Meigen), and *Ae. dorsalis* (Meigen) were recently shown to feed on both cattle and deer in agricultural northern Colorado plains, suggesting high spillover risk given adequate vector competence and dispersal (Hartman et al. 2019). Populations of *Ae. vexans* exhibit some variation in their vector competence (Turell et al. 2010, 2013), while *Ae. dorsalis* from mixed California/Colorado sampling exhibit low vector competence (Turell et al. 2010). Vector competence data were previously lacking for *Ae. melanimon*. Blood meals from cattle and sheep were also identified in field-collected *Culiseta inornata* (Williston) mosquitoes from northern Colorado. Canadian *Cs. inornata* have demonstrated efficient transmission of RVFV (ZH501) previously, as measured by RT-qPCR analysis of saliva samples (Iranpour et al. 2011).

To inform the potential for these mosquito species to transmit RVFV between susceptible North American vertebrate hosts, we conducted vector competence experiments with an epidemic, Kenyan strain (128B-15) of RVFV. We targeted *Ae. melanimon*, *Ae. vexans*, and *Ae. dorsalis* due to the recently documented blood-host choices in Colorado, and to illuminate their competence for transmitting an epidemic strain of RVFV. A local sampling of *Cs. inornata* was included in these experiments to confirm its high susceptibility and transmission efficiency for RVFV 128B-15. We also included *Aedes increpitus* (Dyar) based on high abundances in our sampling sites, and *Cx. tarsalis* to confirm previous vector competence studies while providing a positive control species. For each of these species, we investigated the progression of virus infection throughout mosquito bodies (midgut infection, dissemination to the circulatory system, saliva), as well as potential for vertical transmission of RVFV using infection of ovaries as a

proxy. Finally, we developed a within-host model for the functional analysis of infection patterns, as well as the "barriers" to infection (Houk et al. 1981) for each tissue.

3.2 Methods

3.2.1 Field Collections

Field collections of our target species for vector competence experiments were made using CDC light traps. Three replicates of vector competence challenges were completed with field-collected mosquitoes. The first replicate utilized mosquitoes collected from the Environmental Learning Center (N 40.557º, W 105.017) in Fort Collins, Colorado on 6/14/2019 (Fig 1). For the second replicate we collected in Timnath, Colorado (N 40.532º, W 104.980º) on 7/3/2019. We collected near the McMurray Natural Area (N 40.603º, W 105.091) in northwestern Fort Collins for the third and final replicate on 7/30/2019.

3.2.2 Vector competence for RVFV strain 128B-15

For these studies, RVFV strain KEN128B-15 from the 2006-2007 outbreak in Kenya was used (23,24). Prior to oral challenge with RVFV, mosquitoes were sorted into screened pint-sized ice cream cartons (Huhtamaki, Espoo, Finland) and acclimated to insectary conditions (26 degrees C, 70% relative humidity, 16:8 light/dark cycle) for 2-3 days, and were provided with water and sugar cubes *ad libitum*. Mosquitoes were relocated to an incubator in the Biosafety Level 3 laboratory 24 hours prior to virus challenge, and deprived of sugar and water.

Virus was prepared for oral challenge by infecting Vero cells (ATCC CCL-81, American Type Culture Collection) at a multiplicity of infection (MOI) of 0.01. On day 3 post-inoculation, virus supernatant was collected and mixed 1:1 with fresh defibrinated calf blood (Colorado Serum Company, Denver, CO), and ATP to a final concentration of 8 mM. Virus-blood preparation was presented to mosquitoes using a Hemotek Membrane Feeding System (Hemotek, Blackburn, United Kingdom) for 75 minutes, with a small (~ 9g) mass of dry ice near

each feeder to encourage feeding by releasing CO2. Mosquitoes were cold-immobilized, sorted to separate fully-engorged females, and placed in an incubator at 26 degrees C and 70% relative humidity. One mL of each blood/virus preparation were frozen at -80 C until titration by plaque assay.

After 14 days of incubation, we identified mosquitoes to the species level using two taxonomic keys (Darsie and Ward 2005, Rose et al. 2017), and harvested saliva, legs/wings, ovaries, and carcasses. We collected saliva as a measure of horizontal transmission capacity, legs and wings as a measure of viral dissemination, ovaries to determine potential for vertical transmission, and carcasses to determine midgut infection. Mosquitoes were cold immobilized, and legs and wings were removed from each specimen. Saliva was collected by placing the proboscis in the end of a 10 uL capillary tube of Type B immersion oil (Cargille, Cedar Grove, New Jersey) and allowing to expectorate for 30 minutes, after which the end of the capillary tube was placed in 100 uL of mosquito diluent (DMEM supplemented with 10% fetal bovine serum, 1% Penicillin/Streptomycin, 0.1% Gentamycin, and 0.1% Amphotericin B). Finally, ovaries were dissected, and the remaining carcass was collected. All tissues (legs/wings, ovaries, carcasses) were collected in a microcentrifuge tube containing 2 glass Colirollers beads (MilliporeSigma, Burlington, MA) and 200 uL of mosquito diluent. All samples were frozen at -80 deg C until analysis.

Mosquito saliva samples were thawed, centrifuged at 11,000 RPM for 5 minutes, diluted serially (1:2 – $1:2x10^5$) and plaqued on Vero cells. Tissue samples (bodies, legs/wings, ovaries) were thawed, homogenized using a TissueLyser (Qiagen, Hilden, Germany) at 24 Hz for 1 minute, and centrifuged at 14,000 RPM for 1.5 minutes prior to performing plaque assays. Tissue homogenates were plaqued undiluted, and diluted 1:10 – $1:10^5$. Plaque assays were performed by

plating 125 uL of dilutions of each sample on Vero cell monolayers in 12-well plates in singlicate, adding a 2% agarose/DMEM overlay, and staining with 0.33 % neutral red (Sigma Aldrich, St. Louis, Missouri) 2 days later. Plaques were counted on day 3 post-inoculation. The limit of detection (LOD) for this assay was defined as the corresponding PFU/mL obtained by observing 1 plaque in the least dilute well.

3.2.3 Confirmation of Virus Identity.

Because wild-caught mosquitoes were used for these experiments, mosquito carcasses were screened by RT-qPCR to confirm the presence of RVFV, and exclude possibility of natural West Nile virus (WNV) detection by plaque assay. RNA extractions were performed using the MagMAX -96 Viral RNA Isolation Kit (Applied Biosystems, Waltham, Massachusetts, United States), and reactions were performed with TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems) using fast cycling parameters on a QuantStudio 3 cycler. The qRT–PCR for RVFV quantification utilized the primers RVFL-2912fwdGG and RVFL-2981revAC at 500 nM final concentration, with probe RVFL-probe-2950 at 100 nM final concentration (Bird, Bawiec, et al. 2007). West Nile virus assays were performed using the primers WNENV-forward and WNENV-reverse at final concentrations of 500 nM (each), and probe WNENV-probe at a final concentration of 250 nM (Lanciotti et al. 2000). RT-qPCR reactions were run in singlicate alongside no-template controls. Standard sets were run in duplicate, utilizing serially diluted RVFV MP12 (vaccine strain) or local (Fort Collins) isolates of WNV. Serial dilutions were plaqued in duplicate according the the methods above for relating Ct values to PFU/mL. Default detection thresholds from the Quantstudio 3 software were used.

3.2.4 Within-Vector Model of Arbovirus Infection

We describe the progression of arbovirus infection in a competent mosquito vector as a sequence of independent events, following **Figure 3.1**. First, after ingesting a blood meal from a

viremic host, the virus establishes a midgut infection (**Figure 3.1**, "a"). After replicating in midgut cells, the virus then passes through the midgut epithelium into the circulating hemolymph ("b"). Infection of the salivary glands then takes place. With saliva now containing infectious virions, the mosquito can expectorate virus into a new vertebrate host ("d"). For some arboviruses such as Rift Valley Fever Virus, virus can be transmitted to offspring through the reproductive system. While this may be dependent upon midgut infection, it represents a second path of infection through the tracheal system to the ovaries ("b"), that is independent of viral dissemination through the circulatory system (Romoser et al. 2004).

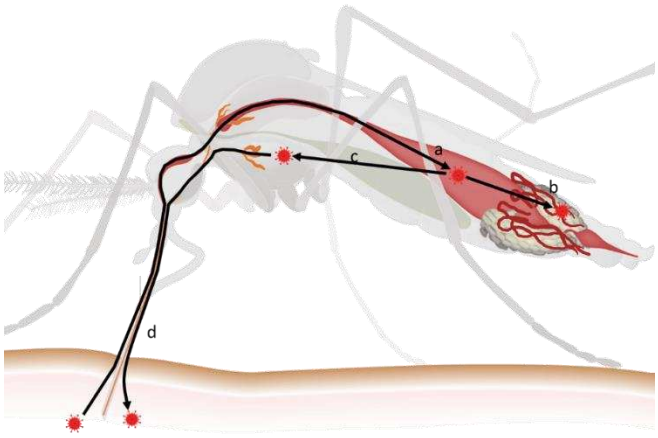


Figure 3.1. Infection of a mosquito vector with an arbovirus. The events a (midgut infection), c (viral dissemination) and d (infection of salivary glands and expectoration of infectious virions) each occur to allow horizontal transmission. The infection of ovaries (b) is independent of viral dissemination (c).

By considering these steps as discrete, with probabilities of success *a*, *b*, *c*, and *d*, we have constructed a simple Bayesian model to estimate susceptibility to infection of mosquito organs collected throughout a vector competence experiment, in which native Colorado mosquitoes were assessed for the competence for Rift Valley Fever Virus (RVFV). This model was constructed based on our sample collection methods, where dissections were performed to

collect saliva, legs/wings (as an indicator for dissemination), ovaries (indicating potential for vertical transmission), and remaining carcass (as an indicator for midgut infection). The following can be modified for more detailed follow-up sample collection, for example, where salivary glands are removed to tease apart salivary gland infection and salivary gland escape barriers.

Infection status of mosquito organs follows a Bernoulli distribution with probability of success represented by p_i. **m** is the data vector of mosquito midguts, where $\mathbf{m}_i = 1$ for an infected midgut from individual *i*, and $\mathbf{m}_i = 0$ for an uninfected midgut. **o** is the ovary data vector, **d** is the vector of leg/wing data and **s** is the vector for the saliva data.

$$\boldsymbol{m}_i \sim Bernoulli(p_1)$$

$$\boldsymbol{o}_i \sim Bernoulli(p_2)$$

$$\boldsymbol{d}_i \sim Bernoulli(p_3)$$

$$\boldsymbol{s}_i \sim Bernoulli(p_4)$$

As p_1 is interpreted as the probability of a mosquito acquiring a midgut infection, it is equal to *a*. p_2, the probability of ovarian infection in a mosquito following a bloodmeal, however is the result of midgut infection establishment and spread to the ovaries. In this case it is the product of the probabilities of those two events, or $a * b$. The probability of a disseminated infection, p_3 is $a * c$, and the probability of virus in saliva (transmission, p_4) is $a * c * d$.

$$p_1 = a$$

$$p_2 = a * b$$

$$p_3 = a * c$$

$$p_4 = a * c * d$$

Uninformative beta prior distributions were chosen to exert the minimum effect on the value of the parameter estimates (*a*, *b*, *c*, *d*).

$$a, b, c, d \sim beta(1,1)$$

As the estimated parameters (*a*,*b*,*c*,*d*) represent the probabilities of infection, we defined the midgut infection barrier as the probability of failure, so that the midgut infection barrier is 1-*a*, the midgut escape barrier is 1-*c*, the salivary gland barrier as 1-*d*, and the ovarian infection barrier as 1-*b*. A complete list of model parameters with definitions is given in **Table 3.1**.

Table 3.1. Parameters of the within-vector model of arbovirus infection, with definitions and expressions.

Parameter	Definition	Mathematical Expression
p_1	Probability of midgut infection	a
p_2	Probability of ovarian infection	a*b
p_3	Probability of infection dissemination	a*c
p_4	Probability of infectious saliva	a*c*d
a	Midgut infection probability	a=p1
b	Probability that established midgut infection spreads to ovaries	a=p2/p1
c	Probability that established midgut infection disseminates to legs and wings	c=p3/p1
d	Probability that disseminated infection produces virus in saliva	d=p4/p3
1-a	Midgut infection barrier	
1-b	Ovarian infection barrier	
1-c	Midgut escape barrier	
1-d	Saliva barrier	

The above model was fit for each species separately using the 'runjags' package (Denwood 2016) in the R environment (R Core Team 2017); two parallel Markov Chain Monte

Carlo (MCMC) chains were run with 5,000 burn-in iterations and 120,000 monitored samples. The JAGS model code is included as **Supplemental Text A1**. Model parameters were fit separately for each species. Statistical significance between parameter estimates was determined by examining 95% credible intervals (CI's) for overlap. These analyses assumed that infectious blood meals administered were consistent enough in titer to have negligible effects on the observed infection outcomes.

3.3 Results

3.3.1 Blood Meal Titers

Infectious blood meals administered to field collected mosquitoes varied only slightly in titer (**Table 3.2**). Mosquito samples from each replicate represent samples for which feeding, incubation, and dissections were completed generating a full sample set (saliva, ovaries, legs/wings, carcasses) reached 52 for *Ae. vexans*, 31 *Ae. melanimon*, 21 *Cx. tarsalis*, 5 *Cs. inornata*, and 3 *Ae. increpitus* (**Table 3.2**).

Table 3.2. Numbers of mosquitoes challenged with RVFV KEN128B-15 by species and replicate. *Titer for each blood meal administered to mosquitoes, in plaque forming units per milliliter (PFU/mL).

Species	Replicate 1 ELC *4.0E6 PFU/mL	Replicate 2 Timnath *2.1E6 PFU/mL	Replicate 3 McMurry *7.8 E6 PFU/mL	Grand Total
Aedes increpitus	0	0	3	3
Aedes melanimon	2	29	0	31
Aedes vexans	12	27	13	52
Culex tarsalis	3	3	15	21
Culiseta inornata	2	1	2	5
Grand Total:	19	60	33	112

3.3.2Midgut Infection

Mosquitoes of each species in this study exhibited viral infections of the midgut, detected by plaque assays of homogenized carcasses. Numbers of positive samples are shown in **Table 3.3**. Model estimates for midgut infection probability were significantly higher for *Cx. tarsalis* than for *Ae. vexans* and *Ae. melanimon* (Fig. 2). Midgut infection probabilities were also significantly higher for *Cs. inornata* than for *Ae. melanimon*. All RT-qPCR testing confirmed the presence of RVFV RNA in samples with positive plaque assays; none of these samples were positive for WNV by RT-qPCR.

Table 3.3. Positive samples by species and sample type (number of positive samples / total number of samples).

Tissue	carcass	legs/wings	ovaries	saliva
Aedes increpitus	1/3	0/3	0/3	0/3
Aedes melanimon	6/31	1/31	1/31	1/31
Aedes vexans	22/52	3/52	6/52	1/52
Culex tarsalis	18/21	9/21	7/21	6/21
Culiseta inornata	4/5	3/5	3/5	3/5

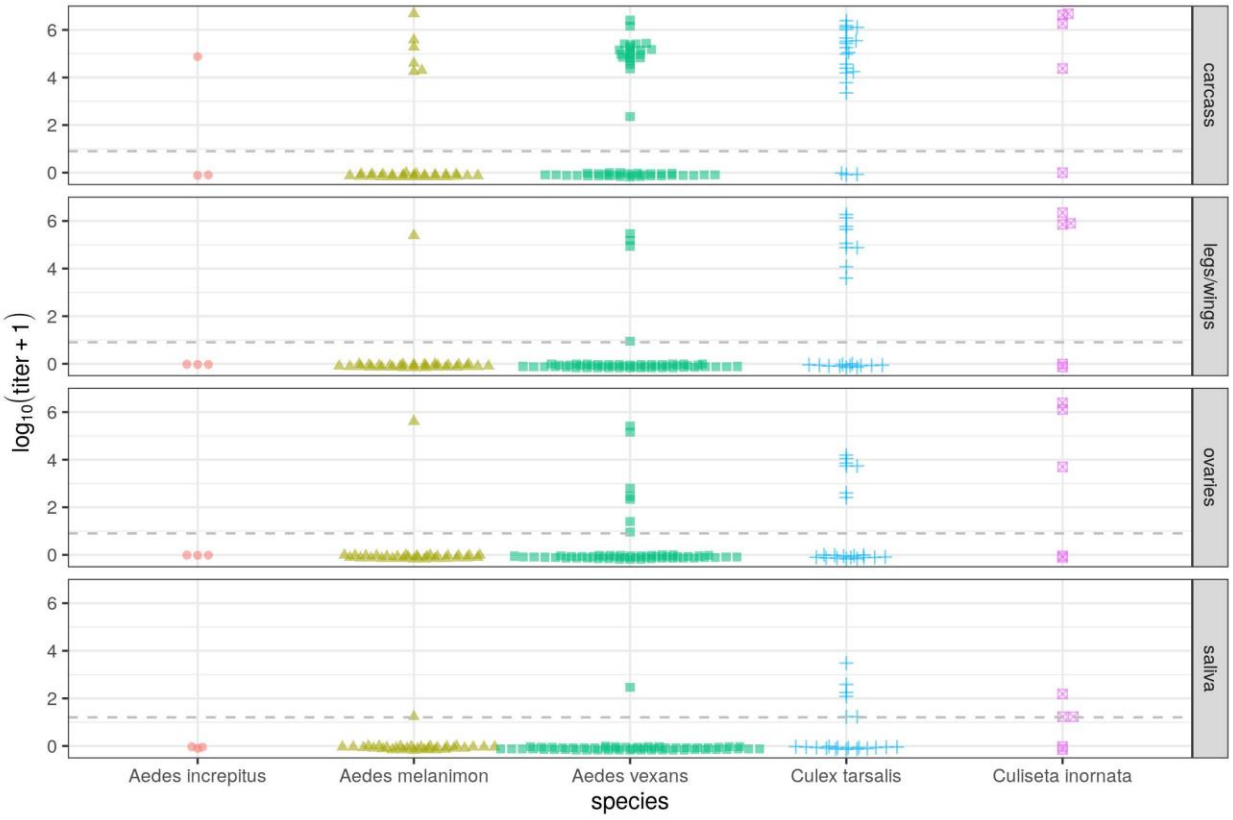


Figure 3.2. PFU/mL RVFV detected in mosquito tissues by plaque assay. Dashed lines represent limits of detection for the assay.

3.3.3Viral Dissemination

Viral dissemination from the midgut to the hemolymph of the mosquito, indicated by virus detection in legs and wings of mosquitoes, occurred in all species with the exception of *Aedes increpitus* (**Figure 3.2**, **Table 3.3**). There were significantly higher model estimates for probability of dissemination among *Cs. inornata* and *Cx tarsalis* than both *Ae. melanimon* and *Ae. vexans* (**Figure 3.3A)**. We did not observe significant differences between model estimates of midgut escape barriers among mosquito species (**Figure 3.3B)**.

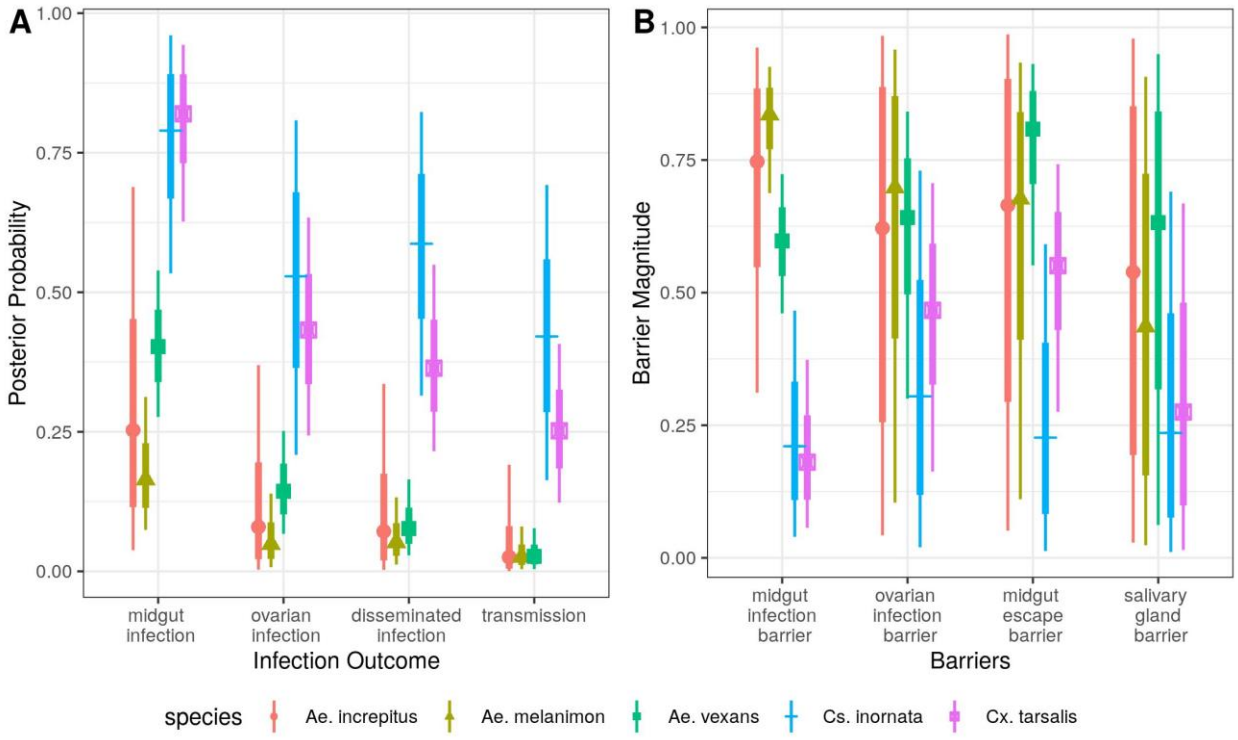


Figure 3.3. Model parameter estimates fit to mosquito organ infection data. A shows the probability of each infection outcome. **B** shows the barriers to infection as independent, step-wise parameters. See also Supplemental Text A1 and Table 1 for the definitions and associated model parameters.

3.3.4 Transmission via Saliva

Infectious virions were detected in the saliva of at least one individual mosquito representing every mosquito species tested in these experiments except for *Ae. increpitus* (**Figure 3.2**, **Table 3.3**). Transmission was assumed for mosquitoes with any measurable virus in saliva by plaque assay, and parameter estimates for p4 (**Table 3.1**) interpreted as the probability of transmission. Transmission probability for *Cs. inornata* was the highest (median = 0.42, lower 95% CI = 0.16, upper 95% CI = 0.69), followed by *Cx. tarsalis* (median = 0.26, lower 95% CI = 0.12, upper 95% CI = 0.40), then *Ae. vexans* (median = 0.027, lower 95% CI = 0.0012, upper 95% CI = 0.068), *Ae. melanimon* (median = 0.026, lower 95% CI = 7.9 E-4, upper 95% CI = 0.070), and *Ae. increpitus* (median = 0.025, lower 95% CI = 3.6 E-7, upper 95% CI = 0.15). Though we did

not detect virus in saliva samples from *Ae. increpitus*, sample sizes were low for this species (n=3) therefore the possibility of transmission by this species cannot be eliminated.

3.3.5Ovarian Infection

Finally, ovaries were tested by plaque assay to investigate the possibility of vertical transmission. Numbers of infected ovary samples are shown in **Table 3.3**. *Cs. inornata* showed the highest probability of ovarian infection (median = 0.53, lower 95% CI = 0.21, upper 95% CI = 0.81), followed by *Cx. tarsalis* (median = 0.43, lower 95% CI = 0.24, upper 95% CI = 0.63), *Ae. vexans* (median = 0.14, lower 95% CI = 0.06, upper95% CI = 0.24), *Ae. increpitus* (median = 0.08, lower 95% CI = 4.2 E-6, upper 95% CI = 0.31), and *Ae. melanimon* (median = 0.05, lower 95% CI = 2.8E-3, upper 95% CI = 0.12) (**Figure 3.3A)**. Interestingly, we observed four *Ae. vexans* mosquitoes for which ovaries tested positive, while corresponding legs/wings were not (**Figure 3.2**, **Table 3.3**).

3.4 Discussion

This study assessed the ability of wild-caught mosquitoes from Colorado to become infected with and transmit an outbreak strain of RVFV. Among the species assessed are those that are documented to feed upon potential local amplifying hosts of RVFV, and two mosquito species for which vector competence had not yet been assessed (*Ae. melanimon* and *Ae. increpitus*). This study also represents the first set of experiments to test several North American mosquitoes for a more recent epidemic strain than that historically used. The data presented here confirm the ability of several of these mosquito species, all with host breadths including RVFV-susceptible vertebrate hosts, to transmit RVFV by bite. In order to understand infection patterns at the organismal level, several tissues were harvested from mosquitoes and tested for infectious virions by plaque assay. While sample sizes are relatively low for *Cs. inornata* and *Ae. increpitus,* we were able to draw credibility intervals on the susceptibilities of these mosquitoes

by using a within-host model. This novel within-host model further allowed us to assess the relative importance of infection and transmission barriers in different species.

3.4.1 Blood Meal Titers

Viral titers encountered by naïve mosquitoes can vary widely depending on the host species, host age, and period of viremia. The viral titers in the blood meals administered to mosquitoes were realistic representations of peak viremias reached in 4-5 month old North American Polypay sheep (Faburay et al. 2016), 5 month old white-tailed deer (Wilson et al. 2018), and 7 dal old calves (Rippy et al. 1992), so our inoculum may represent either transient or peak viremias of these animals (**Table 3.2**). Due to the nature of RVFV blood viremias in these vertebrate hosts, and the demonstrated effect of viremia on vector competence (Turell et al. 2010), the infection probabilities presented here likely represent the higher end of the spectrum. The viremias that may develop in domestic North American cattle are not well investigated at the time of this writing; this should be a research priority given the relationship between viremia and mosquito susceptibility (Golnar et al. 2014).

3.4.2 Midgut Infection Probability

Infection probabilties for *Ae. vexans* in this study were not markedly different from previously reported infections using mixed Colorado/California populations (Turell et al. 2010), and still below infection probabilities for the moderately competent Florida population (Turell et al. 2013). Population-level variation in susceptibility of *Ae. vexans* to infection may reflect genetic factors (Beerntsen et al. 2000), or variation among experimental methods. Infection probabilities of *Ae. increpitus* and *Ae. melanimon* were moderate and did not differ significantly from *Ae. vexans.* Midgut infection probabilities were relatively high for the permanent water breeders *Cx. tarsalis* and *Cs. inornata* (**Figure 3.3**); previous infection rates of *Cx. tarsalis* exposed to a higher dose (7.3 $\log_{10}$ PFU/mL) were lower than reported here. Infection

probabilities of *Cs. inornata* were high, similar to previous experiments using Canadian mosquitoes (Iranpour et al. 2011).

3.4.3Dissemination Probabilities

Viral dissemination from the midgut to the circulatory system, or midgut escape, requires virus particles to pass through the basal lamina of the mosquito gut into the hemolymph. As with midgut infection, disseminated infection probabilities (**Figure 3.3A)** were higher than previously demonstrated with *Ae. vexans* from mixed Colorado/California collections (Turell et al. 2010). This previous work demonstrated strong midgut infection barriers, as well as strong midgut escape barriers in *Ae. vexans*, resulting in overall low transmission efficiency. Data presented here supports this observation; for *Ae. vexans*, the midgut escape barrier was the highest estimated (**Figure 3.3B)**. Disseminated infection probabilities were similar for the other floodwater species, *Ae. increpitus* and *Ae. melanimon* (**Figure 3.3A**). Dissemination also trended higher for *Cx. tarsalis* and *Cs. inornata* compared to the *Aedes spp. Cs. inornata* exhibited an especially low midgut escape barrier (**Figure 3.3B**), in addition to its low midgut infection barrier.

3.4.4Transmission Probabilities

Transmission, defined as detectable virus in saliva, trended higher for *Cx. tarsalis* and *Cs. inornata* than the *Ae. spp* (**Figure 3.3A**). Previous transmission efficiency data for *Cx. tarsalis*, fed on a higher-titered blood meal (7.3 $\log_{10}$ PFU/mL), was lower than presented here (Turell et al. 2010). These previous experiments utilized infected hamsters to infect mosquitoes, and susceptible hamsters to test for transmission, so it is unclear how to relate these data to that generated in our study. The transmission probability estimated for *Aedes vexans* were low as demonstrated previously (Turell et al. 2010), and for *Ae. melanimon*. Given the high abundances for these species in Colorado, as well as their blood-host preferences for susceptible vertebrate

hosts (Hartman et al. 2019), they could contribute significantly to RVFV transmission. The only species we tested that did not show positive saliva for RVFV was *Ae. increpitus*; however, the sample size was small, and Bayesian estimation of transmission probability for these mosquitoes yielded 95% CI's similar to the other floodwater species (**Figure 3.3**), so transmission cannot be ruled out entirely. *Ae. increpitus* mosquitoes have exhibited blood-host preferences including a large proportion of mule deer (Thiemann et al. 2017), and may still make a contribution to RVFV maintenance in the United States.

The results from some individuals that were positive but directly on the limit of detection of the plaque assay are difficult to interpret in terms of biological relevance. This was evident for some saliva samples from *Cs. inornata*, *Cx. tarsalis* and *Ae. melanimon* all of which had a disseminated infection (**Figure 3.2**). Removing these individuals from the data as positives and running the model produces slightly different parameter estimates (**Supplemental Figure A1**) but does not qualitatively change the conclusions made here. While low RVFV titers have been reported elsewhere (Garcia et al. 2001, Wilson et al. 2013, Bergren et al. 2021), contamination cannot be ruled out entirely.

3.4.5Ovarian Infection

RVFV also presents some ecological complexity due to its ability to be vertically transmitted by mosquitoes. There is strong evidence for vertical transmission among *Ae. macintoshi* mosquitoes from Kenya, contributing to viral maintenance through inter-epidemic periods (Linthicum et al. 1985). While infection of ovaries is a prerequisite for vertical transmission, proportions of mosquito ovaries with detectable virus may not relate directly to the proportion of infected progeny. However, these data provide preliminary evidence that vertical transmission may be possible in these mosquito species. Again, there was a trend toward higher

ovarian infections probabilities in *Cx. tarsalis* and *Cs. inornata* compared to *Ae. vexans*, *Ae. melanimon*, and *Ae. increpitus* (**Figure 3.3A**). Though we do not see statistically significant differences among many of the internal infection barrier estimates (**Figure 3.3B**), median ovarian infection barrier estimates were lower for *Cx. tarsalis* and *Cs. inornata*, suggesting that this is not all attributable to differences in midgut infection and escape barriers.

We made an interesting observation with four *Ae. vexans* mosquitoes, for which ovaries were positive for infectious virus in the absence of viral dissemination from the midgut. This observation has been made with experimentally infected *Cx. tarsalis* mosquitoes (Bergren et al. 2021), but it cannot be ruled out that some disseminated infections were missed due to low viral loads in legs/wings relative to the limit of detection. Similar patterns have been observed with La Crosse virus in the vector *Aedes triseriatus* (Say) (Chandler et al. 1998). RVFV has been detected in the tracheal system of mosquitoes, and this has been hypothesized as an alternative route of dissemination to classical midgut escape in which virus passes through the gut and basal lamina (Romoser et al. 2005, 2011, Kading et al. 2014). This route of ovarian infection is recognized for other mosquito-borne diseases (Romoser et al. 2004, Salazar et al. 2007). Independence between these routes of infection is accounted for in our model structure.

3.4.6 Conclusions

Collectively, these results reinforce the hypothesis that transmission of RVFV among various wildlife species and domestic ungulates in the United States would likely involve several mosquito vector species (Golnar et al. 2014). This complexity presents a major challenge for the implementation of vector surveillance and control strategies in the event of an invasion of RVFV. The detection of infectious virus in mosquito ovaries in several of these species is especially troubling. Vertical transmission by *Aedes spp.* would result in additional viral reservoirs in desiccation-resistant egg populations, while vertical transmission by *Culex* and *Culiseta spp.,*

which overwinter as adults, would enhance early season amplification in temperate zones where these mosquitoes diapause. Further studies should investigate the viral tropism in F1 generation mosquitoes to determine any transstadial barriers that may or may not exist.

We developed a within-host model for the analysis of vector competence data. This offers many advantages over qualitative descriptions. First, this model offers mathematical definitions that formalize ideas such as infection barriers. Fitting this model to data offers a holistic, functional analysis to estimate these parameters while producing measures of uncertainty (95% CI's). This is especially useful for studies using wild-caught mosquitoes and select agent pathogens, wherein sample sizes can be small. Finally, this model can be easily extended to include any number of covariates, such as blood meal titer and incubation temperature. We recommend the use of such models for future vector competence work, so that rigorous comparisons can be made between experiments.

CHAPTER 4: MOSQUITO ABUNDANCES AND FEEDING BEHAVIORS SUPPORT THE POTENTIAL FOR ESTABLISHMENT OF RIFT VALLEY FEVER IN NORTH AMERICAN DEER

4.1 Introduction

Rift Valley fever virus (RVFV) is mosquito-borne disease of domestic ungulates that causes high burdens on human and livestock health throughout sub-Saharan Africa, Egypt, and the Arabian Peninsula. Infection of cattle and sheep can result in spontaneous abortions, and high mortality among young animals (Pepin et al. 2010). Infection of humans can occur through mosquito-borne transmission, or by direct contact via handling of infected animal tissues, causing febrile illness, encephalitis, blindness, and mortality albeit at low rates (Bird et al. 2009). Originally focused in eastern Africa, RVFV has spread throughout much of sub-Saharan Africa, Egypt, and the Arabian Peninsula since its description in 1931 (Daubney et al. 1931, Bron et al. 2021). The introduction of Rift Valley fever virus (RVFV) to the United States is a very high threat that would have wide ranging impacts on human, wildlife, and domestic ungulate health (Hartley et al. 2011, Wilson et al. 2018).

In order to inform the most effective preventative measures requires an understanding of risk for each step of the process, from potential sources, pathways of introduction, and potential for establishment of local transmission cycles or "receptivity". Analysis of human and mosquito movement by plane and ship, as well as mammal importation suggest the most likely route of introduction to be the arrival of an infected human by plane, while identifying several high-risk source and arrival cities (Golnar et al. 2017). The receptivity of domestic ungulate populations in an animal agriculture setting has also been analyzed quantitatively, showing seasonal risk of establishment in the California Central Valley (Barker et al. 2013).

Receptivity in a sylvatic context has yet to be addressed. While there is a dearth of data on potential for North American wildlife to serve as amplifying hosts of RVFV, white-tailed deer have been demonstrated to be highly susceptible to infection, while producing exceptionally high blood viremias (Wilson et al. 2018). Hoofed ungulates including cattle, sheep and deer are predicted to be the most likely amplifying hosts of RVFV in North America based on existing reservoir competence data (Golnar et al. 2014). This is a grave concern, given that many North American species have demonstrated the ability to transmit RVFV horizontally by bite (Turell et al. 2010, 2013, 2015, Iranpour et al. 2011, Turell, M.J., Byrd, B.D., Harrison 2013, see also Chapter 3), and have demonstrated some capacity to feed on deer in agricultural areas in northern Colorado including *Aedes vexans* Meigen, *Aedes melanimon* Dyar, and *Aedes dorsalis* Meigen (Chapter 2). Vector abundances and rates of contact with hosts are critically important to determining the overall vectorial capacity of a mosquito species, as they incorporate epidemiologically significant ecological components of virus transmission in addition to the capacity for a mosquito to transmit the virus biologically. *Culex tarsalis* Linnaeus has demonstrated high vector competence for RVFV in the laboratory setting (Turell et al. 2010) as well as the capacity to feed on deer in the wild (Thiemann et al. 2012, 2017). Still, entomological studies specifically investigating the potential for sylvatic establishment of RVFV in populations of wild deer are lacking.

Control of RVFV following introduction would be immensely difficult, if not impossible, in the long term if sylvatic transmission cycles become established. This has been the case for West Nile virus (WNV) since the introduction and establishment of sylvatic transmission in avian populations (Nash et al. 2001). Subsequent identification of mosquito vectors involved in sylvatic transmission, as well as those determined to be bridge vectors between avian species and

humans, have been crucial to the mitigation of human disease. Still, WNV continues to cause human cases annually, due largely to amplification in avian populations and spillover into humans via bridge vectors (Dunphy et al. 2019). While the ecology of RVFV is very different from WNV, the health consequences of zoonotic establishment of an invasive virus in native wildlife and mosquito populations following an introduction are clear. This study investigates the potential ecology of RVFV circulation in a woodland environment in Northern Colorado, with an emphasis on mosquito abundances and blood feeding behaviors on deer and other wildlife species that may contribute to the establishment of RVFV in this ecosystem.

4.2 Methods

4.2.1 Field Collections

Collections were made at the Environmental Learning Center (ELC) in Fort Collins, Colorado. This site lies along the Poudre River in Fort Collins, CO (**Figure 4.1**). The property is the site of walking trails, a length of which runs parallel to the river. The site is characterized by cottonwood stands, with areas of dense brush, and areas of more sparse meadow allowing the growth of cacti of the genus *Opuntia*. Deer are regularly observed at the site, as well as lagomorphs and raccoons. The path also allows access for human recreation, making this site suitable for studying spillover/spillback potential.

Five trapping locations were established (**Figure 4.1**), where collections were made weekly from June 27, 2019 to August 30, 2019. Abundances were measured using 5 CDC Miniature Light Traps (John W. Hock Company, Gainesville, Florida, United States) baited with CO2. Traps were placed along a trail adjacent to the Poudre River, and spaced between 85 and 155 m apart. We deployed square fiber pots (12-3/8 in square x 11-1/8 in. tall; Western Pulp Products Company, Corvalis, Oregon, United States), painted black on the interior, for

collections of blooded mosquitoes (Komar et al. 1995). Thirty pots were placed along the trap line and aspirated twice weekly using an InsectaZooka Field Aspirator (Bioquip, Rancho Dominguez, California). Mosquitoes were killed by freezing and placed at -80 C for blood meal preservation until identification. Blood from the abdomens of *Aedes spp.* mosquitoes that were collected during routine surveillance by Vector Disease Control International (Loveland, Colorado) were also added to the sample set for blood meal identification. Female mosquitoes were identified to the species level using two taxonomic keys (Darsie and Ward 2005, Rose et al. 2017).

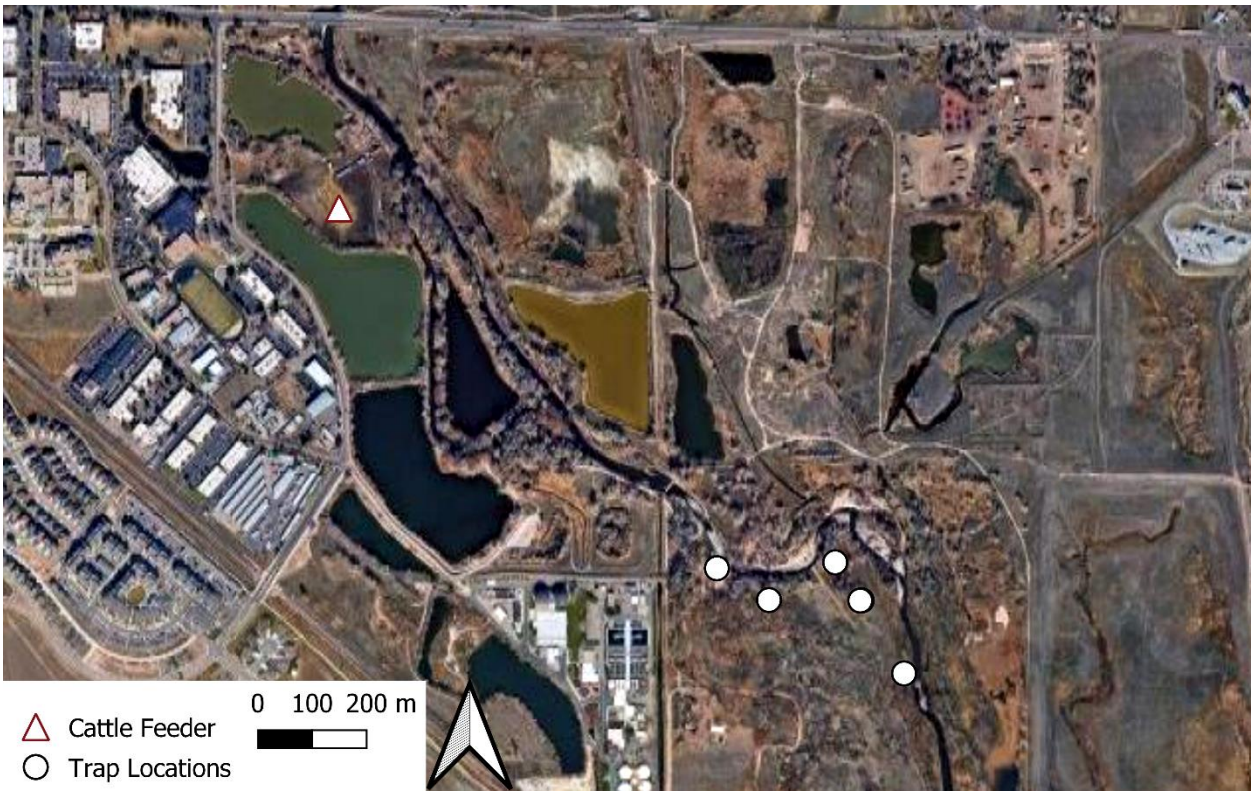


Figure 4.1. Map of the Trap Locations and Nearby Cattle Feeder.

4.2.2 Blood Meal Sequencing

For mosquitoes identified with blood in abdomen, we recorded the Sella stage (Detinova 1962) describing the stage of bloodmeal digestion, following the figure in Santos et al. (2019) The numerical scoring was modified to allow for scoring of partial blood meals; to include these

categorically, empty abdomens were assigned 0 instead of 1, partially blood meals were assigned a value of 1, and the remaining stages were unchanged. Partial blood meals (score=1) were distinguished from those at later stages of digestions by a brighter red color and the lack of egg development, while later stages were darker and egg development was apparent.

DNA was extracted for identification of the vertebrate host source. DNA was extracted using the QIAmp DNA Investigator Kit (Qiagen, Valencia, California), following the recommended protocol for small volumes of blood or saliva. We amplified 700 nucleotides of the *cytochrome c oxidase subunit 1* (*CO1*) gene (Ivanova et al. 2007) according to (Hartman et al. 2019). Those samples that did not amplify PCR products with this primer set were rerun using the primers Mod_RepCOI_F and Mod_RepCOI_R (Reeves et al. 2018), also targeting the *CO1* gene. PCR products were sent to either Quintarabio (Cambridge, Massachusetts) or Genewiz (South Plainfield, New Jersey) for PCR purification and Sanger sequencing using the M13 forward primer (Messing 1983). Chromatograms were checked visually for quality using Geneious Prime version 2019.2.3 (https://www.geneious.com). Vertebrate host identity was identified using the Barcode of Life database (BOLD) as well as GenBank Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) as performed previously (Hartman et al. 2019).

4.3Results

4.3.1Mosquito Abundances

We accomplished a total trapping effort of 49 trap nights considering CDC light traps, as well as 180 fiber pot aspirations throughout the 9-week sampling period. We experienced 2 trap failures; 1 occurred on 7/3/2019 (week 2) and another on 8/9/2019 (week 7). We placed one trap

opportunistically on 8/19/2019, between weeks 8 and 9. We collected a total of 51,422 female mosquitoes belonging to 16 species across 5 genera.

Several floodwater *Aedes spp.* were present, with *Ae. vexans* being the most abundant with 38,700 total mosquitoes collected. Peak abundances for Ae. vexans reached nearly 4000/trap*night on July 19, and maintained high abundances throughout the season relative to the other species measured (**Figure 4.2A, Figure 4.3)**. There was some variability for this species by trap location, with trap 5 collecting more individuals than the other locations. *Ae. increpitus* and *Ae. melanimon* were the next two abundant species with 3,458 and 3,276 total mosquitoes collected, respectively. Abundances for these species peaked with similar timing to *Ae. vexans*, albeit with lower numbers (**Figure 4.2A, Figure 4.3**). *Ae. dorsalis* and *Ae. trivittatus* were also present in low numbers, with 23 and 20 total collections, respectively. *Ae. spencerii idahoensis* was collected, though this represents 2 specimens collected across the sampling period.

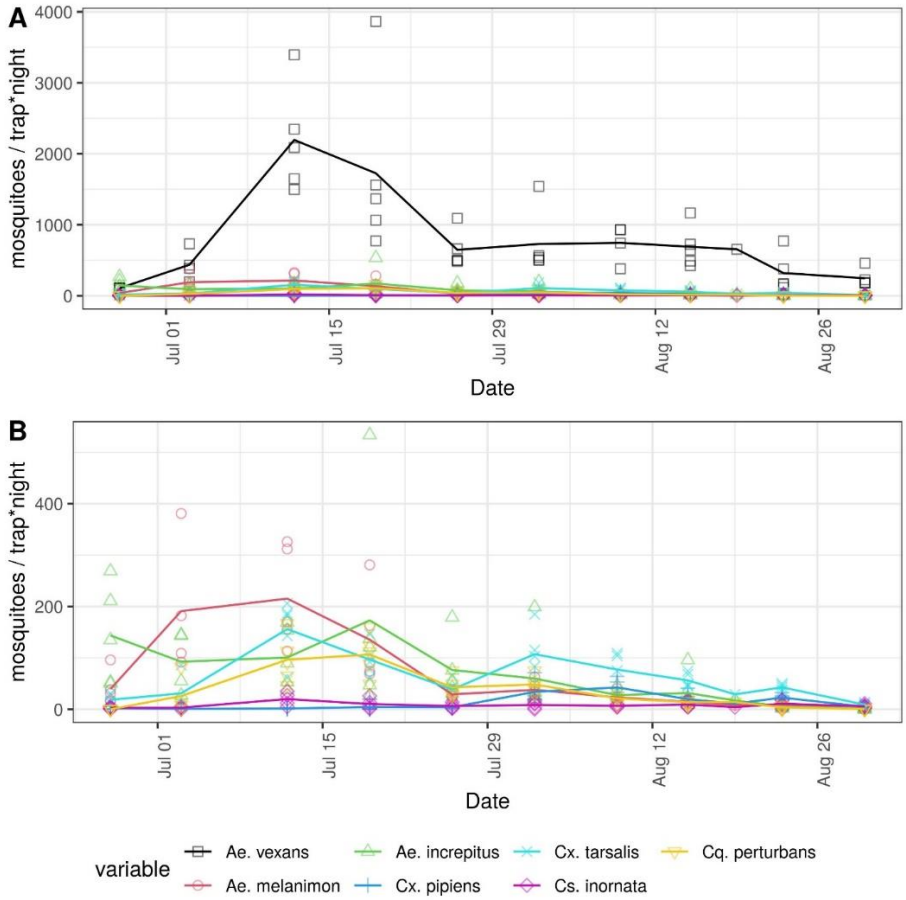


Figure 4.2. Abundances of mosquitoes collected at the Environmental Learning Center. Points show individual trap counts, and lines indicate mean numbers of mosquitoes for the date indicated on the x-axis. Panel A shows all mosquito species with total collections greater than 25 individuals. Panel B shows the same collections of species but without *Ae. vexans*, so that the less abundant species can be visualized.

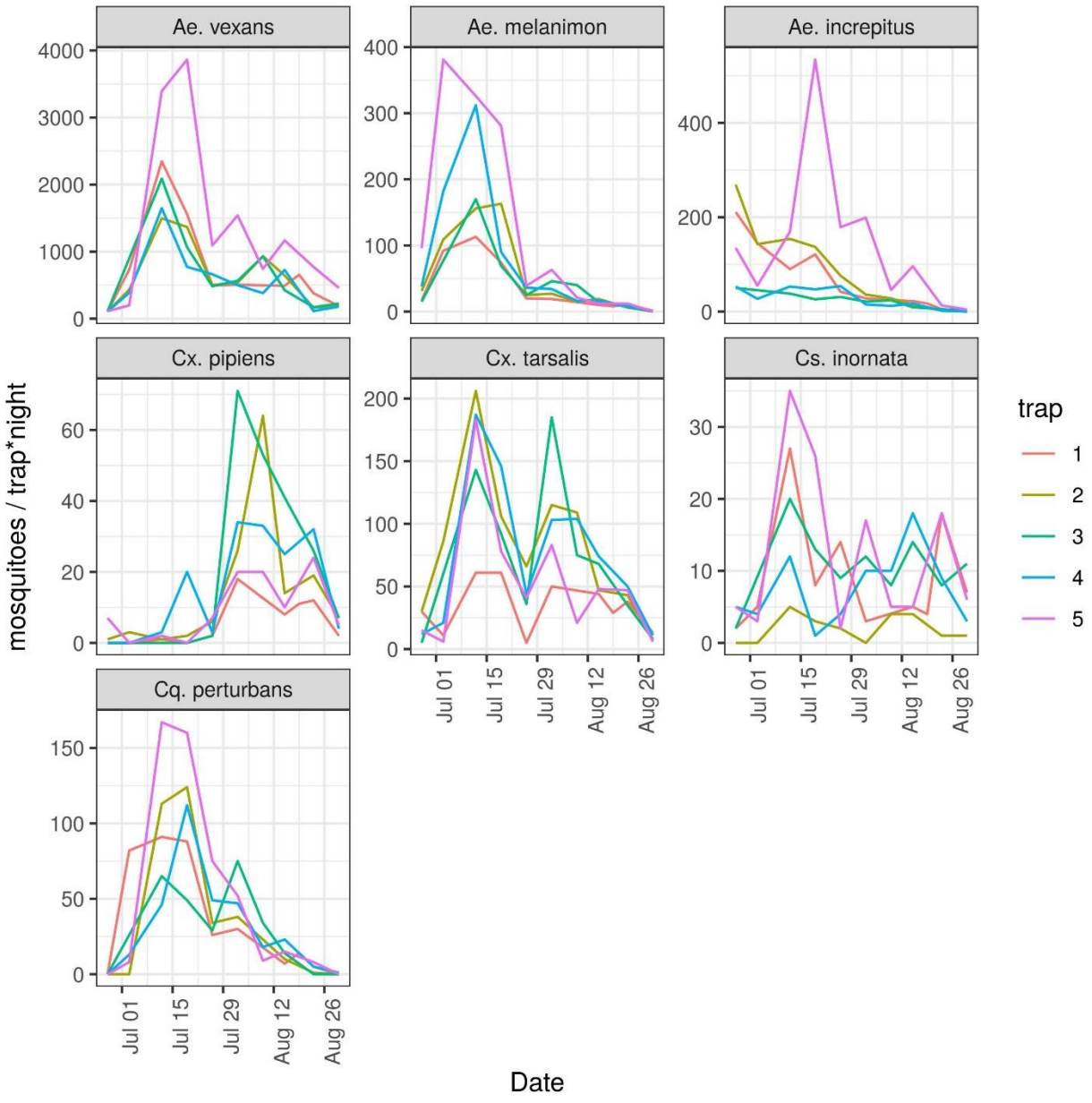


Figure 4.3. Mosquito abundances by species, trap location and date. Each line represents the abundance at a specific trap location, highlighting both the qualitative shape of the abundances through time, as well as potential spatial heterogeneity.

Permanent and semi-permanent water breeding mosquitoes were present at the study site, but generally were generally less abundant. *Culex tarsalis* was the most abundant permanent water mosquito, and the fourth most abundant overall, with 3,084 mosquitoes collected

throughout the sampling period. *Coquillettidia perturbans* was present at the site with 1,760 total mosquitoes collected, followed by *Culex pipiens* and *Culiseta inornata* with 646 and 408 respective total collections. Four additional *Culex* species were collected: *Cx. salinarius*, *Cx. restuans*, *Cx. eurythorax*, and *Cx. territans*, but each with fewer than 15 mosquitoes total. Finally, two Anopheline species were collected: *An. earlei* with 22 total collections, and *An. freeborni/hermsi spp.* with 5 total collections. *Culex tarsalis* abundances appear bimodal across all specific trap locations, with peak abundances around mid-July and early August (**Figure 4.2**, **Figure 4.3**). *Coquillettidia perturbans* abundances peaked at mid-July, while *Cx. pipiens* peaked in early August. *Culiseta inornata* were abundant in mid-July, but maintained a slightly lower level throughout the season (**Figure 4.2**, **Figure 4.3**).

4.3.2Blood Meal Analysis

We sequenced and identified the vertebrate species source of 141 blood meals, including 87 *Ae. vexans*, 46 *Ae. increpitus*, 4 *Ae. melanimon*, 2 *Cx. tarsalis*, and 1 *Cs. inornata* (**Figure 4.4**). Few of these blood fed mosquitoes were collected from the fiber pot resting traps: 5 *Ae. increpitus*, 4 *Ae. vexans*, 2 *Cx. tarsalis*, and 1 *Cs. inornata*. Sanger sequencing of the *co1* gene spanned 7 total vertebrate species, including 5 mammalian species [*Bos taurus* (cow), *Equus caballus* (domestic horse), *Homo sapiens* (human), *Procyon lotor* (raccoon), and *Odocoileus virginianus* (white-tailed deer), 1 lagomorph species *Sylvilagus floridanus* (eastern cottontail rabbit)] and a single bird species (*troglodytes aedon*, house wren).

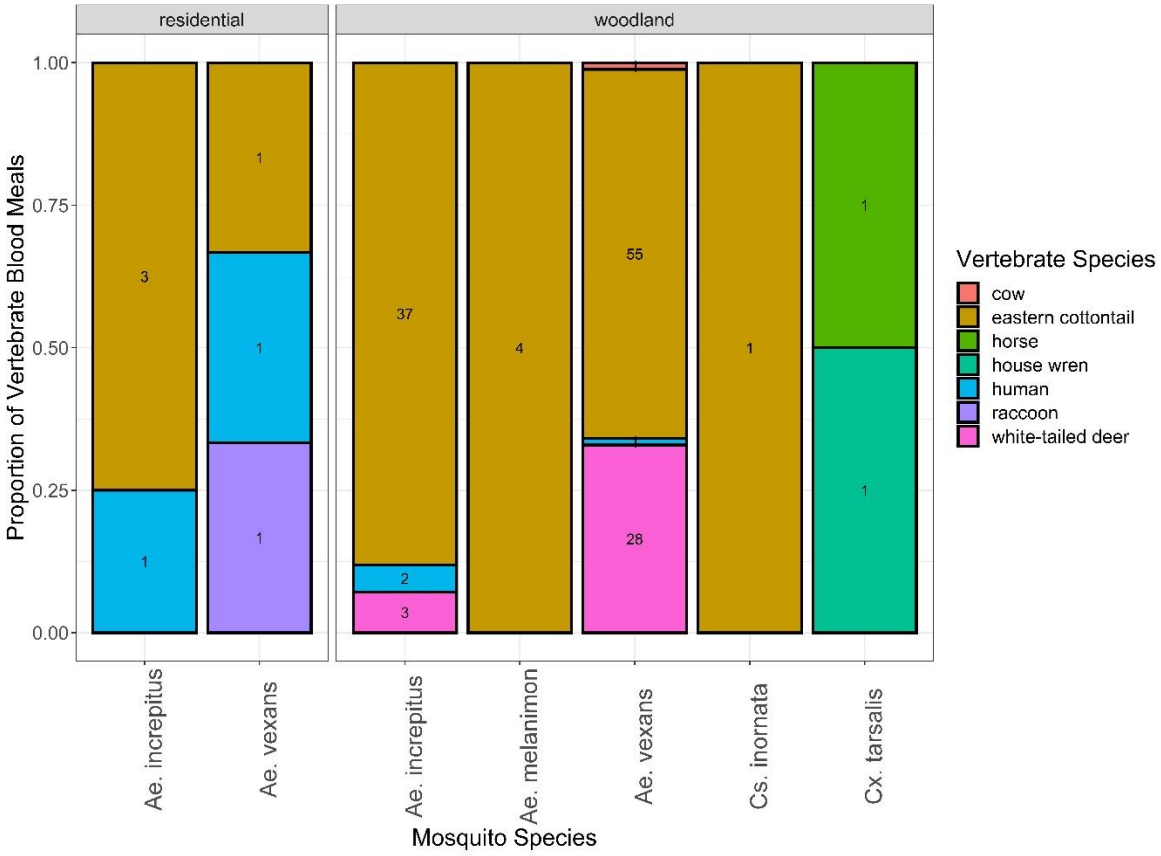


Figure 4.4. Blood Meals Identified to the species level, by habitat type, mosquito species and vertebrate identity. "Woodland" includes mosquitoes collected from the Environmental Learning Center, and "residential" includes mosquitoes collected by Vector Disease Control International (VDCI). Numbers indicate total number of blood meals identified to each vertebrate species.

The recorded Sella stages for collected blood meals for *Ae. vexans* and *Ae. increpitus*, for which many vertebrate sources were identified as eastern cottontail and white-tailed deer. For both of these mosquito species, Sella stages were more variable for eastern cottontail rabbits, compared to white-tailed deer, which were by a much larger margin replete blood meals (Sella => 2). This comparison, and the data plotted in **Figure 4.5** consider only mosquitoes collected in CDC light traps, due to the low numbers of mosquitoes collected in pots, and the possibility for

sampling bias between these two collection methods (Thiemann and Reisen 2012). A complete plot of the Sella scores for all blood-fed mosquitoes is included as **Supplemental Figure A2**.

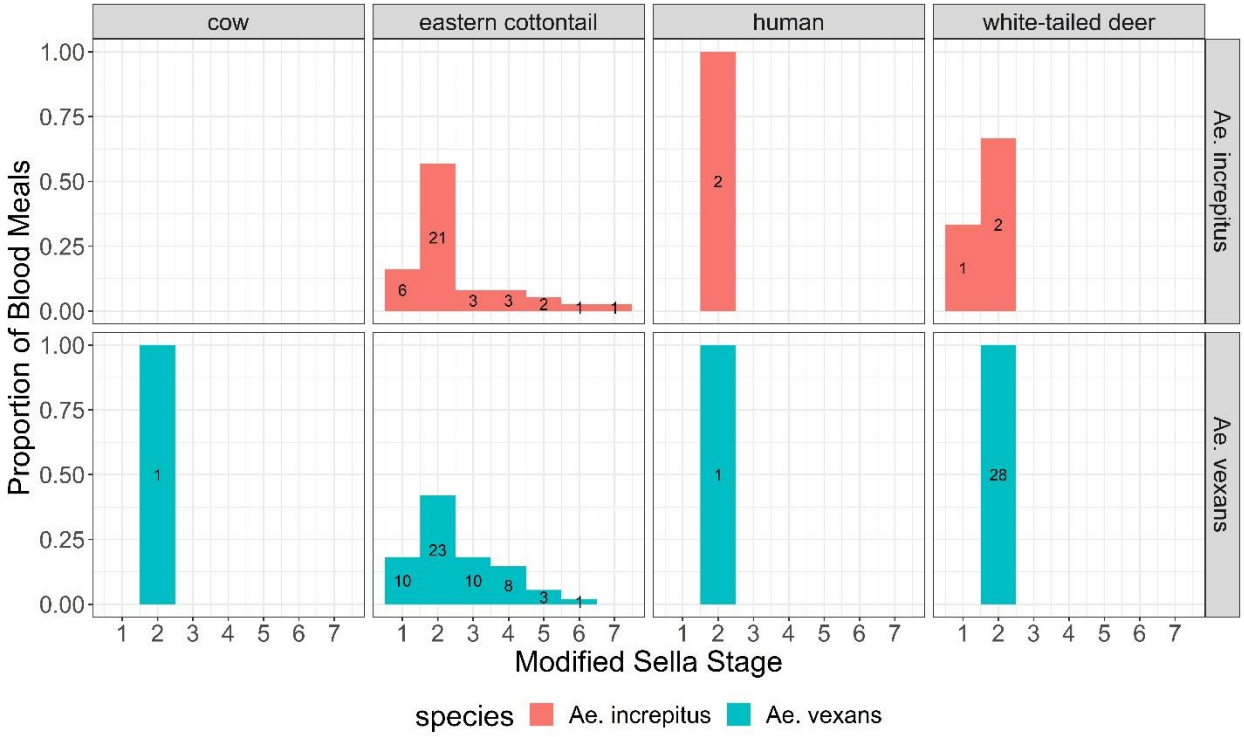


Figure 4.5. Density histograms of modified Sella stages recorded from blood-fed *Ae. increpitus* and *Ae. vexans*. Numbers indicate total samples included for each stage. Data presented only from mosquitoes collected in CDC light traps.

4.4 Discussion

The introduction of Rift Valley fever virus to the United States is a troubling possibility, due to the ramifications for the health of humans, wildlife, and domestic ungulates. This in turn would have grave consequences for the agricultural industry and food security (Hartley et al. 2011). Given the current lack of commercially available vaccines, control may be limited at least initially by vector control (Britch et al. 2007). Given the susceptibility of white-tailed deer to infection (Wilson et al. 2018), the establishment of a sylvatic cycle is a very troubling prospect, adding an additional dimension of complexity to disease control upon arrival of RVFV. We conducted this work to establish mosquito vector species in a sylvatic environment that are most

likely to enable establishment of RVFV in local deer species, while describing their seasonal abundances to inform seasonal risk for establishment.

4.4.1 Mosquito Abundances

Mosquito abundances, and the temporal patterns they exhibit are an important part of understanding their potential to vector arboviruses among wildlife species such as deer. Routine surveillance activities are more likely to be focused near commercial and residential areas where control measures are implemented. Relatively little data are collected on adult mosquito abundances in woodlands, and other natural areas where human densities are lower. Riparian woodlands, and the associated floodwater pools provide ideal habitat for many *Aedes spp.* including those under investigation here.

By far, the most abundant mosquito at the field site was *Ae. vexans*. We were specifically interested in *Ae. vexans, Ae. melanimon, and Ae. dorsalis* due to their feeding on both cattle and white-tailed deer at feedlots and surrounding agricultural areas in northern Colorado (Hartman et al. 2019). These high abundances both at peak and throughout the season indicates a lack of temporal refugia for their preferred vertebrate host species in this woodland habitat. *Aedes increpitus* was also collected in high abundances; this species both exhibited sharp peaks in mid- to late-July, and were relatively less abundant afterwards. Very few *Ae. dorsalis* were observed at this study site; it is possible that we missed potential hatches in May and/or June. Spring and summer, 2019 saw very few of this species overall relative to other years in traps operated by the local vector control district (Will Schlattmann, Vector Disease Control International, personal communication). The data presented here represents a single season of sampling, and there is still a need for long-term data on mosquito ecology in sylvatic habitats.

Permanent and semipermanent water breeding mosquitoes showed different patterns of abundances, and were relatively less abundant compared to the *Aedes spp. Culex tarsalis* was the most competent vector for RVFV present at the study site, so this is a concern. Similarly, *Cq. perturbans* and *Cs. inornata* were present in low abundances, but are able to transmit RVFV biologically (Iranpour et al. 2011), and should be considered potential sylvatic vector species if RVFV is introduced to the United States.

Future studies of mosquito abundances in the context of wildlife disease transmission should also be contextualized in terms of the life history traits of their hosts, such as seasonal breeding and diel activity patterns. Finally, we observed some fine-scale spatial heterogeneity in mosquito abundances. Sympatry with spatial resource selection patterns of deer should be investigated to inform heterogeneity in contacts that may impact transmission.

4.4.2 Mosquito Blood Meal Identification

We analyzed blood-fed mosquitoes collected in light traps, while utilizing fiber pot resting traps to supplement the sample set and limit sampling bias (Thiemann and Reisen 2012). Very few mosquitoes were collected in fiber pots (2 *Cx. tarsalis*, 5 *Ae. increpitus*, 4 *Ae. vexans*). This sample size did not permit analysis of vertebrate host identity as a function of collection method, although they did provide both blood-fed *Cx. tarsalis* mosquitoes from the woodland site.

Overall, the results from the blood meal analysis demonstrate the feeding of the floodwater *Aedes spp*. on each host of concern for RVFV transmission: human, deer, and cow. The detection of human blood meals in *Ae. vexans* and *Ae. increpitus* at both the residential areas, as well as the woodland site is epidemiologicaly relevant to the potential circulation of RVFV in this area. The most likely route of introduction into the United States of RVFV is

predicted to be the arrival of an infected human (Golnar et al. 2017). The feeding of humans at both the woodland site as well as the residential area represents both the risk of establishment in deer, as well as transmission back to humans following such an establishment. The detection of a cow blood meal in *Ae. vexans* was an unexpected result, and prompted a follow-up visit to the study site. A search of the surrounding area did result in the location of a small cattle feeder near Prospect Ponds Natural Area, 950m from the main site, the Environmental Learning Center. This is well within the documented dispersal capability of *Ae. vexans* (Bogojević et al. 2007), but we cannot say definitively that this feeding operation was the source of this blood meal. Overall, these results highlight the risk that is represented for multidirectional human-deer-cattle transmission of RVFV near areas of mixed residential and agricultural land use. In addition to these hosts, we found a large proportion of blood meals taken from eastern cottontail rabbits (*Sylvilagus floridanis*) by all *Aedes spp.* collected (*Ae. vexans*, *Ae. increpitus*, *Ae. melanimon*). Host susceptibility data are lacking for this species and should be a research priority.

The blood meal results for permanent water breeding mosquitoes *Cx. tarsalis* and *Cs. inornata* were few. While *Cx. tarsalis* exhibits feeding preferences for avian hosts, their feeding behaviors can be plastic based on local availability (Reeves et al. 1965, Kent et al. 2009, Campbell et al. 2013). In norther Colorado, *Cx. tarsalis* shifts feeding behaviors from a diversity hosts in semi-agricultural areas to mostly cattle feeding on feedlots (Chapter 2). Blood feeding of *Cx. tarsalis* on deer has been documented at Yolo Bypass Natural Area in California, as well as an oak woodland in California, but represented a small portion of a diverse vertebrate representation in these areas (Thiemann et al. 2012, 2017). Due to the high vector competence of this species (Turell et al. 2010), feeding event on deer may be very consequential in this species, even if rare, as it may allow for cross-species transmission.

The Sella stage recorded of blood-fed mosquitoes captured in this study may provide some insight into the behavioral interactions between mosquito species and their hosts. We modified the Sella score in order to include partial blood meals (score=1). Blood meals of eastern cottontail origin appeared more likely to be greater than 2, compared to white-tailed deer, nearly all of which were scored as 2 (replete blood feed). This may reflect host defensive behaviors exhibited by eastern cottontail rabbits against *Aedes spp.* mosquitoes, preventing the acquisition of full blood meals. The implications of partial blood feeding for susceptibility of mosquitoes to RVFV infection are unclear, and should be a research priority of eastern cottontails are determined to be competent amplifying hosts. Interrupted blood feeding could also be responsible for the large numbers of blood meals obtained from eastern cottontails in CDC light traps, if partial blood meals do not inhibit host-seeking behaviors of the mosquito. This could confound the interpretation of the blood meal data as proportional feeding, or actual host-preferences by mosquitoes.

4.5 Conclusions

The high abundances of several floodwater Aedes spp. in sympatry with white-tailed deer, and their blood host breadth including humans, deer, and cattle is troubling. While these species (*Ae. vexans*, *Ae. melanimon*, *Ae. increpitus*) have not shown very high vector competence in the laboratory setting (Chapter 3, Turell et al. 2010), their high abundances and host contact rates could compensate for this in the context of vectorial capacity, allowing them to amplify RVFV among deer while also enabling zoonotic spillover into the human population. The feeding of Ae. vexans on three RVFV-susceptible hosts is especially troubling. Furthermore, Sella scores for *Ae. vexans* and *Ae. increpitus* blood meals identified from deer indicate the lack

of interrupted blood feeding relative to eastern cottontails; however, it is unclear whether the acquisition of partial blood meals reduces the probability of infection.

The presence of *Cx. tarsalis* in a recreational woodland habitat frequently utilized by humans is troubling because it facilitates the interaction between humans weith potential RVFV amplifying hosts and competent mosquito vectors. *Cx tarsalis* is the primary vector of WNV in this area, and frequently feeds on humans in addition to many avian and mammalian hosts including humans (Kent et al. 2009, Chapter 2) Even if not highly abundant in riparian woodlands, the high vector competence and plastic blood host selection by this species may also contribute to sylvatic transmission of RVFV with transmission to humans. *Coquilletidia perturbans* was also present in low numbers, but exhibits efficient transmission in the laboratory (Iranpour et al. 2011), and feeds on deer (Molaei et al. 2008). This species vectors Jamestown Canyon Virus, a related virus (Order: Bunyavirales) among deer (Heard et al. 1991, Andreadis et al. 2008), and would likely contribute significantly to RVFV among deer as well.

CHAPTER 5: CONCLUSIONS

5.1 Towards a Consensus Illustration of RVFV in the United States

Differences between the Horn of Africa and the United States are large and numerous, considering differences between environments, hosts, vectors and agricultural practices. The classical model of the RVFV transmission cycle modeled after this region is informative but offers questionable relevance to the potential transmission cycle in the United States. While we cannot gain an empirical snapshot of RVFV ecology in the United States, we may be able to start a working illustration that can help guide control efforts as rapidly as possible after an introduction. The work included in this dissertation seeks to provide the most relevant entomological information to the potential ecology of RVFV in the United States with respect to establishment in wildlife and impact on livestock health. As transmission will primarily be vector-borne, entomological data can provide good indications of amplification potential in vertebrate populations, and between species.

Environmental conditions are a strong determinant of RVFV ecology throughout its current range. Precipitation patterns are especially informative for understanding transmission risk, and in some cases can even predict outbreak conditions months in advance for the Horn of Africa (Anyamba et al. 2009) and South Africa (Williams et al. 2016). The hydrological conditions out our study sites in northern Colorado are affected by the conditions throughout the Poudre watershed, including precipitation but also snowmelt from the higher elevations during the spring months. Conditions of flooding along the Poudre River create temporary larval habitat for *Aedes spp.* mosquitoes. Abundances of these species, especially *Ae. vexans* and *Ae. increpitus* (Chapter 4) were some of the highest population numbers observed throughout this work, and

this may compensate for their low vector competence allowing for efficient transmission. This idea has been proposed by Turell et al. (2015) with regards to Psorophora spp.: *Ps. ciliata* and *Ps. columbiae* exhibited a strong salivary gland barrier to transmission of RVFV, but given their exorbitant numbers and feeding preferences for large mammals, these species may still play a role in enzootic amplification of RVFV. In these studies, we showed that floodwater mosquitoes are highly abundant in residential, semi-agricultural, and woodland areas with feeding preferences for both wild and domestic ungulates (Chapters 2, 4) Vertical transmission is generally assumed for *Aedes spp.*, and while this has yet to be demonstrated in the laboratory, it could be a component of establishment and cryptic circulation of RVFV in the United States.

Along the northern Colorado Front Range, irrigation is a major source of larval habitat for *Culex spp*. mosquitoes, particularly *Culex tarsalis*. Irrigation primarily influences abundances of *Culex tarsalis* mosquitoes during August (Schurich et al. 2014), when we observe the highest abundances in Colorado near agriculture and feedlots (Chapter 2). The bimodal abundances of *Cx. tarsalis* in the wooded habitat (Chapter 4) stand in contrast to the unimodal, late-season abundances in the agricultural/residential locations. It is likely that the differences observed in these different studies is due to differences in the availability and quality of larval habitats. Irrigation-driven populations of *Culex spp.* mosquitoes have been associated with outbreaks in Saudi Arabia (*Culex tritaeniorrhynchus*), Egypt (*Culex pipiens*) and South Africa (*Cx. theileri*) (Hoogstraal et al. 1979, CDC 2000c, Williams et al. 2016).

The risk of transmission by *Culex tarsalis*, as determined by mosquito abundances and blood feeding behaviors will likely be much higher later in the summer in agricultural areas, and possibly more constant throughout spring and summer in natural wooded habitats. While we did not detect deer blood meals from deer by this species near feedlots or in the woodlands, there is

evidence of *Cx. tarsalis* feeding on deer elsewhere (Thiemann et al. 2012, 2017). We did detect opportunistic blood feeding behaviors by Cx. tarsalis mosquitoes in agricultural areas (Chapter 2) where cattle made up a large proportion of host choices on feedlots. Further work needs to be done to bolster the collective sample sizes and fully describe the full host breadth of *Cx. tarsalis* across habitats and land uses, including areas of sympatry with deer. In contrast to local *Aedes spp.* mosquitoes, vertical transmission has been demonstrated in this *Cx. tarsalis* (Bergren et al. 2021). We detected high probabilities of ovarian infection after a single blood meal in this species (Chapter 3), adding support for vertical transmission by this species using field-collected mosquitoes. Because *Culex spp.* oviposit egg rafts directly to standing water for development, egg populations will not provide a long-term reservoir via vertical transmission. Vertical transmission by this species may aid in the establishment potential of RVFV, however as this species exhibits a high degree of plasticity in its host preferences.

Agricultural systems are closely associated with mosquito populations in Colorado. The largest feedlot site in the field investigations was located with neighboring irrigated fields where feed is grown for the animals (feedlot site 2, Chapter 2). This creates ideal conditions for amplification of RVFV among large concentrations of cattle at feedlots. Given adequate dispersal of these mosquitoes to nearby wildlife habitat, or if feedlots are host to wild animals that are susceptible to RVFV, they will also represent connectivity for cross-species transmission to and from wild vertebrates such as deer. We did not study deer explicitly at our study sites during the time of mosquito collections, but they are known to frequent livestock feeding operations where they contribute significantly to bovine tuberculosis transmission (Wilber et al. 2019).

Given what we know, and what we have learned from these works, it is clear that the illustration of the United States transmission cycle would be complex, including several vectors,

which are capable of vertical as well as horizontal transmission (**Figure 5.1**). Vector competence experiments and studies of mosquito abundances and blood-host utlilization show that local Colorado vectors should include *Aedes spp.*, *Culex tarsalis*, and *Culiseta inornata*. More work is needed to demonstrate vertical transmission by *Aedes spp.* and *Cs. inornata* under controlled conditions, but mosquito ovaries are readily infected following ingestion of infectious blood meals. These mosquitoes have demonstrated the capacity to feed on all deer, humans, and domestic livestock in the preceding chapters or elsewhere in the literature.

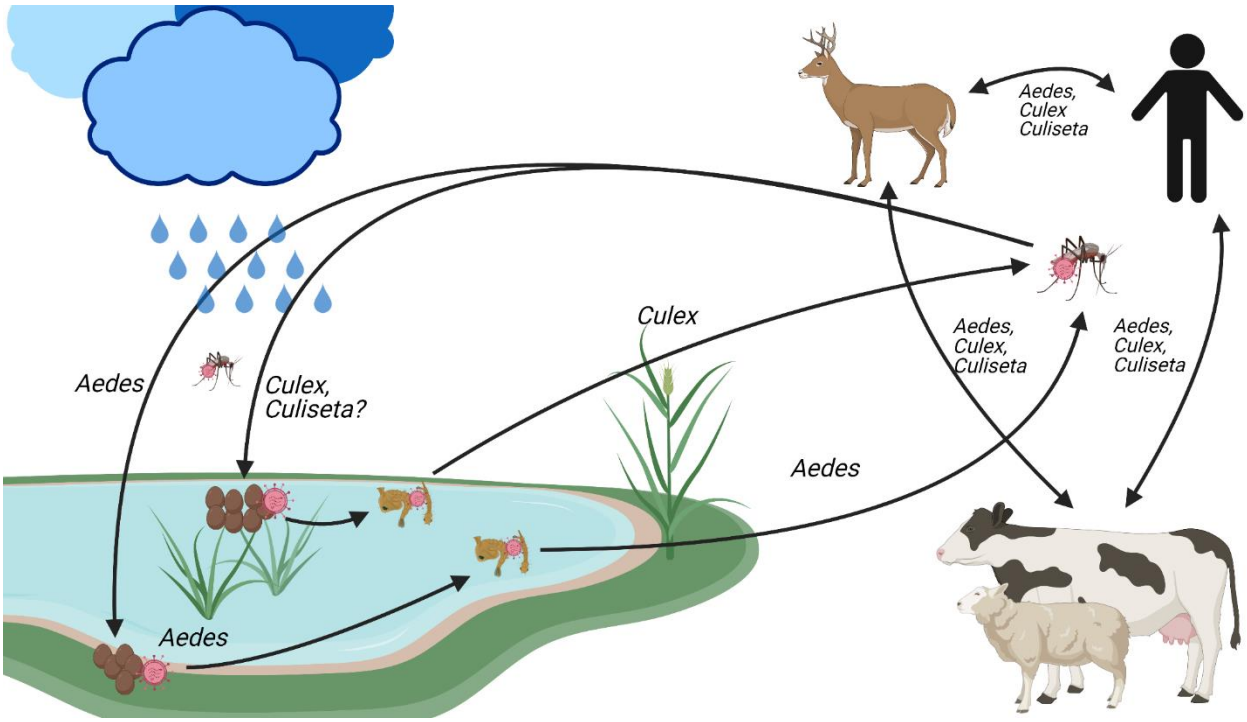


Figure 5.1. Working illustration of Rift Valley fever virus transmission in the United States.

5.2 Future Directions

This illustration is a working model, and more work is needed to measure the parameters that would be used to prioritize mosquito control efforts. Large gaps still exist in the data for

vector competence experiments. Many species have been tested, but with small sample sizes in most cases. This is in large part due to the difficulty of testing transmission of select agent pathogens with wild-caught mosquitoes. Low numbers of field collections, low feeding rates in the laboratory, and mortality during incubation result in low numbers of mosquitoes to dissect and analyze. These experiments bear repeating in order to gain better estimates of vector competence across a range of viremias. We have created a model that we hope will promote this initiative, as Bayesian estimation provides a natural, intuitive way to integrate new data with informative prior distributions to refine these parameter estimates across multiple experiments.

Given an introduction of RVFV to the United States, it is also critical to know how the virus may overwinter. This seems to occur during the cool months in Egypt and South Africa, though these locations represent very different climates. For Culex mosquitoes that overwinter as adults, infected mosquitoes emerging from diapause in the spring might initiate a seasonal transmission cycle. *Aedes* eggs may also be able to maintain RVFV through winter conditions.

At this time, the most urgent entomological research to maintain a proactive stance against a RVFV introduction is to repeat these experiments, but focusing on ports of entry. These locations have already been prioritized (Golnar et al. 2017), but this work focused on direct flights rather than destinations. Given the recent infected traveler who maintained a 30-day viremia in China (Liu et al. 2017), and the likelihood of RVFV entering the United States by an infected human, passenger level data may be able to point towards some of the more likely destinations.

BIBLIOGRAPHY

Abd El-Rahim, I. H. A., U. Abd El-Hakim, and M. Hussein. **1999**. An epizootic of Rift Valley fever in Egypt in 1997. Rev Sci Tech OIE. 18: 741–748.

Ahmed Kamal, S. 2011. Observations on rift valley fever virus and vaccines in Egypt. Virol J. 8: 532.

Al-Afaleq, A. I., and M. F. Hussein. **2011**. The status of Rift Valley fever in animals in Saudi Arabia: A mini review. Vector Borne Zoonotic Dis. 11: 1513–1520.

Al-Azraqi, T. A., A. A. El Mekki, and A. A. Mahfouz. **2012**. Rift Valley fever in southwestern Saudi Arabia: A sero-epidemiological study seven years after the outbreak of 2000–2001. Acta Tropica. 123: 111–116.

Al-Hazmi, M., E. A. Ayoola, M. Abdurahman, S. Banzal, J. Ashraf, A. El-Bushra, A. Hazmi, M. Abdullah, H. Abbo, A. Elamin, E.-T. Al-Sammani, M. Gadour, C. Menon, M. Hamza, I. Rahim, M. Hafez, M. Jambavalikar, H. Arishi, and A. Aqeel. **2003**. Epidemic Rift Valley fever in Saudi Arabia: a clinical study of severe illness in humans. Clin Infect Dis. 36: 245–52.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. **1990**. Basic Local Alignment Search Tool. J Mol Biol. 215: 403–410.

Anderson, E. C., and L. W. Rowe. **1998**. The prevalence of antibody to the viruses of bovine virus diarrhoea, bovine herpes virus 1, Rift Valley fever, ephemeral fever and bluetongue and to *Leptospira* sp in free-ranging wildlife in Zimbabwe. Epidemiol Infect. 121: 441–449.

Andreadis, T. G., J. F. Anderson, P. M. Armstrong, and A. J. Main. **2008**. Isolations of Jamestown Canyon virus (Bunyaviridae: *Orthobunyavirus*) from field-collected mosquitoes (Diptera: Culicidae) in Connecticut, USA: A ten-year analysis, 1997–2006. Vector Borne Zoonotic Dis. 8: 175–188.

Andriamandimby, S. F., A. E. Randrianarivo-Solofoniaina, E. M. Jeanmaire, L. Ravololomanana, L. T. Razafimanantsoa, T. Rakotojoelinandrasana, J. Razainirina, J. Hoffmann, J. P. Ravalohery, J. T. Rafisandratantsoa, P. E. Rollin, and J. M. Reynes. **2010**. Rift valley fever during rainy seasons, Madagascar, 2008 and 2009. Emerg Infect Dis. 16: 963–970.

Anyamba, A., J.-P. Chretien, J. Small, C. J. Tucker, P. B. Formenty, J. H. Richardson, S. C. Britch, D. C. Schnabel, R. L. Erickson, and K. J. Linthicum. **2009**. Prediction of a Rift Valley fever outbreak. PNAS. 106: 955–959.

Barker, C. M., B. G. Bolling, W. C. Black, C. G. Moore, and L. Eisen. **2009**. Mosquitoes and West Nile virus along a river corridor from prairie to montane habitats in eastern Colorado. J Vector Ecol. 34: 276–293.

Barker, C. M., T. Niu, W. K. Reisen, and D. M. Hartley. **2013**. Data-driven modeling to assess receptivity for Rift Valley fever virus. PLoS Negl Trop Dis. 7: e2515.

Beechler, B. R., R. Bengis, R. Swanepoel, J. T. Paweska, A. Kemp, P. J. van Vuren, J. Joubert, V. O. Ezenwa, and A. E. Jolles. **2013**. Rift Valley fever in Kruger National Park: Do buffalo play a role in the inter-epidemic circulation of virus? Transbound Emerg Dis. 62: 24–32.

Beehler, J. W., and M. Mulla. **1995**. Effects of organic enrichment on temporal distribution and abundance of Culicine egg rafts. J Am Mosq Control Assoc. 11: 167–171.

Beerntsen, B. T., A. A. James, and B. M. Christensen. **2000**. Genetics of mosquito vector competence. Microbiol Mol Biol Rev. 64: 115–137.

van den Bergh, C., E. H. Venter, R. Swanepoel, and P. N. Thompson. **2019**. High seroconversion rate to Rift Valley fever virus in cattle and goats in far northern KwaZulu-Natal, South Africa, in the absence of reported outbreaks. PLoS Negl Trop Dis. 13: e0007296.

Bergren, N. A., E. M. Borland, D. A. Hartman, and R. C. Kading. **2021**. Laboratory demonstration of the vertical transmission of Rift Valley fever virus by *Culex tarsalis* mosquitoes. PLoS Negl Trop Dis. 15: e0009273.

Bergren, N. A., and R. C. Kading. **2018**. The Ecological significance and implications of transovarial transmission among the vector-borne Bunyaviruses: A review. Insects. 9: 173.

Bicout, D. J., and P. Sabatier. **2004**. Mapping Rift Valley fever vectors and prevalence using rainfall variations. Vector Borne Zoonotic Dis. 4.

Bird, B. H., D. A. Bawiec, T. G. Ksiazek, T. R. Shoemaker, and S. T. Nichol. **2007**. Highly sensitive and broadly reactive quantitative reverse transcription-PCR assay for high-throughput detection of Rift Valley fever virus. J Clin Microbiol. 45: 3506–3513.

Bird, B. H., J. W. K. Githinji, J. M. Macharia, J. L. Kasiiti, R. M. Muriithi, S. G. Gacheru, J. O. Musaa, J. S. Towner, S. A. Reeder, J. B. Oliver, T. L. Stevens, B. R. Erickson, L. T. Morgan, M. L. Khristova, A. L. Hartman, J. A. Comer, P. E. Rollin, T. G. Ksiazek, and S. T. Nichol. **2008**. Multiple virus lineages sharing recent common ancestry were associated with a large Rift Valley fever outbreak among livestock in Kenya during 2006-2007. J Virol. 82: 11152–11166.

Bird, B. H., M. L. Khristova, P. E. Rollin, T. G. Ksiazek, and S. T. Nichol. **2007**. Complete genome analysis of 33 ecologically and biologically diverse Rift Valley fever virus strains reveals widespread virus movement and low genetic diversity due to recent common ancestry. J Virol. 81: 2805–2816.

Bird, B. H., T. G. Ksiazek, S. T. Nichol, and N. J. MacLachlan. **2009**. Rift Valley fever virus. J. Am. Vet. Med. 234: 883–893.

Bogojević, M. S., T. Hengl, and E. Merdić. **2007**. Spatiotemporal monitoring of floodwater mosquito dispersal in Osijek, Croatia. J Am Mosq Control Assoc. 23: 99–108.

Boiro, I., O. Konstaninov, and A. Numerov. **1987**. [Isolation of Rift Valley fever virus from bats in the Republic of Guinea]. Bulletin de la Societe de pathologie exotique et de ses filiales. 80: 62—67.

Britch, S. C., K. J. Linthicum, and Rift Valley Fever Working Group. **2007**. Developing a research agenda and a comprehensive national prevention and response plan for Rift Valley fever in the United States. Emerg Infect Dis. 13.

Bron, G. M., K. Strimbu, H. Cecilia, A. Lerch, S. M. Moore, Q. Tran, T. A. Perkins, and Q. A. ten Bosch. **2021**. Over 100 years of Rift Valley fever: A patchwork of data on pathogen spread and spillover. Pathogens. 10: 708.

Campbell, G. L., C. S. Ceianu, and H. M. Savage. **2006**. Epidemic West Nile encephalitis in Romania: Waiting for history to repeat itself. Ann NY Acad Sci. 951: 94–101.

Campbell, R., T. C. Thiemann, D. Lemenager, and W. K. Reisen. **2013**. Host-selection patterns of *Culex tarsalis* (Diptera: Culicidae) determine the spatial heterogeneity of West Nile virus enzootic activity in Northern California. J Med Entomol. 50: 1303–1309.

Cao-Lormeau, V.-M., C. Roche, A. Teissier, E. Robin, A.-L. Berry, H.-P. Mallet, A. A. Sall, and D. Musso. **2014**. Zikav virus, French Polynesia, South Pacific, 2013. Emerg Infect Dis. 20: 1085–1086.

Carroll, S. A., J.-M. Reynes, M. L. Khristova, S. F. Andriamandimby, P. E. Rollin, and S. T. Nichol. **2011**. Genetic evidence for Rift Valley fever outbreaks in Madagascar resulting from virus introductions from the East African mainland rather than enzootic maintenance. J Virol. 85: 6162–6167.

CDC. **2000a**. Outbreak of Rift Valley Fever — Saudi Arabia, August-October, 2000. MMWR. 49: 905–908.

CDC. **2000b**. Outbreak of Rift Valley Fever - Yemen, Augus-October 2000. MMWR 2000. 49: 1065–1066.

CDC. **2000c**. Update: Outbreak of Rift Valley Fever - Saudi Arabia, August-November 2000. 49: 982–985.

Chandler, L. J., C. D. Blair, and B. J. Beaty. **1998**. La Crosse virus infection of *Aedes triseriatus* (Diptera: Culicidae) ovaries before dissemination of virus from the midgut. J Med Entomol. 35: 567–572.

Corwin, A., M. Habib, D. Watts, J. Olson, M. Darwis, R. Hibbs, B. Botros, M. Kleinosky, R. Shope, and M. Kilpatrick. **1993**. Prevalence of antibody to Rift Valley fever virus in the Nile river delta of Egypt, 13 years after a major outbreak. Trans R Soc Trop Med Hyg. 87: 161.

Crabtree, M. B., R. C. Kading, J.-P. Mutebi, J. J. Lutwama, and B. R. Miller. **2013**. Identification of host blood from engorged mosquitoes collected in western Uganda using cytochrome oxidase I gene sequences. J Wildl Dis. 49: 611–626.

Darsie, R. F., and R. A. Ward. **2005**. Identification and geographical distribution of the mosquitoes of North America, North of Mexico. University Press of Florida, Gainesville, Florida.

Daubney, R., J. R. Hudson, and P. C. Garnham. **1931**. Enzootic hepatitis or Rift Valley fever, an undescribed virus disease of sheep, cattle and man from East Africa. J Pathol Bacteriol. 34: 545–579.

Davies, F. G. 1975. Observations on the epidemiology of Rift Valley fever in Kenya. J Hyg (Lond). 75: 219–230.

Davies, F. G. 2006. Risk of a Rift Valley fever epidemic at the haj in Mecca, Saudi Arabia. Rev. Sci. Tech. OIE. 25: 137–147.

Davies, F. G., and L. Karstad. **1981**. Experimental infection of the African buffalo with the virus of Rift Valley fever. Trop Anim Health Prod. 13: 185–188.

Davies, F. G., K. J. Linthicum, and A. D. James. **1985**. Rainfall and epizootic Rift Valley fever. Bull World Health Organ. 63: 941–943.

Denwood, M. J. 2016. runjags: An R package providing interface utilities, model templates, parallel computing methods and additional distributions for MCMC models in JAGS. J Stat Soft. 71.

Detinova, T. S. 1962. Age-grouping methods in Diptera of medical importance with special reference to some vectors of malaria. Monogr Ser World Health Organ. 47: 13–191.

Digoutte, J. P. 1981. Viruses identified from 1963-1981. Rapport Sur Le Fonctionnement de L'Institut Pasteur de Dakar.

Digoutte, J. P., R. Cordellier, Y. Robin, F. Pajot, and B. Geoffroy. **1974**. Zinga virus (ArB 1976), a new arbovirus isolated in Central Africa. Ann Microbiol (Inst Pasteur). 125B: 107–118.

Digoutte, J. P., J. C. Jacobi, Y. Robin, and V. J. Gagnard. **1974**. [Zinga virus infection in man]. Bull Soc Pathol Exot Filiales. 67: 451–457.

Drake, J. M., A. N. Hassan, and J. C. Beier. **2013**. A statistical model of Rift Valley fever activity in Egypt. J Vector Ecol. 38: 251–259.

Duffy, M. R., T.-H. Chen, W. T. Hancock, A. M. Powers, J. L. Kool, R. S. Lanciotti, M. Pretrick, M. Marfel, S. Holzbauer, C. Dubray, L. Guillaumot, A. Griggs, M. Bel, A. J. Lambert, J. Laven, O. Kosoy, A. Panella, B. J. Biggerstaff, M. Fischer, and E. B. Hayes. **2009**. Zika Virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 360: 2536–2543.

Dunphy, B. M., K. B. Kovach, E. J. Gehrke, E. N. Field, W. A. Rowley, L. C. Bartholomay, and R. C. Smith. **2019**. Long-term surveillance defines spatial and temporal patterns implicating *Culex tarsalis* as the primary vector of West Nile virus. Sci Rep. 9: 6637.

El Mamy, A. B. O., M. O. Baba, Y. Barry, K. Isselmou, M. L. Dia, B. Hampate, M. Y. Diallo, M. O. B. El Kory, M. Diop, M. M. Lo, Y. Thiongane, M. Bengoumi, L. Puech, L. Plee, F. Claes, S. de La Rocque, and B. Doumbia. **2011**. Unexpected Rift Valley fever outbreak, northern Mauritania. Emerg Infect Dis. 17: 1894–1896.

Elfadil, A. A., K. A. Hasab-Allah, O. M. Dafa-Allah, and A. A. Elmanea. **n.d.** The persistence of Rift Valley fever in the Jazan region of Saudi Arabia. Rev Sci Tech Off Int Epiz. 25: 1131–1136.

Evans, A., F. Gakuya, J. T. Paweska, M. Rostal, L. Akoolo, P. J. Van Vuren, T. Manyibe, J. M. Macharia, T. G. Ksiazek, D. R. Feikin, R. F. Breiman, and M. Kariuki Njenga. **2008**. Prevalence of antibodies against Rift Valley fever virus in Kenyan wildlife. Epidemiol Infect. 136: 1261–1269.

Faburay, B., N. N. Gaudreault, Q. Liu, A. S. Davis, V. Shivanna, S. Y. Sunwoo, Y. Lang, I. Morozov, M. Ruder, B. Drolet, D. Scott McVey, W. Ma, W. Wilson, and J. A. Richt. **2016**. Development of a sheep challenge model for Rift Valley fever. Virology. 489: 128–140.

Favier, C., K. Chalvet-Monfray, P. Sabatier, R. Lancelot, D. Fontenille, and M. A. Dubois. **2006**. Rift Valley fever in West Africa: the role of space in endemicity. Trop Med Int Health. 11: 1878–1888.

Fischer-Tenhagen, C., C. Hamblin, S. Quandt, and K. Frölich. **2000**. Serosurvey for selected infectious disease agents in free-ranging black and white rhinoceros in Africa. J Wildl Dis. 36: 316–323.

Fontenille, D. 1989. Etude des circuits de vection d'arbovirus, à Madagascar [Transmission cycles of arboviruses in Madagascar]. Arch Inst Pasteur Madagascar. 55: 7–317.

Fontenille, D., M. Traore-Lamizana, M. Diallo, J. Thonnon, J. P. Digoutte, and H. G. Zeller. **1998**. New vectors of Rift Valley fever in West Africa. Emerg Infect Dis. 4: 289–293.

Garcia, S., J. M. Crance, A. Billecocq, A. Peinnequin, A. Jouan, M. Bouloy, and D. Garin. **2001**. Quantitative real-time PCR detection of Rift Valley fever virus and its application to evaluation of antiviral compounds. J Clin Microbiol. 39: 4456–4461.

Getz, W. M., C. H. King, P. C. Cross, A. Glinka, and A. D. LaBeaud. **2011**. Rift Valley fever virus infection in African buffalo (*Syncerus caffer*) herds in rural South Africa: Evidence of interepidemic transmission. Am J Trop Med Hyg. 84: 641–646.

Golnar, A. J., R. C. Kading, and G. L. Hamer. **2017**. Quantifying the potential pathways and locations of Rift Valley fever virus entry into the United States. Transbound Emerg Dis. 65: 85–95.

Golnar, A. J., M. J. Turell, A. D. LaBeaud, R. C. Kading, and G. L. Hamer. **2014**. Predicting the mosquito species and vertebrate species involved in the theoretical transmission of Rift Valley fever virus in the United States. PLoS Negl Trop Dis. 8: e3163.

Gould, E., J. Pettersson, S. Higgs, R. Charrel, and X. de Lamballerie. **2017**. Emerging arboviruses: Why today? One Health. 4: 1–13.

Hartley, D. M., J. L. Rinderknecht, T. L. Nipp, N. P. Clarke, and G. D. Snowder. **2011**. Potential Effects of Rift Valley Fever in the United States. Emerg Infect Dis. 17.

Hartman, D. A., L. M. Rice, J. DeMaria, E. M. Borland, N. A. Bergren, A. C. Fagre, L. L. Robb, C. T. Webb, and R. C. Kading. **2019**. Entomological risk factors for potential transmission of Rift Valley fever virus around concentrations of livestock in Colorado. Transbound Emerg Dis. 66: 1709–1717.

Hassell, J. M., M. Begon, M. J. Ward, and E. M. Fèvre. **2017**. Urbanization and Disease Emergence: Dynamics at the Wildlife–Livestock–Human Interface. Trends Ecol Evol. 32: 55–67.

Hayes, E. B., N. Komar, R. S. Nasci, S. P. Montgomery, D. R. O'Leary, and G. L. Campbell. **2005**. Epidemiology and transmission dynamics of West Nile virus disease. Emerg Infect Dis. 11: 1167–1173.

Heard, P. B., M. B. Zhang, and P. R. Grimstad. **1991**. Laboratory transmission of Jamestown Canyon and snowshoe hare viruses (Bunyaviridae: California serogroup) by several species of mosquitoes. J Am Mosq Control Assoc. 7: 94–102.

Hill, M. O. 1973. Diversity and evenness: a unifying notation and its consequences. Ecology. 54: 427–432.

Hoogstraal, H., J. M. Meegan, G. M. Khalil, and F. K. Adham. **1979**. The Rift Valley fever epizootic in Egypt 1977–1978 2. Ecological and entomological studies. Trans. R. Soc. Trop. Med. Hyg. 73: 624–629.

Hopken, M. W., T. M. Lum, P. M. Meyers, and A. J. Piaggio. **2015**. Molecular assessment of translocation and management of an endangered subspecies of white-tailed deer (*Odocoileus virginianus*). Conserv Genet. 16: 635–647.

Houk, E. J., J. L. Hardy, S. B. Presser, and L. D. Kramer. **1981**. Dissemination barriers for western equine encephalomyelitis virus in *Culex tarsalis* infected after ingestion of low viral doses. Am J Trop Med Hyg. 30: 190–197.

Hsieh, T. C., K. H. Ma, and A. Chao. **2016**. iNEXT: Interpolation and extrapolation for species diversity.

Huang, Y.-M. 1985. A new African species of *Aedes* (Diptera: Culicidae). Mosquito Systematics. 17: 13.

Hui, F. K. C. 2016. BORAL – Bayesian ordination and regression analysis of multivariate abundance data in R. Methods Ecol Evol. 7: 744–750.

Iranpour, M., M. J. Turell, and L. R. Lindsay. **2011**. Potential for Canadian mosquitoes to transmit Rift Valley fever virus. J Am Mosq Control Assoc. 27: 363–9.

Ivanova, N. V., T. S. Zemlak, R. H. Hanner, and P. D. N. Hebert. **2007**. Universal primer cocktails for fish DNA barcoding. Molecular Ecology Notes. 7: 544–548.

Jansen van Vuren, P., J. Kgaladi, V. Msimang, and J. T. Paweska. **2019**. Rift Valley fever reemergence after 7 years of quiescence, South Africa, May 2018. Emerg Infect Dis. 25: 338–341.

Jouan, A., B. L. Guenno, J. P. Digoutte, B. Philippe, O. Riou, and F. Adam. **1988**. An RVF epidemic in Southern Mauritania. Annales de l'Institut Pasteur / Virologie. 139: 307–308.

Jupp, P. G. 2009a. Preliminary studies on the overwintering stages of *Culex* mosquitoes (Diptera: Culicidae) in the highveld region of South Africa. J Ent Soc Sth Afr. 32: 91–98.

Jupp, P. G. 2009b. Further studies on the overwintering stages of *Culex* mosquitoes (Diptera: Culicidae) in the highveld region of South Africa. J Ent Soc Sth Afr. 38: 89–97.

Jupp, P. G., A. Kemp, A. Grobbelaar, P. Leman, F. J. Burt, A. M. Alahmed, D. A. Mujalli, M. A. Khamees, and R. Swanepoel. **2002**. The 2000 epidemic of Rift Valley fever in Saudi Arabia: mosquito vector studies: Rift Valley fever in Saudi Arabia. Med Vet Entomol. 16: 245–252.

Kading, R. C., M. B. Crabtree, B. H. Bird, S. T. Nichol, B. R. Erickson, K. Horiuchi, B. J. Biggerstaff, and B. R. Miller. **2014**. Deletion of the NSm virulence gene of Rift Valley fever virus inhibits virus replication in and dissemination from the midgut of *Aedes aegypti* mosquitoes. PLoS Negl Trop Dis. 8: 17–18.

Kenawy, M. A., Y. M. Abdel-Hamid, and J. C. Beier. **2018**. Rift Valley fever in Egypt and other African countries: Historical review, recent outbreaks and possibility of disease occurrence in Egypt. Acta Tropica. 181: 40–49.

Kent, R., L. Juliusson, M. Weissmann, S. Evans, and N. Komar. **2009**. Seasonal blood-feeding behavior of *Culex tarsalis* (Diptera: Culicidae) in Weld County, Colorado, 2007. J Med Entomol. 46: 380–390.

Komar, N., R. J. Pollack, and A. Spielman. **1995**. A nestable fiber pot for sampling resting mosquitoes. J Am Mosq Control Assoc. 11: 463–467.

Kruschke, J. 2014. Doing Bayesian data analysis: A tutorial with R, JAGS, and Stan. Academic Press.

Ksiazek, T. G., A. Jouan, J. M. Meegan, B. Le Guenno, M. L. Wilson, C. J. Peters, J. P. Digoutte, M. Guillaud, N. O. Merzoug, and E. M. Touray. **1989**. Rift Valley fever among domestic animals in the recent West African outbreak. Res Virol. 140: 67–77.

Lancelot, R., M. Béral, V. M. Rakotoharinome, S.-F. Andriamandimby, J.-M. Héraud, C. Coste, A. Apolloni, C. Squarzoni-Diaw, S. de La Rocque, P. B. H. Formenty, J. Bouyer, G. R. W. Wint, and E. Cardinale. **2017**. Drivers of Rift Valley fever epidemics in Madagascar. Proc Natl Acad Sci U S A. 114: 938–943.

Lanciotti, R. S., A. J. Kerst, R. S. Nasci, M. S. Godsey, C. J. Mitchell, H. M. Savage, N. Komar, N. a Panella, B. C. Allen, K. E. Volpe, S. Brent, and J. T. Roehrig. **2000**. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. J Clin Microbiol. 38: 4066–4071.

Lanciotti, R. S., J. T. Roehrig, V. Deubel, J. Smith, M. Parker, K. Steele, B. Crise, K. E. Volpe, M. B. Crabtree, J. H. Scherret, R. A. Hall, J. S. MacKenzie, C. B. Cropp, B. Panigrahy, E. Ostlund, B. Schmitt, M. Malkinson, C. Banet, J. Weissman, N. Komar, H. M. Savage, W. Stone, T. McNamara, and D. J. Gubler. **1999**. Origin of the West

Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science. 286: 2333–2337.

Laughlin, L. W., J. M. Meegan, L. J. Strausbaugh, D. M. Morens, and R. H. Watten. **1979**. Epidemic Rift Valley fever in Egypt: observations of the spectrum of human illness. Trans R Soc Trop Med Hyg. 73: 630–633.

Linthicum, K. J., A. Anyamba, C. J. Tucker, P. Kelley, M. Myers, and C. J. Peters. **1999**. Climate and satellite indicators to forecast Rift Valley fever epidemics in Kenya. Science. 285: 397–400.

Linthicum, K. J., F. G. Davies, A. Kairo, and C. L. Bailey. **1985**. Rift Valley fever virus (family Bunyaviridae, genus Phlebovirus). Isolations from Diptera collected during an inter-epizootic period in Kenya. J Hyg (Lond). 95: 197–209.

Liu, J., Y. Sun, W. Shi, S. Tan, Y. Pan, S. Cui, Q. Zhang, X. Dou, Y. Lv, X. Li, X. Li, L. Chen, C. Quan, Q. Wang, Y. Zhao, Q. lv, W. Hua, H. Zeng, Z. Chen, H. Xiong, C. Jiang, X. Pang, F. Zhang, M. Liang, G. Wu, G. F. Gao, W. J. Liu, A. Li, and Q. Wang. **2017**. The first imported case of Rift Valley fever in China reveals a genetic reassortment of different viral lineages. Emerg Microbes Infect. 6: e4.

Loaiza, J. R., L. C. Dutari, J. R. Rovira, O. I. Sanjur, G. Z. Laporta, J. Pecor, D. H. Foley, G. Eastwood, L. D. Kramer, M. Radtke, and M. Pongsiri. **2017**. Disturbance and mosquito diversity in the lowland tropical rainforest of central Panama. Scientific Reports. 7: 7248.

Madani, T. A., Y. Y. Al-Mazrou, M. H. Al-Jeffri, A. A. Mishkhas, A. M. Al-Rabeah, A. M. Turkistani, M. O. Al-Sayed, A. A. Abodahish, A. S. Khan, T. G. Ksiazek, and O. Shobokshi. **2003**. Rift Valley fever epidemic in Saudi Arabia: Epidemiological, clinical, and laboratory characteristics. Clin Infect Dis. 37: 1084–1092.

Mangili, A., T. Vindenes, and M. Gendreau. **2021**. Infectious risks of air travel. Microbiol Spectrum. 3: IOL5-0009–2015.

Manore, C. A., and B. R. Beechler. **2015**. Interepidemic and between season persistence of Rift Valley fever: vertical transmission or cryptic cycling? Transbound Emerg Dis. 62: 13–23.

McIntosh, B., P. Jupp, I. dos Santos, and B. Barnard. **1980**. Vector studies on Rift Valley fever virus in South Africa. S Afr Med J. 58: 127–132.

McIntosh, M., P. G. Jupp, I. dos Santos, and Rowe, A C. **1983**. Field and laboratory evidence implicating *Culex zombaensis* and *Aedes circumluteolus* as vectors of Rift Valley Fever virus in Coastal South Africa (. S Afr J Sci. 79: 61–64.

Meegan, J. M., and C. L. Bailey. **1989**. Rift Valley Fever, pp. 51–76. *In* The Arboviruses: Epidemiology and Ecology. Volume 4. CRC Press, Boca Raton.

Meegan, J. M., J. P. Digoutte, C. J. Peters, and R. E. Shope. **1983**. Monoclonal antibodies to identify Zinga virus as Rift Valley fever virus. The Lancet. 321: 641.

Meegan, J. M., G. M. Khalil, H. Hoogstraal, and F. K. Adham. **1980**. Experimental transmission and field isolation studies implicating *Culex pipiens* as a vector of Rift Valley fever virus in Egypt. Am J Trop Med Hyg. 29: 1405–1410.

Messing, J. 1983. New M13 vectors for cloning. Methods in Enzymology. 101: 20–78.

Métras, R., C. Jewell, T. Porphyre, P. N. Thompson, D. U. Pfeiffer, L. M. Collins, and R. G. White. **2015**. Risk factors associated with Rift Valley fever epidemics in South Africa in 2008–11. Sci Rep. 5.

Mohamed, A. M., A. M. Ashshi, A. H. Asghar, I. H. A. Abd El-Rahim, A. G. El-Shemi, and T. Zafar. **2014**. Seroepidemiological survey on Rift Valley fever among small ruminants

and their close human contacts in Makkah, Saudi Arabia, in 2011. Rev. Sci. Tech. 33: 903–915.

Molaei, G., T. G. Andreadis, P. M. Armstrong, and M. Diuk-Wasser. **2008**. Host-feeding patterns of potential mosquito vectors in Connecticut, USA: Molecular analysis of bloodmeals from 23 species of <i>Aedes</>, *Anopheles*, *Culex*, *Coquillettidia*, *Psorophora*, and *Uranotaenia*. J Med Entomol. 45: 9.

Morvan, J., D. Fontenille, J. F. Saluzzo, and P. Coulanges. **1991**. Possible Rift Valley fever outbreak in man and cattle in Madagascar. Trans R Soc Trop Med Hyg. 85: 108.

Morvan, J., J. F. Saluzzo, D. Fontenille, P. E. Rollin, and P. Coulanges. **1991**. Rift Valley fever on the east coast of Madagascar. Research in Virology. 142: 475–482.

Msimang, V., P. N. Thompson, P. Jansen van Vuren, S. Tempia, C. Cordel, J. Kgaladi, J. Khosa, F. J. Burt, J. Liang, M. K. Rostal, W. B. Karesh, and J. T. Paweska. **2019**. Rift Valley fever virus exposure amongst farmers, farm workers, and veterinary professionals in central South Africa. Viruses. 11.

Murithi, R. M., P. Munyua, P. M. Ithondeka, J. M. Macharia, A. Hightower, E. T. Luman, R. F. Breiman, and M. K. Njenga. **2011**. Rift Valley fever in Kenya: history of epizootics and identification of vulnerable districts. Epidemiol. Infect. 139: 372–380.

Nash, D., D. O'Leary, and M. Sherman. **2001**. The outbreak of West Nile virus infection in the New York City area in 1999. N Engl J Med. 8.

Nepomichene, T. N. J. J., F. N. Raharimalala, S. F. Andriamandimby, J.-P. Ravalohery, A.-B. Failloux, J.-M. Heraud, and S. Boyer. **2018**. Vector competence of *Culex antennatus* and *Anopheles coustani* mosquitoes for Rift Valley fever virus in Madagascar: Vector competence for RVFV in Madagascar. Med Vet Entomol. 32: 259–262.

Ngoshe, Y. B., A. Avenant, M. K. Rostal, W. B. Karesh, J. T. Paweska, W. Bagge, J. Van Vuren, A. Kemp, C. Cordel, V. Msimang, and P. N. Thompson. **2020**. patterns of Rift Valley fever virus seropositivity in domestic ruminants in central South Africa four years after a large outbreak. Scientific Reports. 10: 5489.

(NIAID Emerging Infectious Diseases/ Pathogens | NIH) . **2021**. NIAID Emerging Infectious Diseases/ Pathogens | NIH. (https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens).

Patz, J. A., T. A. Burke, and J. M. Balbus. **1996**. Global climate change and emerging infectious diseases. J Am Med Assoc. 275: 217–223.

Paweska, J. T., E. Mortimer, P. A. Leman, and R. Swanepoel. **2005**. An inhibition enzyme-linked immunosorbent assay for the detection of antibody to Rift Valley fever virus in humans, domestic and wild ruminants. Journal of Virological Methods. 127: 10–18.

Paweska, J. T., P. J. van Vuren, A. Kemp, P. Buss, R. G. Bengis, F. Gakuya, R. F. Breiman, M. K. Njenga, and R. Swanepoel. **2008**. Recombinant nucleocapsid-based ELISA for detection of IgG antibody to Rift Valley fever virus in African buffalo. Veterinary Microbiology. 127: 21–28.

Pepin, M., M. Bouloy, B. H. Bird, A. Kemp, and J. Paweska. **2010**. Rift Valley fever virus (Bunyaviridae: Phlebovirus): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. Vet Res. 41: 61.

Petersen, L. R., D. J. Jamieson, A. M. Powers, and M. A. Honein. **2016**. Zika virus. N Engl J Med. 374: 1552–1563.

Pienaar, N. J., and P. N. Thompson. **2013**. Temporal and spatial history of Rift Valley fever in South Africa: 1950 to 2011. Onderstepoort J Vet Res. 80: 13 pages.

Purdue, J. R., M. H. Smith, and J. C. Patton. **2000**. Female philopatry and extreme spatial genetic heterogeneity in white-tailed deer. Journal of Mammalogy. 81: 179–185.

R Core Team. **2017**. R: a language and environment for statistical computing.

Ratnasingham, S., and P. D. N. Hebert. **2007**. BOLD: The barcode of life data system (http://www.barcodinglife.org). Molecular Ecology Notes. 7: 355–364.

Ratovonjato, J., M.-M. Olive, L. M. Tantely, L. Andrianaivolambo, E. Tata, J. Razainirina, E. Jeanmaire, J.-M. Reynes, and N. Elissa. **2011**. Detection, isolation, and genetic characterization of Rift Valley fever virus from *Anopheles* (*Anopheles*) *coustani* , *Anopheles* (*Anopheles*) *squamosus* , and *Culex* (*Culex*) *antennatus* of the Haute Matsiatra region, Madagascar. Vector Borne Zoonotic Dis. 11: 753–759.

Reeves, L. E., J. L. Gillett-Kaufman, A. Y. Kawahara, and P. E. Kaufman. **2018**. Barcoding blood meals: New vertebrate-specific primer sets for assigning taxonomic identities to host DNA from mosquito blood meals. PLoS Negl Trop Dis. 12.

Reeves, W. C. 1958. Arthropods as Vectors and Reservoirs of Animal Pathogenic Viruses, pp. 177–202. *In* Hallauer, C., Meyer, K.F. (eds.), Handbuch Der Virusforschung. Springer Vienna, Vienna.

Reeves, W. C., R. E. Bellamy, C. H. Tempelis, and M. F. Lofy. **1965**. A three-year study of the feeding habits of *Culex tarsalis* in Kern County, California. Am J Trop Med Hyg. 14: 170–177.

Rippy, M. K., M. J. Topper, C. A. Mebus, and J. C. Morrill. **1992**. Rift Valley Fever virus-induced encephalomyelitis and hepatitis in calves. Vet Pathol. 29: 495–502.

Robb, L. L., D. A. Hartman, L. Rice, J. Demaria, N. A. Bergren, E. M. Borland, and R. C. Kading. **2019**. Continued evidence of decline in the enzootic activity of Western Equine Encephalitis virus in Colorado. J Med Entomol. 56: 584–588.

Roehrig, J. T. 2013. West Nile virus in the United States — A historical perspective. Viruses. 5: 3088–3108.

Rolin, A. I., L. Berrang-Ford, and M. a Kulkarni. **2013**. The risk of Rift Valley fever virus introduction and establishment in the United States and European Union. Emerging Microbes & Infections. 2: e81.

Romoser, W. S., M. N. Oviedo, K. Lerdthusnee, L. A. Patrican, M. J. Turell, D. J. Dohm, K. J. Linthicum, and C. L. Bailey. **2011**. Rift Valley fever virus-infected mosquito ova and associated pathology: possible implications for endemic maintenance. Res Rep Trop Med. 2: 121–127.

Romoser, W. S., M. J. Turell, K. Lerdthusnee, M. Neira, D. Dohm, G. Ludwig, and L. Wasieloski. **2005**. Pathogenesis of Rift Valley fever virus in mosquitoes — tracheal conduits & the basal lamina as an extra-cellular barrier, pp. 89–100. *In* Peters, C.J., Calisher, C.H. (eds.), Infectious Diseases from Nature: Mechanisms of Viral Emergence and Persistence. Springer Vienna, Vienna.

Romoser, W. S., L. P. Wasieloski, P. Pushko, J. P. Kondig, K. Lerdthusnee, M. Neira, and G. V. Ludwig. **2004**. Evidence for arbovirus dissemination conduits from the mosquito (Diptera: Culicidae) midgut. J Med Entomol. 41: 467–475.

Rose, D. A., B. C. Kondratieff, and M. J. Weissmann. **2017**. Colorado Mosquitoes (Diptera: Culicidae). *In* Insects of Western North America. Contributions of the C.P. Gillette Museum of Arthropod Diversity, Department of Bioagricultural Sciences and Pest Management, Colorado State University.

Rostal, M. K., A. L. Evans, R. Sang, S. Gikundi, L. Wakhule, P. Munyua, J. Macharia, D. R. Feikin, R. F. Breiman, and M. K. Njenga. **2010**. Identification of potential vectors of and detection of antibodies against Rift Valley fever virus in livestock during interepizootic periods. Am J Vet Res. 71: 522–526.

Ryan, S. J., C. J. Carlson, E. A. Mordecai, and L. R. Johnson. **2019**. Global expansion and redistribution of *Aedes*-borne virus transmission risk with climate change. PLoS Negl Trop Dis. 13: e0007213.

Salazar, M. I., J. H. Richardson, I. Sánchez-Vargas, K. E. Olson, and B. J. Beaty. **2007**. Dengue virus type 2: Replication and tropisms in orally infected *Aedes aegypti* mosquitoes. BMC Microbiology. 7.

Saluzzo, J. F., J. P. Digoutte, C. Chartier, D. Martinez, and R. Bada. **1987**. Focus of Rift Valley fever virus transmisson in southern Mauritania. the Lancet. 329: 504.

Samy, A. M., A. T. Peterson, and M. Hall. **2017**. Phylogeography of Rift Valley fever virus in Africa and the Arabian Peninsula. PLoS Negl Trop Dis. 11.

Sang, R., P. M. Nguku, S. C. Britch, J.-M. Reynes, M. A. Mohamed, P. Formenty, A. Anyamba, R. F. Breiman, S. de La Rocque, K. Haagsma, R. Swanepoel, M. Latham, J.-P. Chretien, J. Small, A. W. Hightower, K. J. Linthicum, H. B. Lewandowski, D. Schnabel, S. O. Magdi, E. Pak, and C. J. Tucker. **2010**. Prediction, assessment of the Rift Valley fever activity in east and southern Africa 2006–2008 and possible vector control strategies. Am J Trop Med Hyg. 83: 43–51.

Santos, C. S., M. R. Pie, T. C. da Rocha, and M. A. Navarro-Silva. **2019**. Molecular identification of blood meals in mosquitoes (Diptera, Culicidae) in urban and forested habitats in southern Brazil. PLoS ONE. 14: e0212517.

Schurich, J. A., S. Kumar, L. Eisen, and C. G. Moore. **2014**. Modeling *Culex tarsalis* abundance on the northern Colorado Front Range using a landscape-level approach. J Am Mosq Control Assoc. 30: 7–20.

(Select Agents and Toxins List | Federal Select Agent Program) . **2020**. Select Agents and Toxins List | Federal Select Agent Program. (https://www.selectagents.gov/sat/list.htm).

Sharaf El-Deen, M. E. E. 1987. Some studies on Rift Valley fever virus (Master Thesis).

Shoemaker, T., C. Boulianne, M. J. Vincent, L. Pezzanite, M. M. Al-Qahtani, Y. Al-Mazrou, A. S. Khan, P. E. Rollin, R. Swanepoel, T. G. Ksiazek, and S. T. Nichol. **2002**. Genetic analysis of viruses associated with emergence of Rift Valley fever in Saudi Arabia and Yemen, 2000-01. Emerg Infect Dis. 8: 1415–1420.

Steele, K. E., M. J. Linn, R. J. Schoepp, N. Komar, T. W. Geisbert, R. M. Manduca, P. P. Calle, B. L. Raphael, T. L. Clippinger, T. Larsen, J. Smith, R. S. Lanciotti, N. A. Panella, and T. S. McNamara. **2000**. Pathology of fatal West Nile virus infections in native and exotic birds during the 1999 outbreak in New York City, New York. Vet Pathol. 37: 208–224.

Tantely, L. M., S. Boyer, and D. Fontenille. **2015**. A review of mosquitoes associated with Rift Valley fever virus in Madagascar. Am J Trop Med Hyg. 92: 722–729.

Terasaki, K., and S. Makino. **2015**. Interplay between the virus and host in Rift Valley fever pathogenesis. J Innate Immun. 7: 450–458.

Thiemann, T. C., D. A. Lemenager, S. Kluh, B. D. Carroll, H. D. Lothrop, and W. K. Reisen. **2012**. Spatial variation in host feeding patterns of <i>Culex tarsalis</> and the *Culex pipiens* complex (Diptera: Culicidae) in California. J Med Entomol. 49: 903–916.

Thiemann, T. C., and W. K. Reisen. **2012**. Evaluating sampling method bias in <i>Culex tarsalis</> and *Culex quinquefasciatus* (Diptera: Culicidae) bloodmeal identification studies. J. Med. Entomol. 49: 143–149.

Thiemann, T. C., S. S. Wheeler, C. M. Barker, and W. K. Reisen. **2011**. Mosquito host selection varies seasonally with host availability and mosquito density. PLoS Neglected Tropical Diseases. 5: e1452.

Thiemann, T. C., D. L. Woodward, Y. Fang, B. M. Ryan, B. M. Nelms, J. J. Scott, and W. K. Reisen. **2017**. Abundance and bloodfeeding patterns of mosquitoes (Diptera: Culicidae) in an oak woodland on the eastern slope of the Northern Coast Range of California. J Med Entomol. 54: 1344–1353.

Tong, C., E. Javelle, G. Grard, A. Dia, C. Lacrosse, T. Fourié, P. Gravier, S. Watier-Grillot, R. Lancelot, F. Letourneur, F. Comby, M. Grau, L. Cassou, J.-B. Meynard, S. Briolant, I. Leparc-Goffart, and V. Pommier de Santi. **2019**. Tracking Rift Valley fever: From Mali to Europe and other countries, 2016. Euro Surveill. 24.

Tucker, C. J., K. A. Melocik, A. Anyamba, K. J. Linthicum, S. F. Fagbo, and J. L. Small. **2020**. Reanalysis of the 2000 Rift Valley fever outbreak in Southwestern Arabia. PLoS ONE. 15: e0233279.

Turell, M. J., C. L. Bailey, and J. R. Beaman. **1988**. Vector competence of a Houston, Texas strain of *Aedes albopictus* for Rift Valley fever virus. J. Am. Mosq. Control Assoc. 4: 94–96.

Turell, M. J., S. C. Britch, R. L. Aldridge, D. L. Kline, C. Boohene, and K. J. Linthicum. **2013**. Potential for mosquitoes (Diptera: Culicidae) from Florida to transmit Rift Valley fever virus. J. Med. Entomol. 50: 1111–1117.

Turell, M. J., S. C. Britch, R. L. Aldridge, R.-D. Xue, M. L. Smith, L. W. Cohnstaedt, and K. J. Linthicum. **2015**. Potential for *Psorophora columbiae* and *Psorophora ciliata* mosquitoes (Diptera: Culicidae) to transmit Rift Valley fever virus. Journal of Medical Entomology. 52: 1111–1116.

Turell, M. J., M. L. O'Guinn, D. J. Dohm, and J. W. Jones. **2001**. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. Journal of Medical Entomology. 38: 130–134.

Turell, M. J., W. C. Wilson, and K. E. Bennett. **2010**. Potential for North American mosquitoes (Diptera: Culicidae) to transmit Rift Valley fever virus. J Med Entomol. 47: 884–889.

Turell, M.J., Byrd, B.D., Harrison, B. A. 2013. Potential for populations of *Aedes J. japonicus* to transmit rift valley fever virus in the USA. J. Am. Mosq. Control Assoc. 29: 133–137.

Wang, Y., U. Naumann, S. T. Wright, and D. I. Warton. **2012**. mvabund - an R package for model-based analysis of multivariate abundance data. Methods in Ecology and Evolution. 3: 471–474.

Wilber, M. Q., K. M. Pepin, H. Campa, S. E. Hygnstrom, M. J. Lavelle, T. Xifara, K. C. VerCauteren, and C. T. Webb. **2019**. Modelling multi-species and multi-mode contact networks: Implications for persistence of bovine tuberculosis at the wildlife–livestock interface. J Appl Ecol. 56: 1471–1481.

Williams, R., J. Malherbe, H. Weepener, P. Majiwa, and R. Swanepoel. **2016**. Anomalous high rainfall and soil saturation as combined risk indicator of Rift Valley fever outbreaks, South Africa, 2008–2011. Emerging Infectious Diseases. 22: 2054–2062.

Wilson, W. C., I. J. Kim, J. D. Trujillo, S. Y. Sunwoo, L. E. Noronha, K. Urbaniak, D. S. McVey, B. S. Drolet, I. Morozov, B. Faburay, E. E. Schirtzinger, T. Koopman, S. V.

Indran, V. Balaraman, and J. A. Richt. **2018**. Susceptibility of white-tailed deer to Rift Valley fever virus. Emerg. Infect. Dis. 24: 1717–1719.

Wilson, W. C., M. Romito, D. C. Jasperson, H. Weingartl, Y. S. Binepal, M. R. Maluleke, D. B. Wallace, P. Jansen van Vuren, and J. T. Paweska. **2013**. Development of a Rift Valley fever real-time RT-PCR assay that can detect all three genome segments. Journal of Virological Methods. 193: 426–431.

Zeller, H. G., D. Fontenille, M. Traore-Lamizana, Y. Thiongane, and J. P. Digoutte. **1997**. Enzootic activity of Rift Valley fever virus in Senegal. The American Journal of Tropical Medicine and Hygiene. 56: 265–272.

APPENDIX A: SUPPLEMENTAL MATERIALS

Supplemental Table A1. Blood meal data from mosquitoes collected at feedlots and nearby sites in northern Colorado.

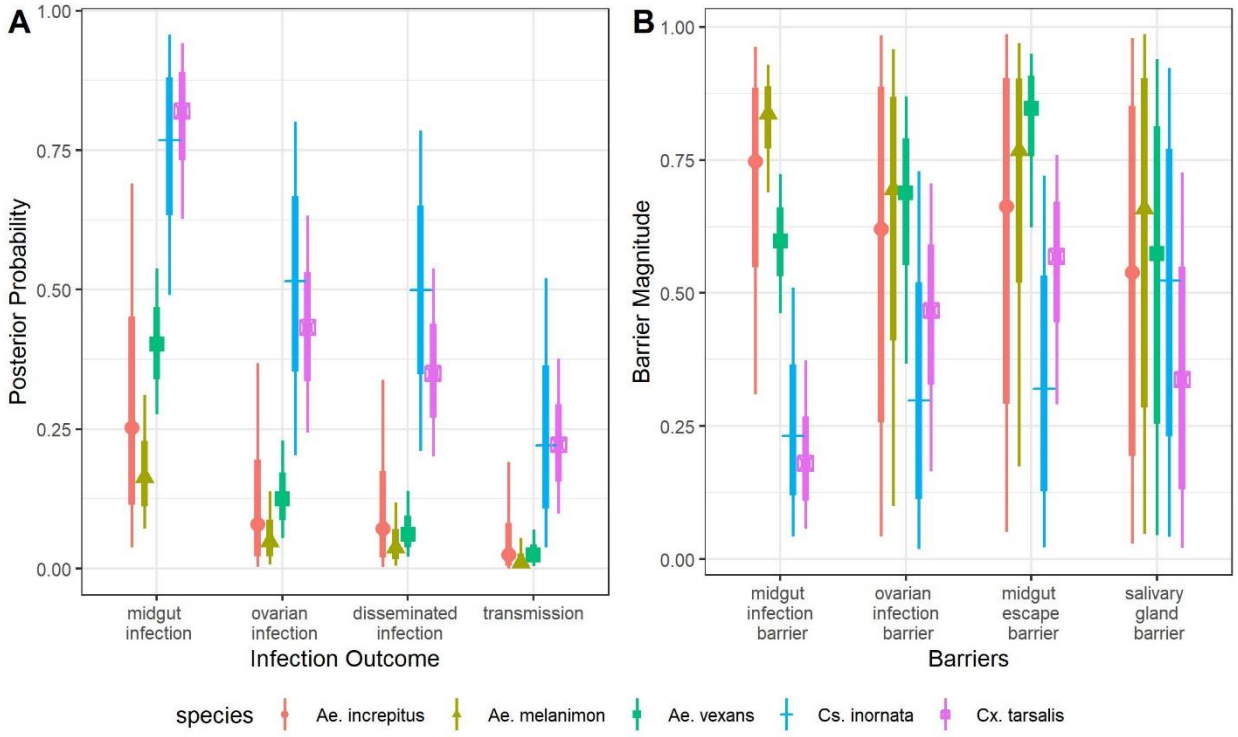
Site Type	Mosquito Species	cattle	desert cottontail	dog	eastern cottontail	goat	great horned owl	horse	house mouse	house sparrow	house wren
Feedlot Sites	Ae. dorsalis	7 (0.88)	0	0	0	0	0	1 (0.12)	0	0	0
	Ae. melanimon	2 (0.4)	0	0	0	0	0	0	0	0	0
	Ae. nigromaculis	4 (1)	0	0	0	0	0	0	0	0	0
	Ae. vexans	8 (0.53)	0	0	0	0	0	0	0	0	0
	Cs. inornata	4 (0.8)	0	0	0	0	0	0	0	0	0
	Cx. pipiens	5 (1)	0	0	0	0	0	0	0	0	0
	Cx. tarsalis	67 (0.92)	1 (0.01)	0	0	0	0	1 (0.01)	0	3 (0.04)	0
Non-Feedlot Sites	Ae. dorsalis	4 (0.21)	1 (0.05)	0	0	0	0	11 (0.58)	0	0	0
	Ae. melanimon	2 (0.22)	1 (0.11)	0	0	0	0	5 (0.56)	0	0	0
	Ae. nigromaculis	2 (1)	0	0	0	0	0	0	0	0	0
	Ae. vexans	14 (0.33)	5 (0.12)	0	2 (0.05)	0	0	17 (0.4)	0	0	0
	Cs. inornata	4 (0.33)	0	1 (0.08)	0	0	0	6 (0.5)	0	0	0
	Cx. pipiens	0	1 (0.5)	0	0	0	0	0	0	1 (0.5)	0
	Cx. tarsalis	6 (0.19)	3 (0.1)	0	0	1 (0.03)	1 (0.03)	12 (0.39)	1 (0.03)	1 (0.03)	1 (0.03)

Supplemental Table A1, continued.

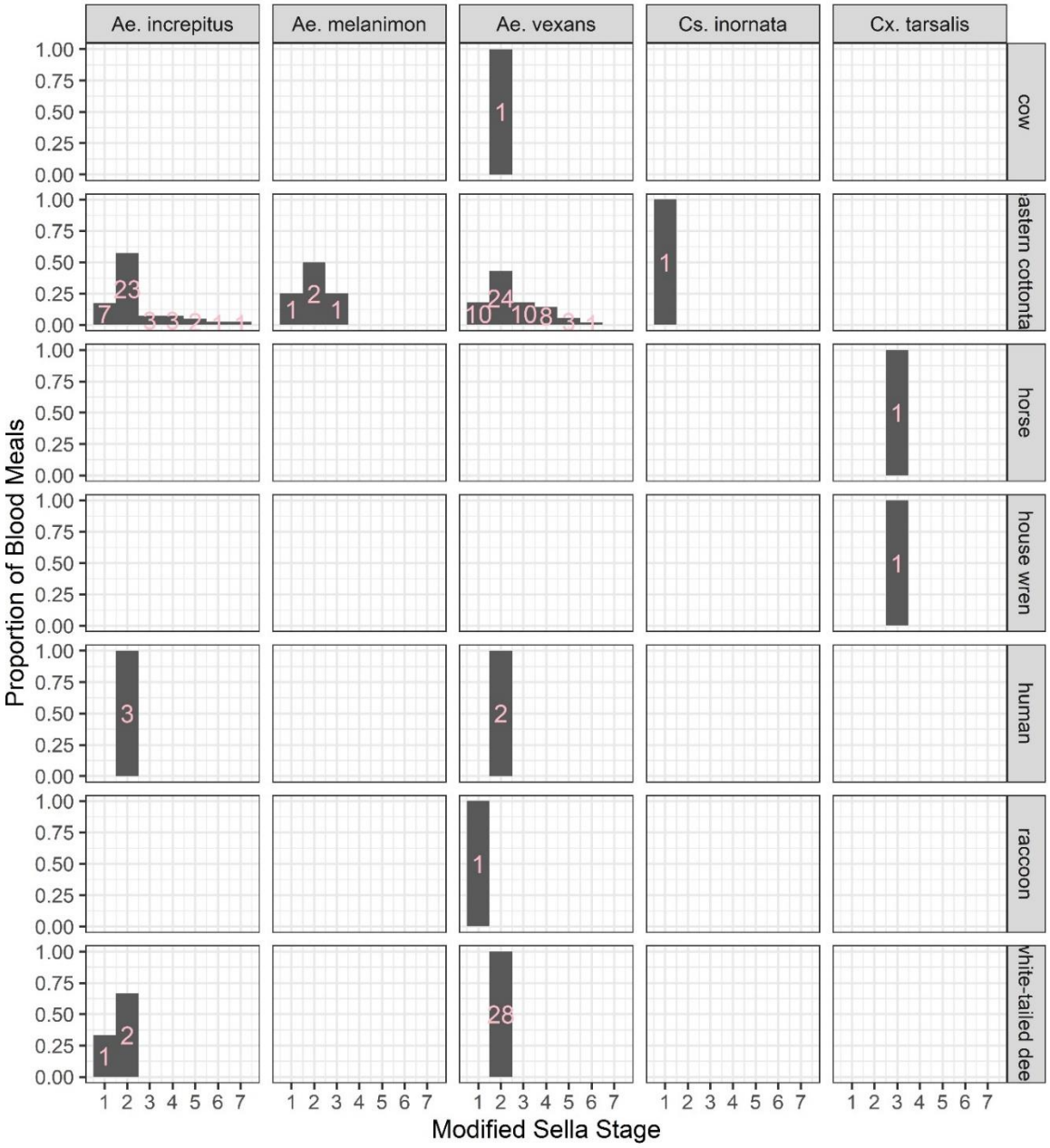
Site Type	Mosquito Species	llama	mourning dove	mule deer	raccoon	sheep	vesper sparrow	white-tailed deer
Feedlot Sites	Ae. dorsalis	0	0	0	0	0	0	0
	Ae. melanimon	0	0	0	0	3 (0.6)	0	0
	Ae. nigromaculis	0	0	0	0	0	0	0
	Ae. vexans	1 (0.07)	0	0	0	6 (0.4)	0	0
	Cs. inornata	0	0	0	0	1 (0.2)	0	0
	Cx. pipiens	0	0	0	0	0	0	0
	Cx. tarsalis	0	0	0	0	0	1 (0.01)	0
Non-Feedlot Sites	Ae. dorsalis	0	0	0	0	1 (0.05)	0	2 (0.11)
	Ae. melanimon	0	0	1 (0.11)	0	0	0	0
	Ae. nigromaculis	0	0	0	0	0	0	0
	Ae. vexans	0	0	1 (0.02)	0	0	0	3 (0.07)
	Cs. inornata	0	0	0	0	1 (0.08)	0	0
	Cx. pipiens	0	0	0	0	0	0	0
	Cx. tarsalis	0	4 (0.13)	0	1 (0.03)	0	0	0

Supplemental Table A2. Blood meal data from mosquitoes collected at the Environmental Learning Center (woodland) and residential Fort Collins as counts for each vertebrate species, as well as relative proportions in parentheses.

habitat	Mosquito Species	cow	eastern cottontail	horse	house wren	human	raccoon	white-tailed deer
residential	Ae. increpitus	0	3 (0.75)	0	0	1 (0.25)	0	0
	Ae. vexans	0	1 (0.33)	0	0	1 (0.33)	1 (0.33)	0
	Ae. increpitus	0	37 (0.88)	0	0	2 (0.05)	0	3 (0.07)
	Ae. melanimon	0	4 (1)	0	0	0	0	0
woodland	Ae. vexans	1 (0.01)	55 (0.65)	0	0	1 (0.01)	0	28 (0.33)
	Cs. inornata	0	1 (1)	0	0	0	0	0
	Cx. tarsalis	0	0	1 (0.50)	1 (0.50)	0	0	0



Supplemental Figure A1. Probability estimates for each infection outcome, as well as the internal barriers to infection of mosquitoes, while considering samples detectable on the limit of detection to be negative.



Supplemental Figure A2. **Density Histograms of Sella scores from all blood-fed mosquitoes collected throughout the sampling period.** Numbers indicate the absolute counts contributing to each score.

Supplemental Text A1. Code specifying the vector competence model in the JAGS language for Markov Chain Monte Carlo estimation of parameter values.

```
# Priors
a ~ dbeta(1, 1)
b ~ dbeta(1, 1)
c ~ dbeta(1, 1)
d ~ dbeta(1, 1)

# The data model
for(i in 1:length(M) ) {
  M[i] ~ dbern(a)
}
for(i in 1:length(O) ) {
  O[i] ~ dbern(a*b)
}
for(i in 1:length(D) ) {
  D[i] ~ dbern(a*c)
}
for(i in 1:length(S) ) {
  S[i] ~ dbern(a*c*d)
}
p1=a
p2=a*b
p3=a*c
p4=a*c*d
```