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Examining Various Aspects of Zika Virus Dissemination in Aedes aegypti

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EXAMINING VARIOUS ASPECTS OF ZIKA VIRUS DISSEMINATION IN *AEDES AEGYPTI*

A Thesis

by

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ABSTRACT

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South Texas is one of the few locations where Zika virus has been locally transmitted in the U.S. It has a climate which is distinct to other areas with autochthonous transmission, with extremely hot, dry summers and moderate winter temperatures. Studying mosquito transmission of Zika in a range of temperatures conditions replicating where virus transmission is occurring is essential in order to have a better understanding of transmission patterns. These factors were examined by infecting mosquitoes and monitoring the dissemination status through real-time PCR analysis.

To further characterize dissemination of Zika virus within south Texas mosquitoes a time series immunohistochemical analysis was conducted. In this proof of concept experiment infected mosquitoes were fixed days post infection and processed for antibody staining before being imaged with a confocal microscope. More reliable experimental methodology will result in more accurate assessment of transmission risk and prediction of transmission of Zika virus transmission.

DEDICATION

Thank you for all the support from my mom (Jean Lozano), grandmother (Juanita Rodriguez) and sister (Ariana Lozano-Coco) throughout this masters degree. I greatly appreciate all the help from my advisors and professors before, during and after my masters. Furthermore, I extend gratitude to the UTRGV staff which also played a great part in helping make this possible. Without the help and guidance of all these individuals none of this work would have been possible.

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CHAPTER I

INTRODUCTION

Zika Virus

Zika virus is a single stranded RNA virus which was first found in the Zika Forest of Uganda in 1947 (Hills, Fischer, & Petersen, 2017). The virus is composed of several structural proteins including an envelope, pre membrane protein, and capsid (Rabaan et. al, 2017; Wang et. al, 2017). The virus is also contains various nonstructural proteins such as NS1, NS2, 2K, NS4, and NS5 (Rabaan et al., 2017; Wang et al., 2017). Zika is member of the Flaviviridae family which includes West Nile virus, yellow fever virus and dengue virus (Atif et. al, 2016; Paixão et. al, 2018). There are two lineages of Zika virus, the African and the Asian lineage, with the Asian lineage being linked to cases of microcephaly in humans (Simonin et. al, 2017).

Approximately 80% of people infected with Zika virus will be asymptomatic leading to decreased number of reported cases (Jamali Moghadam et al., 2016; Gorshkov et al., 2019). Symptoms in the symptomatic patients will range from mild to severe. Mild symptoms include rash, fever, and conjunctivitis (Basarab et. al, 2016; Gorshkov et al., 2019). In most cases Zika is non-fatal although there has been associations with Guillain Barre syndrome, and microcephaly in fetuses (Basarab et al., 2016; Paixão et al., 2018).

Zika virus can be transmitted multiple ways, including vector transmission, maternal-fetal transmission, blood transfusion transmission, and sexual transmission (Abushouk et, al, 2016;

Alaniz, 2019). This represents a departure from other flaviviruses which do not exhibit this variety of routes of transmission (Abushouk et al., 2016; Alaniz, 2019).

Autochthonous transmission of Zika virus was recorded in countries in North America, Latin America and the Caribbean (Colón-González et. al, 2017; Theel & Hata, 2018; Alaniz, 2019). Throughout those areas more than a million people became infected with the virus (Theel & Hata, 2018). Considering the lack of vaccinations or specific treatments most efforts for limiting Zika virus transmission rely on vector control (Vasilakis & Weaver, 2017; Musso et. al, 2019).

Zika virus Vectors

The predominate vector for Zika virus is *Aedes aegypti* and the secondary vector which has been implicated is *Aedes albopictus* (Epelboin, Y., et. al, 2017; Reinhold et. al, 2018). *Aedes aegypti* is found throughout areas of the Americas including in Central America, the Caribbean and the United States (Eisen & Moore, 2013). The distribution of *Ae. aegypti* in the United States is from coast to coast in the southern region of the country (Eisen & Moore, 2013; Diaz, 2016). *Ae. albopictus* on the other hand has a wider distribution range in the U.S. in comparison (Diaz, 2016). Climatic conditions in some northern regions of the U.S. limit the northern establishment of both *Ae. aegypti* and to a lesser extent *Ae. albopictus.*

Aedes aegypti mosquitoes can survive a temperature range from the lower limit of ~10°C, where the mosquitoes are immobilized, to the upper thermal limit of ~40°C (Jansen & Beebe, 2010; Reinhold et. al, 2018). The fitness, survivability and development for this species is most favorable at temperatures between 22°C and 32°C (Marinho et al., 2015). Females were found to be most efficient at blood feeding at temperatures which were between 26°C and 35°C (Huber et.

al, 2018; Reinhold et. al, 2018). Mosquitoes optimum temperature range can either limit or enhance important biological processes such as life cycle, behavior and virus dissemination (Lambrechts et al., 2011; Carrington et. al, 2013a; Carrington, 2013b; Reinhold et. al, 2018).

Aedes albopictus has greater probability to transmit virus in cooler temperatures than *Ae. aegypti* (Mordecai et al., 2017). *Ae. aegypti* is more likely to be able to transmit virus at higher temperatures because it can survive these temperatures better than *Ae. albopictus* (Mordecai et al., 2017). Peak transmission for *Ae. albopictus* and *Ae. aegypti* is likely due to their adaptation to different temperature ranges (Chang et. al, 2007; Brady et. al, 2013).

Vector Transmission

Aedes aegypti feeds predominately on humans and, to a lesser extent on other vertebrate hosts (Eisen & Moore, 2013; Muktar et. al, 2016). It often feeds on multiple host and ingests only a small blood meal per host in order to produce the necessary nutrients to produce its eggs, a behavior often referred to as sip feeding (Eisen & Moore, 2013; Muktar et al., 2016). This feeding behavior increases the probability a female mosquito will transmit the virus to multiple hosts.

Transmission from host to naïve mosquito occurs when a mosquito feeds on an infected person and ingests the virus. The virus then replicates in the midgut before entering the hemocoel of the mosquito and reaching the salivary gland (Muktar et al., 2016; Epelboin et al., 2017; Lim et. al, 2018). This is referred to as the extrinsic incubation period, is the length of time it takes for a mosquito to become infectious after ingestion of an infected blood meal (Black et al., 2002; Christofferson & Mores, 2016). On average the extrinsic incubation period for Zika virus is 5-10 days (Abushouk et al., 2016; Atif et al., 2016; Gangulyet. al, 2016). Extrinsic incubation period

and probability of disseminations are used to analyze transmission. Vector competence is the ability of a vector to become infected, sustain virus replication in the midgut and transmit the virus through infected saliva (Mercado-Curiel et. al, 2008; Lim et al., 2018). Dissemination is often measured as the percentage of infected mosquitoes which have virus infected legs relative to infected bodies (Alto et. al, 2008). Mosquitoes with non-disseminated infections will be identified as those in which the body is infected (due to the virus found in the midgut), but the legs are not. Lack of dissemination is often attributed, to the virus being unable to pass through the midgut barrier (Alto et al., 2008).

Environment and Transmission

Temperature changes the interaction between mosquitoes and arboviruses (Alto & Bettinardi, 2013; Samuel et al., 2016). For instance, changes in temperature may shape the mosquitoes immune response to the virus (C. C. Murdock et al., 2012). Viruses have different replication rates and structural stability with variations in temperature as well (Reiter, 2001; Goo et. al, 2016). The midgut and salivary gland (which are barriers for dissemination of the virus) may be modified by temperature influencing the dissemination of the virus (Alto & Bettinardi, 2013).

Zika Virus in South Texas

Prior to 2016 all Zika virus cases in the continental United States were associated with travel to countries with ongoing Zika virus transmission (Walker et al., 2016). In the U.S. and its territories there were close to 37,000 cases of Zika virus reported from 2015 to 2016, with majority of cases being from Puerto Rico (Theel & Hata, 2018). The lack of local transmission changed when local mosquito-borne transmission was established in both Texas and Florida. In 2016, the states of Florida and Texas combined had more than 200 cases of locally transmitted

Zika virus infection, including cases in the Texas city of Brownsville (CDC Newsroom, 2016; Hills et. al, 2017; Theel & Hata, 2018). Brownsville is located in Cameron county, which along with Hidalgo country, Starr country and Willacy country comprise the lower Rio Grande Valley (also known as the south Texas area).

Both south Texas and Florida have similar climates. Their climates are identified as subtropical, although they are not identical. South Texas has a warmer climate and more arid conditions (Champion & Vitek, 2014). There are geographic differences as well, such as south Texas sharing a border with Mexico (a country with past documented cases of dengue and Zika) (Ramos et al., 2008; Hernández-Ávila et al., 2018). Florida does not have a neighboring area or country which has endemic vector borne cases, which may impact the number of cases reported (Champion & Vitek, 2014). Extending transmission findings for Florida to south Texas may not reflect accurately the transmission of virus for this area due to these differences. I propose to study south Texas climatic conditions effect on dissemination of Zika virus. In addition, I will examine the effect constant and fluctuating south Texas temperatures had on virus dissemination. I hypothesize there will be differences in dissemination of the virus between constant and fluctuating south Texas temperatures. Environmental chambers will be used to replicate south Texas temperatures, with both constant average temperatures based on seasonal values as well a 24-hour fluctuating temperature range (representing a high mid-day temperature and a low mid-night temperature). Mosquito will be maintained in these chambers as adult. Following an infected blood meal, dissemination will be measured using mosquito's body and legs, Virus will be detected through the use of RT-PCR.

In order to further characterize dissemination of Zika virus within south Texas mosquitoes a time series immunohistochemical analysis was conducted. In this proof of concept

experiment infected mosquitoes were fixed days post infection and processed for antibody staining before being imaged with a confocal microscope. The potential purpose for this analysis could allow for the comparison of transmission for geographically distinct *Aedes* populations particularly examining when tissues are showing virus presence indicative of different transmission criteria. This would allow for the comparison of transmission in an alternate matter without the need for some of the costly reagents and machinery.

CHAPTER II

FLUCTUATING TEMPERATURES V.S. CONSTANT TEMPERATURES

Abstract

Zika virus has been recently introduced into the United Stated, but it is unclear how local environmental conditions may influence transmission. Studying mosquito transmission of Zika in a range of temperatures conditions replicating where virus transmission is occurring is essential in order to have a better understanding of transmission patterns. South Texas is one of the few locations where Zika virus has been locally transmitted in the United States. South Texas also has a climate which is distinct to other areas with autochthonous transmission, with extremely hot, dry summers and moderate winter temperatures. The standard laboratory practice of using constant temperatures for mosquito colonies, does not reflect the fluctuating temperatures that vectors experience in natural conditions on a daily basis and which may alter transmission patterns. These factors were examined by infecting *Aedes aegypti* mosquitoes and monitoring the dissemination status. Infection and dissemination were assessed through real-time PCR analysis. Dissemination occurred earlier in the constant temperature groups when compared to the fluctuating groups in the same seasonal temperatures. A decrease in infected mosquito survivability was observed for the constant temperatures by nearly half the days in comparison to the fluctuating temperatures. The mid temperature treatments showed a difference in dissemination which was not seen in the high or low temperatures. There was a significant difference overall between the dissemination of Zika virus in constant and fluctuating South

Texas temperatures. While these results do not suggest seasonal variation in dissemination rates, they do indicate laboratory methods of fluctuating versus constant temperatures may influence results. More reliable experimental methodology will result in more accurate assessment of transmission risk and prediction of transmission of Zika virus transmission.

Introduction

Mosquitoes are impacted by the daily ambient temperatures they experience due to them being unable to self-regulate their temperature (Alto & Bettinardi, 2013; Samuel et. al, 2016; Reinhold et. al 2018). The ambient temperature changes they are exposed to are a range of temperatures which fluctuate throughout the day (C. C. Murdock et al., 2012; Reinhold, et. al, 2018). These temperature changes could modify both the mosquito and virus altering key aspects of transmission (Alto & Bettinardi, 2013; Samuel et al., 2016).

The majority of studies conducted on dissemination and transmission were conducted in static temperatures although recent studies have begun examining these variables in more realistic ambient temperatures (Watts et al., 1987; Rohani et. al, 2009; Lambrechts et al., 2011; Carrington et. al, 2013b; Xiao et al., 2014; Alto et al., 2018; Tesla et al., 2018; Onyango et al., 2020). Some studies show that fluctuating temperatures increased dissemination when compared to the constant temperatures (Carrington et, al, 2013b; Alto et. al 2018). While there are differences between fluctuating and constant temperatures the results vary.

Intriguingly, studies have found transmission was increased with higher temperatures for Zika and dengue (Rohani et. al, 2009; Xiao et al., 2014; Tesla et al., 2018). This has generally been attributed to shorter incubation period and faster replication of the virus, although there was

also increased mortality for the mosquitoes (Rohani et. al, 2009; Xiao et al., 2014). However, some studies have shown contradictory results. Transmission rates were decreased for higher diurnal average temperature range, although decreased mosquito survivorship was also observed (Lambrechts et al., 2011; Danforth et. al, 2016). Despite these conflicting findings, all studies suggest that temperature variation may have a strong influence on dissemination and transmission.

It is important to analyze the effect that the variation in climatic temperatures of south Texas may have on dissemination. Understanding aspects of mosquito transmission is essential in order to improve control efforts (Yang et. al, 2009). In the present study, I examine south Texas temperatures effect on the dissemination of Zika virus within *Ae. aegypti* mosquitoes. In addition, I investigated the effects of both constant and fluctuating daily temperatures on dissemination. I hypothesize there will be differences in dissemination of the virus between constant and fluctuating south Texas temperatures.

Methods

Temperature selection

Temperatures for this study were selected for the city of McAllen, TX. McAllen was selected because it is in the south Texas region which is a region with past Zika cases. The method used to select the temperatures for this experiment was categorizing months based on their similar average temperatures. Temperatures were determined using the U.S. Climate Data (McAllen U.S. Climate Data, 2017) based on the temperatures over the last five years. For each of the seasons an average high and low temperature was obtained for the daily fluctuations. For the static temperatures the average of both the high and low temperature determined and used as

a constant temperature. The final temperature selections for the low fluctuating temperature was an average high (Day) of 22.58°C and an average low (Night) of 11.29°C. For the mid-range fluctuating temperature, the average high temperature (Day) was 30.55°C and the average low temperature (Night) was 18.97°C. The high fluctuating temperature selected was an average high (Day) of 36.1°C and an average low (Night) of 24.6°C. The daily fluctuating temperature range used was a difference of about 11.3°C to 11.5°C for the three fluctuating temperature experiments. For the constant temperatures, the low temperature was set to 16.94°C, the mid-range temperature was set to 24.76°C and the high temperature was 30.35°C. For all the different temperature treatments humidity was kept constant at 73%.

Rearing and infecting mosquitoes

Lab colony *Ae. aegypti* F4 and F5 generation mosquito larvae were placed in plastic trays in a density of approximately 500 larvae in each tray. To maintain the larvae two scoops of liver powder were given every other day. Mosquitoes were reared to pupae stage under constant room temperatures. Pupae were then placed in environmental chambers with the experimental temperatures and humidity was held constant at 73% relative humidity. They were sustained prior to infection on 10% sugar water and water. Upon reaching adult stage, mosquitoes were left at the experimental temperatures for about two weeks. Several uninfected female mosquitoes were removed from the colony cage and were placed into a sterile 1.5 mL microcentrifuge tube which contained a media source (DMEM+10% FBS 2X anti-anti filter sterilized) (Gibco, Invitrogen, USA). With the use of an aspirator mosquitoes were removed from the cage and anesthetized by placing them in the -20°C freezer for 1 minute to 1 minute and thirty seconds (since some mosquitoes became acclimated to the colder temperatures). Post anesthetization mosquitoes were placed on a chill plate to separate female and male mosquitoes. On average 25-

30 females were placed into five-cylinder containers with mesh netting. The female mosquitoes were starved for twenty-four hours prior to blood feeding and were blood fed with the use of a Hemotek© (Hemotek, UK) (membrane feeding system) and an adult mice skin membrane. Mice were thawed, skinned with a scalpel and fur was removed to ease access of mosquitoes to blood. Bovine blood was infected with 10^6 of ZIKV isolate PRVABC059 and was added to the membrane. The Hemotek© (Hemotek, UK) was warmed to human body temperature (37.4° C) before allowing mosquitoes to fed. Mosquitoes feed for two hours at room temperature through the mesh netting. Post blood feeding females were anesthetized in the -20°C freezer for thirty seconds to one minute. On the chill table mosquitoes were separated based on whether they were engorged or not fully engorged with the use of a chill plate. The engorged mosquitoes were placed into new cylinder containers and placed back into the respective experimental temperature environmental chamber.

Testing infection Status

Following initial infection 3-5 mosquitoes were removed every other day, up to the point until there were no more surviving mosquitoes. Three females were only processed for each of the days due to reagent cost. Legs were removed and dissection tools were placed in a 70% ethanol solution in between sample processing. Legs were cut leaving behind ¼ of the base of the leg. After the leg and body of the mosquitoes were separated, they were placed into a 1.5 mL microcentrifuge tube containing 1.0mL of sterile media. Following mosquito appendage removal, sterile steel beads were added to each 1.5 mL microcentrifuge tube containing either mosquito body or legs. All mosquitoes were then tissuelyzed with the use of a TissueLyzer II (Qiagen, Valencia, CA) for a total of 2 minutes at a frequency of 250 Hz. Then they were centrifuged for 3 minutes at 13,000 RPM and transferred to new sterile 1.5 mL microcentrifuge

tubes. Viral RNA extraction was performed on the media sources and mosquito bodies which were in media. After the samples underwent Qiagen Viral RNA extraction (Qiagen, Valencia, CA) the samples were analyzed through the use of an Applied Biosystems 7500 Fast Dx Real Time PCR (Thermo Fisher Scientific, USA).

Statistical Analysis

Statistical analysis was conducted with JMP 13 software. Comparison of mosquitoes with disseminated infections between treatments was done with an ANOVA test.

Results

The high constant temperature mosquitoes had viral dissemination throughout the 10 days they survived as compared with the high fluctuating temperature mosquitoes which survived until day 16 and only had four days of disseminated infection (Figure 2-1). The two high temperature groups also differed in the number of days post infection when dissemination was found. High constant mosquitoes showed dissemination first occurring around day 1 and high fluctuating initially showed dissemination on day 6. The dissemination of virus is first seen in day 4 for the low fluctuating and in the low constant it is seen in very low amounts during day 2 (Figure 2-2). In the two low temperature treatments the dissemination of the virus is reduced when compared to that of the other two temperature groups. Both low fluctuating and constant temperature mosquitoes showed only 2 days of dissemination. For the mid fluctuating and constant treatments there is a similar pattern seen, with dissemination being detected throughout the mosquito's survival except for one day around day 10 when there is no dissemination (Figure 2-3). In the mid constant the day when there is no dissemination occurs earlier in day 8 instead of day 10. The mid fluctuating treatment mosquitoes lasted the longest from any of the other

treatments in this study reaching up to day 22. Control colony cage mosquitoes which were removed prior to infection showed no amplification for Zika virus. Media samples which were collected at the beginning and end of nearly every temperature experiment were found to have no amplification neither. These were collected to show there had been no virus contamination of the media (which was used throughout the processing of the mosquitoes). Dissemination occurs earlier in the constant groups when compared to the fluctuating groups in the temperature regimes. The comparison in dissemination of the mid temperature treatments was significantly different (df=1, F=7.1459, p=0.0150). The high and low temperature treatments were found not to be significantly different between the constant and fluctuating temperatures (High temperature-df=1, F=0.6445, p=0.4365) (Low temperature-df=1, F=0.4424, p=0.5185). Upon examining the overall effect of constant and fluctuating temperatures these were found to be significant differences (df=5, F= 5.4247, p= 0.0006).

Discussion

Temperatures above 36°C have reduced transmission efficiency in Zika virus and this phenomenon is seen for the high fluctuating temperature which had a day temperature of 36.1°C (Tesla et al., 2018). Due to this elevated fluctuating temperature causing the mosquitoes to decrease in survivability before completing transmission (Tesla et al., 2018). The high constant temperature was held at a temperature of 30.35°C, which is close to the optimum temperature for Zika transmission showed dissemination at an earlier time point. Dissemination also seemed to be sustained for the duration of the study. The low constant dissemination of Zika virus to the legs was decreased in comparison to the high constant mosquitoes

Similar to past studies (Carrington et. al, 2013b) conducted on dengue, our study found for Zika virus a decrease in mosquito survivorship in the constant temperatures as opposed to the fluctuating temperatures. This reduction in the constant temperature survivability was diminished by nearly half the days in comparison to the fluctuating temperatures. These results suggest there is a difference in survival between the static temperatures sometimes used in research and the fluctuating temperatures which are seen in nature. Using fluctuating temperatures in experiments with mosquitoes will be more likely to yield similar results to those seen outside the lab in terms of survival.

Examining ANOVA results indicate only the mid temperatures had a significant difference between the three treatments (Table 2-1). The high and low temperature treatments did not show a significant difference between treatments. This may be attributed to the smaller sample size used in those treatments in comparison. The sample size was decreased due to lack of surviving mosquitoes for the study. Future studies should have larger sample sizes to account for the larger number of mosquitoes which will perish due to the temperatures effect. The overall analysis between constant and fluctuating temperatures did show a statistically significant difference between the virus dissemination of these treatments. Accentuating the overall results and statistic show there was a significant difference overall between the dissemination of Zika virus in constant and fluctuating South Texas temperatures. These differences can be used to increase the accuracy of Zika virus modeling of transmission for the area. Before this study there was no dissemination data for Zika virus specifically in the south Texas region.

It is essential to note this study does not examine the extrinsic incubation period due to the lack of saliva extraction from the infected mosquitoes. For a complete assessment of extrinsic incubation, saliva or infectiousness should also be examined. In addition, the data

suggests contamination issues may have influenced the results. Dissemination was observed in the first day of the experiment, which is highly unlikely. In addition, in some instances mosquitoes had positively infected legs with no positive infection in the body, which is also highly unlikely (these are seen in Table 2-2 with asterisks). The most likely explanation of these results is contamination of samples, which may have occurred during the processing of mosquitoes or testing of samples. Future studies could decrease the chance for contamination by processing samples in smaller subsets with the legs and bodies processed separately.

One aspect which bears further investigation is whether there is a difference in Zika virus dissemination between south Texas and Florida temperatures, as well as local mosquito strains from each local. Future research which could be used to decipher whether there is in fact a significant difference in dissemination for the high and low temperature regimes could use the study we concluded as a foundation. In addition, the study could be expanded to include mosquitoes from the geographically distinct area of Florida and compare their dissemination for Zika virus.

Our study shows how fluctuating and constant south Texas temperatures caused differences in dissemination overall. These findings will be fundamental for future studies on Zika transmission for the south Texas region. They will begin to give a better understanding of the transmission patterns of the area and could allow for better modelling of future outbreaks.

Source	DF	F Ratio	Prob > F
Mid Constant *Mid Fluctuating	1	7.1459	0.0150
High Constant * High Fluctuating	1	0.6445	0.4365
Low Constant * Low Fluctuating	1	0.4424	0.5185
Constant *Fluctuating Treatments	**5**	5.4247	0.0006

Table 2-1. Comparison of Fluctuating and Constant Temperature Dissemination. ANOVA for temperature effects on dissemination of Zika virus.

Day	1	2	4	6	8	10	12	14	16	18	20	22
number positive Body (#/3) Mid Constant	(3/3)	(2/3)	(3/3)	(3/3)	(2/3)	(3/3)	(2/3)	(2/3)	(1/3)			
number positive Leg (#/3) Mid Constant	(2/3)*	(1/3)	(1/3)	(1/3)	(0/3)	(1/3)	(1/3)	(1/3)	(1/3)			
number positive Body (#/3) High Constant	(3/3)	(3/3)	(2/3)	(1/3)	(1/3)	(2/3)						
number positive Leg (#/3) High Constant	(1/3)*	(1/3)	(2/3)	(1/3)	(1/3)	(2/3)						
number positive Body (#/3) Low Constant	(3/3)	(2/3)	(1/3)	(0/3)*	(2/3)							
number positive Leg (#/3) Low Constant	(0/3)	(1/3)	(0/3)	(1/3)*	(0/3)							
number positive Body (#/3) Low Fluctuating	(3/3)	(3/3)	(2/3)	(3/3)	(1/3)	(1/3)	(1/3)	(0/3)	(0/3)			
number positive Leg (#/3) Low Fluctuating	(0/3)	(0/3)	(1/3)	(1/3)	(0/3)	(0/3)	(0/3)	(0/3)	(0/3)			
number positive Body (#/3) High Fluctuating	(1/3)	(1/3)	(1/3)	(2/3)	(0/3)	(1/3)	(1/3)	(2/3)	(2/3)			
number positive Leg (#/3) High Fluctuating	(0/3)	(0/3)	(0/3)	(1/3)	(0/3)	(3/3)*	(1/3)	(0/3)	(3/3)*			
number positive Body (#/3) Mid Fluctuating	(3/3)	(3/3)	(3/3)	(2/3)	(3/3)	(3/3)	(3/3)	(3/3)	(3/3)	(2/3)	(2/3)	(3/3)
number positive Leg (#/3) Mid Fluctuating	(2/3)*	(2/3)	(1/3)	(2/3)	(1/3)	(0/3)	(3/3)	(2/3)	(2/3)	(3/3)*	(2/3)	(2/3)

Table 2-2. Number of positive *Ae. aegypti* bodies and legs for each treatment. * show potential contamination issues.

(A) **(B)**

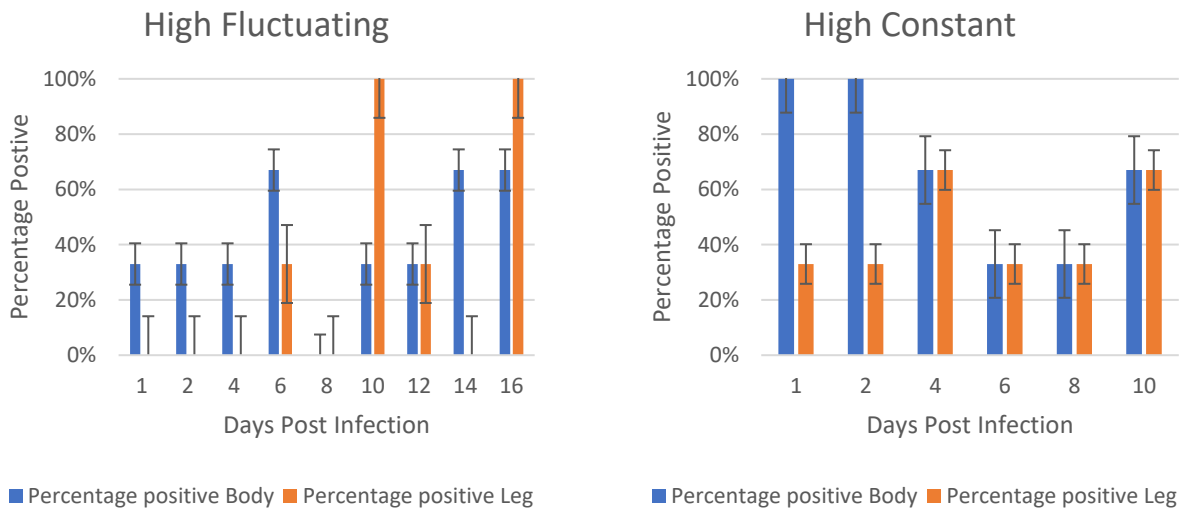
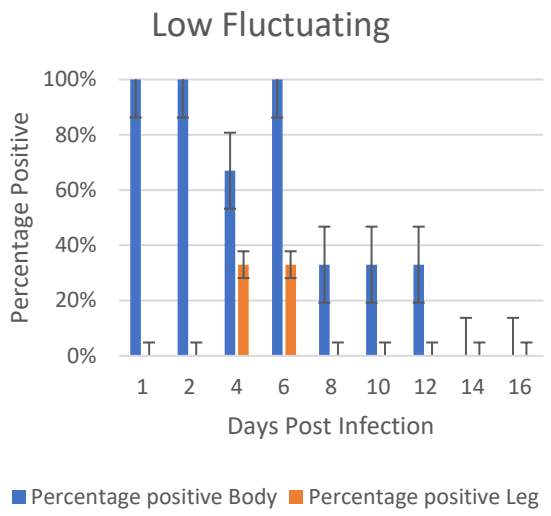


Figure 2-1. High Temperature Comparison-(A) High Fluctuating, (B) High Constant. The high constant temperature mosquitoes had virus dissemination throughout the days they survived as compared with the high fluctuating temperature mosquitoes which only had four days of disseminated infection. Error bars show standard error of the mean.

(A)



(B)

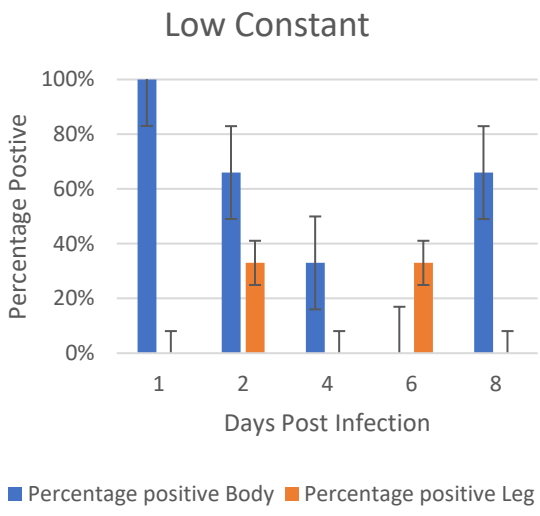


Figure 2-2. Low Temperature Comparison-(A) Low Fluctuating, (B) Low Constant. Both low fluctuating and constant temperature mosquitoes showed only 2 days of viral dissemination. Error bars show standard error of the mean.

(A) **(B)**

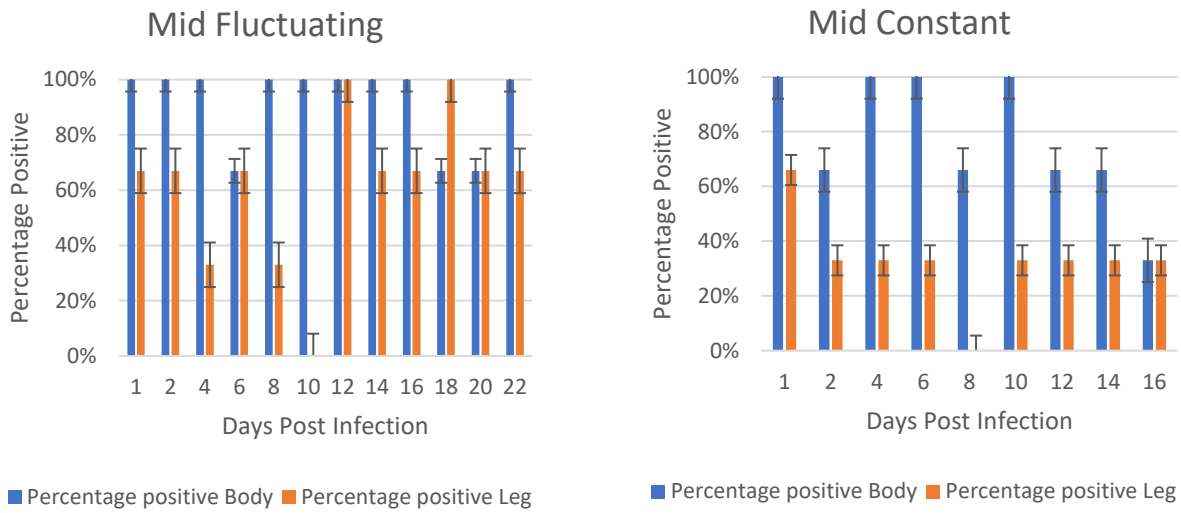


Figure 2-3. Mid Temperature Comparison-(A) Mid Fluctuating, (B) Mid Constant. For the mid fluctuating and constant treatments there is a similar pattern seen, with dissemination being detected throughout the mosquito's survival except for one day when there is no dissemination. Error bars show standard error of the mean.

CHAPTER III

TIME SERIES ANALYSIS

Abstract

Understanding the dissemination process in mosquitoes is essential to improve disease modelling, as well as determining the potential for increased vectorial capacity. Gaining greater understanding of how transmission occurs with relation to time could yield some essential information increasing the understanding of the vector-virus relationship. This study will use immunostaining to examine Zika virus replication within mosquitoes. Mosquitoes were fixed days post infection and processed for antibody staining before being imaged with a confocal microscope. The overall dissemination pattern observed in the mosquitoes was similar to those previously described (Carrington & Simmons, 2014; Fontaine et. al, 2016). With the dissemination pattern for this study showing virus initially replicating in the midgut epithelium cells, overcoming the escape barrier to show dissemination in the portion where the legs were attached, and ultimately reaching the head region where the salivary glands are located. This method will provide a layout for which tissues in mosquitoes have virus replication in relation to time since infection.

Introduction

Understanding the dissemination process in mosquitoes is essential to improve disease modelling, as well as determining the potential for vectorial capacity. Dissemination of virus through mosquitoes occurs by an infected blood meal entering the midgut and replicating in the epithelium cells within that area (Carrington & Simmons, 2014). The virus must overcome the 'midgut escape barrier' in order to enter the hemocoel and reach the salivary glands (Carrington & Simmons, 2014; Fontaine et. al, 2016). If the virus is unable to conquer the midgut barrier or get to the salivary glands this is indicative of the mosquito being unable to transmit the virus (Carrington & Simmons, 2014; Fontaine et al., 2016).

Multiple studies have attempted to gather a better understanding of the relationship between vector and pathogens using imaging techniques and immunohistochemistry (Dinglasan et al., 2007; Ziegler et al., 2011; Chahad-Ehlers et. al, 2013; Rossi et al., 2015; Fontaine et al., 2016; Cui et. al, 2019). One study used antibody staining and fluorescent in situ hybridization to illustrate the regions where two species of bacteria could be present within a mosquito (Rossi et al., 2015). Other studies have used different methods to examine the effects arboviruses have on the midgut region, but none have attempted to analyze the dissemination of flaviviruses in other regions of the mosquito with relation to time.

Envelope and NS1 are two distinct proteins from Zika virus which have been used for molecular identification of the virus. The envelope protein is a glycoprotein used for binding to host cells and only has a shared amino acid homology of around 30% with dengue (Beaver et. al, 2018; Theel & Hata, 2018). NS1 or nonstructural protein 1 is involved in assisting in self

cleaving peptidase and has structural differences to dengue (Beaver et al., 2018; Theel & Hata, 2018). These proteins and others have been used to research aspects of virus transmission such as dissemination, vector competence and extrinsic incubation periods.

Gaining greater understanding of how transmission occurs with relation to time could yield some essential information increasing the understanding of the vector-virus relationship. Illustrating tissues which have virus replication will allow to examine various transmission criteria. Such as for example the extrinsic incubation period which would show disseminated infection expected around the head region. The present study used immunostaining to examine Zika virus replication within mosquitoes. This data should indicate which tissues in mosquitoes have virus replication in relation to time since infection.

Methods

Mosquitoes and tissue preparation

Lab colony *Ae. aegypti* F5 generation mosquitoes were reared to pupae stage in average room temperatures. Pupae were placed in environmental chambers with the temperatures and humidity held constant at 24.76°C and 73% relative humidity. They were sustained prior to infection on 10% sugar water and water. With the use of an aspirator mosquitoes were removed from the cage and anesthetized by placing them in the -20^{o}C freezer for 1 minute to 1 minute and thirty seconds. Post anesthetization mosquitoes were placed on a chill plate to separate female and male mosquitoes. On average 50 females were placed into five-cylinder containers with mesh netting. The female mosquitoes were starved for twenty-four hours prior to blood feeding and were blood fed with the use of a Hemotek© (Hemotek, UK) and an adult mice skin membrane. Mice were thawed, skinned with a scalpel and fur was removed to ease access of mosquitoes to blood. Bovine blood was infected with 10^6 of ZIKV PRVABC059 (the pfu were acquired

through plaque assay) and was added to the membrane. The Hemotek© (Hemotek, UK) was warmed to human body temperature (37.4^0 C) before allowing mosquitoes to feed. Mosquitoes feed at room temperature for two hours through the mesh netting. Post blood feeding females were anesthetized in the -20^0C freezer for thirty seconds to one minute. On the chill table mosquitoes were separated based on whether they were engorged or not fully engorged with the use of a chill plate. The engorged mosquitoes were placed into new cylinder containers and placed back into the environmental chamber. Following this every other day the same number of mosquitoes were removed up to the point there were no more surviving mosquitoes. Mosquitoes which were removed were put into freshly prepared 4% PFA fixative for thirty minutes. Following the fixation, the mosquitoes were washed and dehydrated. The dehydration was done by slowly converting from the Pbtween (sterile PBS and tween 20) to the 100% ethanol concentration with intermediate washes in between increasing ethanol concentrations. Upon reaching 100% ethanol concentration the samples were either stored in the -20^0C or prepared for the next stage. This was done by doing different ratios of OCT with sterile PBS until 100% OCT was reached and washing in between increasing OCT concentrations. Upon completing the 100% OCT, the samples were left in the solution either overnight in the 4^0C or 2 hours at room temperature. Cutting of the tissues was done with a cryostat (Leica Biosystems, USA) and 10-20 µm seemed to produce the best slices. The tissue slices were placed on Superfrost Plus Microscope slides (Thermo Fisher Scientific, USA) these slides were chosen because they helped the tissue adhere more efficiently.

After forming a barrier around the tissues on the slide with a hydrophobic barrier pen. The tissues were blocked in the same solution which the antibodies were going to be diluted in. Blocking was done for an hour with PBTween (sterile PBS and tween 20) and 2% BSA solution

in a high humidity environment for one hour. The primary antibody Envelope or NS1(Arigo Biolaboratories, Taiwan) was added to one well of the slide and the other well was left without the primary antibody on the slides in order to have negative controls. The primary antibody was left for a total of two hours in a high humidity environment. Following a number of washes the secondary antibody was the Alexa Flor 546 or 647 (Alexa Flour 546 or 647-Thermo Fisher Scientific, USA) and it was incubated for an additional two hours. Finally, after a few subsequent washes the phalloidin and DAPI (Thermo Fisher Scientific, USA) were added and an additional set of washes were conducted. Then the slide cover was mounted and sealed with nail polish. Imaging was done with the use of the Olympus FV10i confocal microscope. The magnification which were used for this experiment were the 10X and 60X magnification.

Results

For the infected mosquitoes in hour 0 just after infection was noticeable the presence of virus in a small amount of the tissues in the head region and the most pronounced areas with virus replication is shown in the midgut especially in the surface cells (Figure 3-2, Figure 3-3). Intriguingly, day 4 had significant virus presence in some areas within the abdomen region (Figure 3-4). This is continued to be observed in day 6 with more areas of virus presence in the abdomen region (Figure 3-5B). On day 6 there was no virus seen within either the head or the thorax region (Figure 3-5A). By day 12 there was no significant virus presence in the abdomen area (Figure 3-6). Day 14 showed there was virus in the head region and the legs (Figure 3-7A). For this day there was no virus in the abdomen region which could be seen (Figure 3-7B). The negative control mosquitoes were infected but did not have primary antibody added (Figure 3-1). This step is conducted in order to show there are no tissues which are fluorescing

nonspecifically. The negative controls showed no presence of the NS1 or envelope antibody (Figure 3-1).

Discussion

This study allowed for the investigation of the dissemination of Zika virus with relation to time. The overall dissemination pattern observed in our study was similar to those previously described (Carrington & Simmons, 2014; Fontaine et. al, 2016). With the dissemination pattern for this study showing virus initially replicating in the midgut epithelium cells, overcoming the escape barrier to show dissemination in the portion where the legs were attached, and ultimately reaching the head region where the salivary glands are located. Something which needs more evaluation is examining the step where the infection disseminates into the hemocoel due to the staining only occurring in the tissues this step may be more difficult to observe. Another aspect which needs more inspection would be if these results are seen in a larger scale of staining and if other viruses are able to be observed in the tissues also.

The importance of this study is it gives a more accurate depiction of which tissues have virus still present with relation to time. Knowing which tissues still have virus present with time could yield more accurate models because it allows for the interpretation of more transmission variables to be examined. This method could be used to analyze differences in virus transmission between geographically distinct mosquito populations, temperature treatments, infection studies and more.

Time Post Infection	Tissues with Virus Present
Hour 0	Residual virus seen in upper regions from just being blood feed. Midgut shows significant virus presence, specifically in lining of midgut cells.
Day 4	Continued virus replication in abdomen area.
Day 6	Replication in abdomen is still observed but no virus presence is seen in the head region.
Day 12	No pronounced virus replication seen in abdomen section.
Day 14	Neck region and legs show some virus presence which are indicative of dissemination.

Table 3-1. Overview of Zika Dissemination Time Series Analysis. The table shows where there is the presence of virus in relation to time.

(A) **(B)**

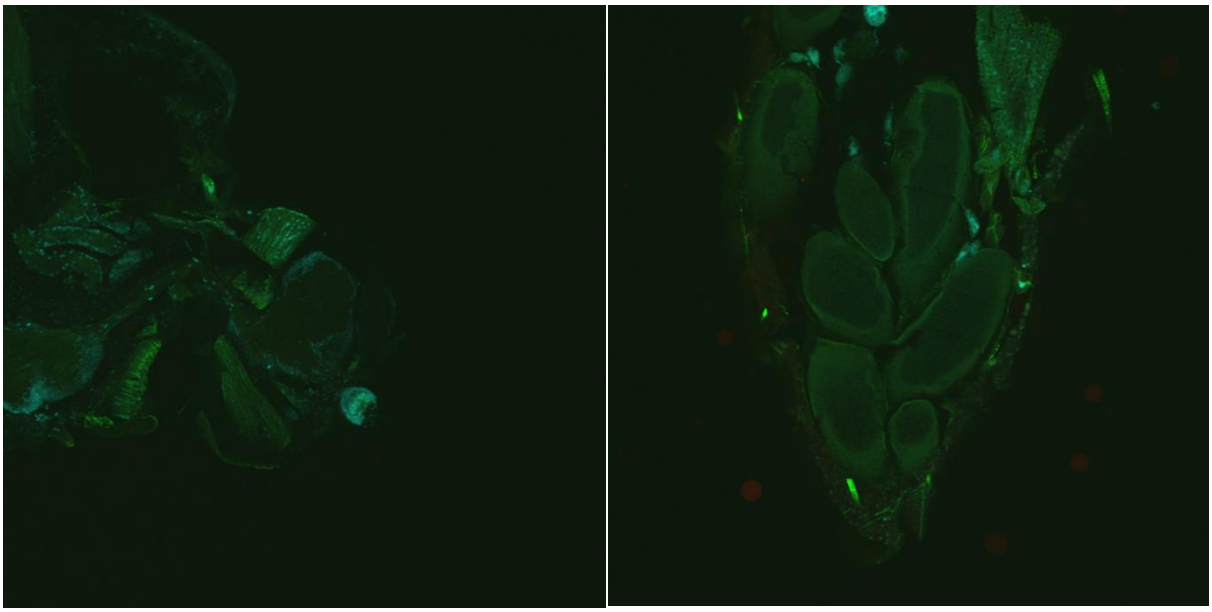


Figure 3-1. Negative Control-Head(A) and Abdomen(B) Regions (10X). Nuclei stained blue, and cytoskeleton is green. Immunofluorescence staining of an infected mosquito without primary antibody.

(A) **(B)**

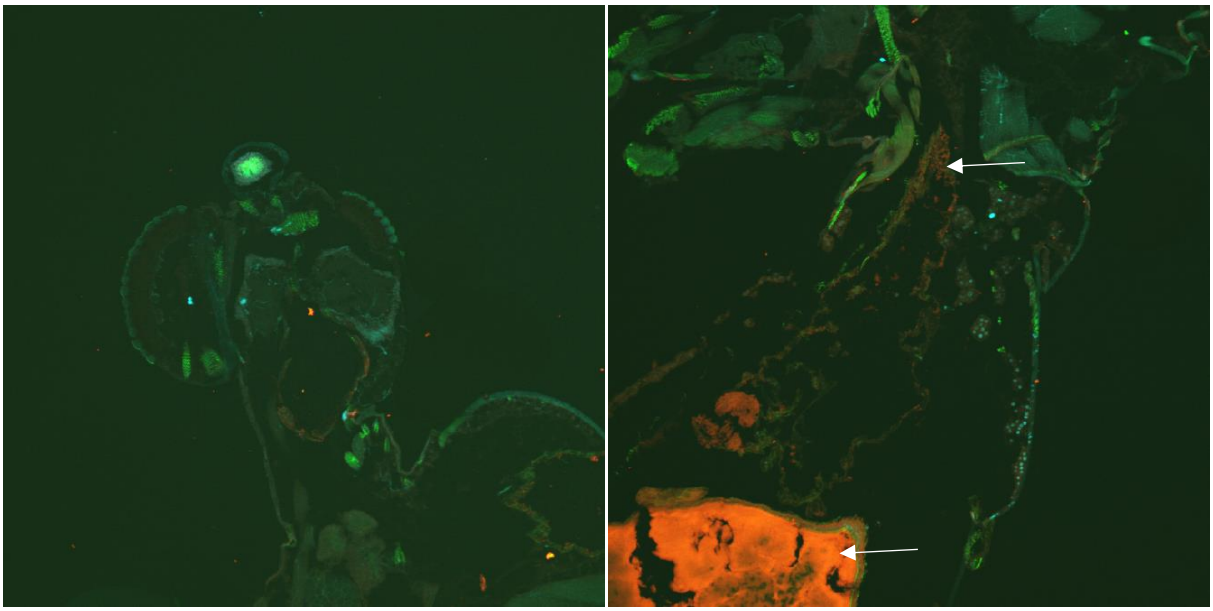


Figure 3-2. Hour 0 (Post Infection-. P.I.)-Head(A) and Thoracic(B) Regions (10X). Nuclei stained blue, cytoskeleton is green, and anti-ZIKV NS1 antibody is red. Immunohistochemical analysis of infected mosquito showing abdominal region with significant virus presence from just being blood fed. White arrows denote virus presence.

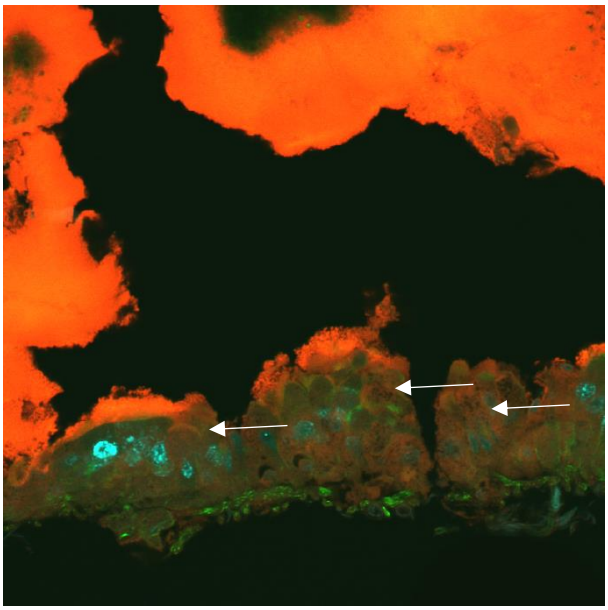


Figure 3-3. Hour 0 .P.I.-Abdomen Region (60X). Nuclei stained blue, cytoskeleton is green, and anti-ZIKV NS1 antibody is red. Immunofluorescence staining showing virus initially replicating in the midgut epithelium cells. White arrows denote virus presence.

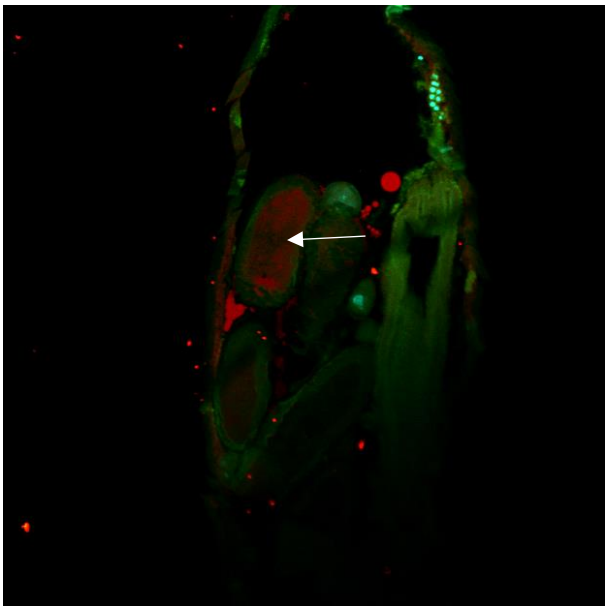


Figure 3-4. Day 4 P.I.-Abdomen Region (10X). Nuclei stained blue, cytoskeleton is green, and anti-ZIKV NS1 antibody is red. Immunohistochemical analysis showing continued virus replication in the abdomen. White arrows denote virus presence.

(A) **(B)**

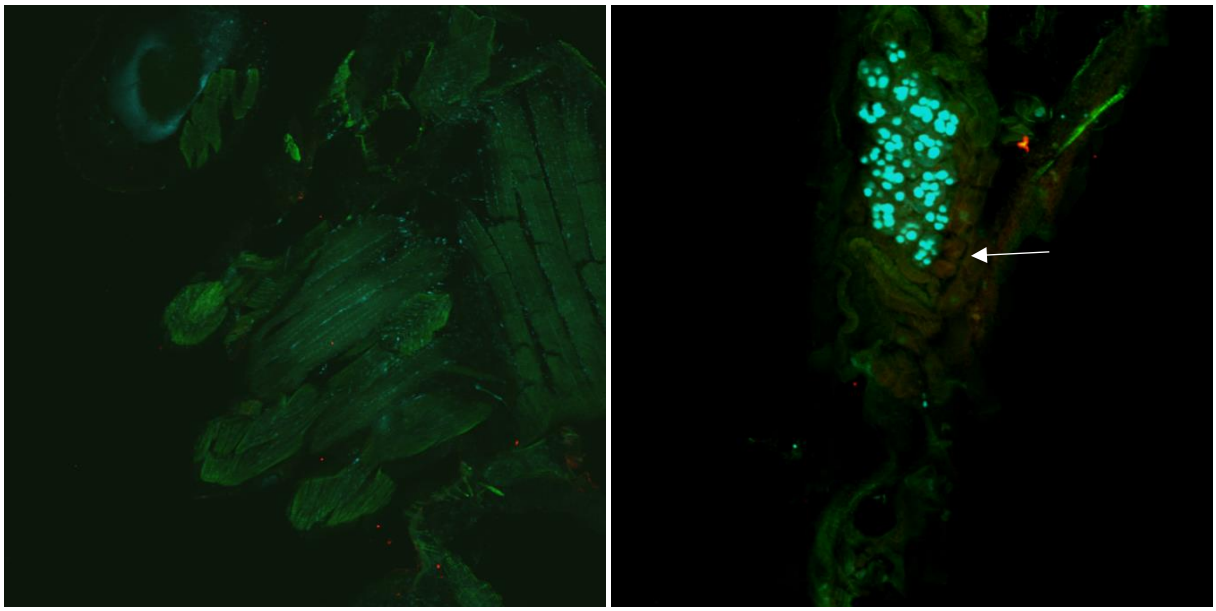


Figure 3-5. Day 6 P.I.-Head(A) and Abdomen(B) Regions (10X). Nuclei stained blue, cytoskeleton is green, and (A. Envelope antibody is red, B. anti-ZIKV NS1 antibody is red). Immunofluorescence staining showing replication in abdomen and no virus replication seen in head region. White arrows denote virus presence.

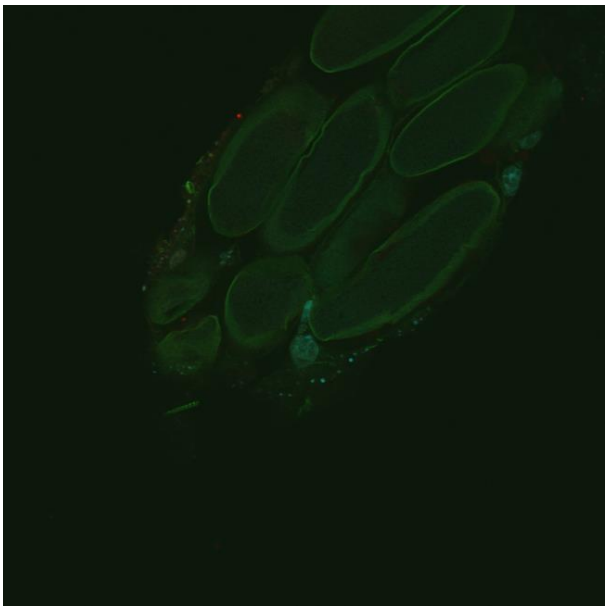


Figure 3-6. Day 12 P.I.-Abdomen Region (10X). Nuclei stained blue, cytoskeleton is green, and Envelope antibody is red. Immunohistochemical analysis shows no pronounced virus replication seen in abdomen.

(A) **(B)**

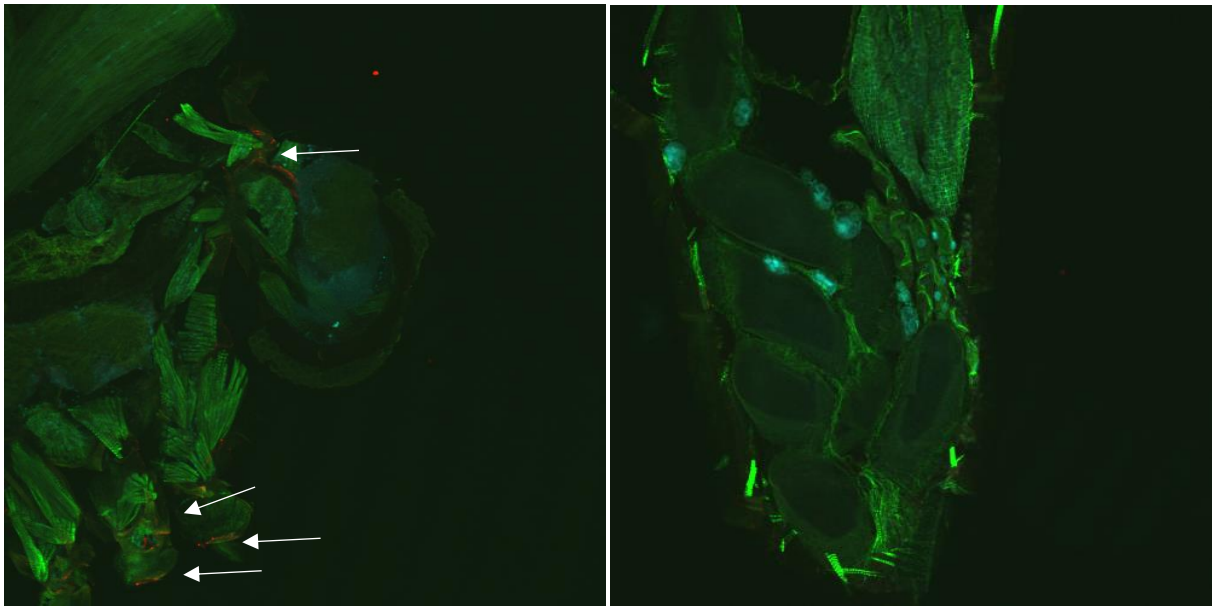


Figure 3-7. Day 14 P.I.-Head(A) and Abdomen(B) Regions (10X). Nuclei stained blue, cytoskeleton is green, and Envelope antibody is red. Immunofluorescence staining show virus dissemination in both the portion where the legs were attached, and the head region where the salivary glands are located. White arrows denote virus presence.

CHAPTER IV

CONCLUSION

The results and statistics emphasized there was a significant difference in the dissemination of Zika virus between the fluctuating and constant south Texas temperatures. These findings will help give a better understanding of the dissemination pattern for the area. Although more research needs to be conducted to have a complete understanding of the climatically unique area of south Texas. More specifically there needs to be further studies comparing the differences in dissemination of Zika virus in the south Texas and Florida regions with past autochthonous Zika transmission.

On the other hand, survivability did show a difference when comparing the constant and fluctuating temperatures. It showed there was a reduction in the infected mosquitoes survival for the constant temperature of almost half the days when compared to the fluctuating temperatures. This is essential because it shows how fluctuating temperatures were associated with differences in survivability which could be used in future studies for the south Texas area.

In the time series analysis, the dissemination pattern was found to be nearly what was expected this is critical because it allows a realistic view of when these events occur with time. This study opens a lot of doors for future experiments for expanding our understanding of what affects dissemination patterns and which tissues are affected to a greater extent. Doing this same analysis for geographically distinct mosquitoes will allow to draw more conclusions for the differences in transmission of different areas.

One caveat of the study conducted in chapter two (comparison of dissemination for fluctuating and constant temperatures) was the mosquito legs and bodies for the experiments were processed together. In future studies the bodies, legs and saliva should be processed separately when extracting RNA and doing RT-PCR in order to reduce the probability of potential cross contamination. In addition, gathering saliva samples from the mosquitoes from this experiment would have provided additional sources of information on the effect temperature has on transmission. The saliva for these experiments was only gathered in the initial stage and was stopped due to issues with getting the mosquitoes to salivate into the capillary tube. In future studies examining other methods to collect saliva from infected mosquitoes will be investigated.

Future studies could examine the interactions between flavivirus co-infections in mosquitoes using the methods discussed in chapter 3 with the addition of in situ hybridization analysis. In addition, we could also use this method to further analyze changes in virus penetration of the midgut barriers with fluctuating and constant temperature regimes

It was found *Ae. aegypti and Ae. albopictus* had differences in the transmission of Zika virus depending on the specific strain of the virus they were infected with (Ciota et. al, 2017). Examining if our findings for chapter 2 are preserved for other isolates or strains of Zika virus such as the Mexican isolate could allow the expansion of information which could be useful in constructing more accurate mathematical models.

REFERENCES

Abushouk, A. I., Negida, A., & Ahmed, H. (2016). An updated review of Zika virus. *Journal of Clinical Virology*, *84*, 53–58. https://doi.org/10.1016/j.jcv.2016.09.012

Alaniz, A. J. (2019). *Zika virus: A compendium of the state of knowledge*. *Encyclopedia of Environmental Health* (2nd ed.). Elsevier Inc. https://doi.org/10.1016/B978-0-12-409548-9.11019-X

Alto, B. W., & Bettinardi, D. (2013). Temperature and dengue virus infection in mosquitoes: Independent effects on the immature and adult stages. *American Journal of Tropical Medicine and Hygiene*, *88*(3), 497–505. https://doi.org/10.4269/ajtmh.12-0421

Alto, B. W., Reiskind, M. H., & Lounibos, L. P. (2008). Size alters susceptibility of vectors to dengue virus infection and dissemination. *American Journal of Tropical Medicine and Hygiene*. https://doi.org/10.4269/ajtmh.2008.79.688

Alto, B. W., Wiggins, K., Eastmond, B., Ortiz, S., Zirbel, K., & Lounibos, L. P. (2018). Diurnal Temperature Range and Chikungunya Virus Infection in Invasive Mosquito Vectors. *Journal of Medical Entomology*, *55*(1), 217–224. https://doi.org/10.1093/jme/tjx182

Atif, M., Azeem, M., & Rehan, M. (2016). Zika virus disease : a current review of the literature. *Infection*, *44*(6), 695–705. https://doi.org/10.1007/s15010-016-0935-6

Basarab, M., Bowman, C., Aarons, E. J., & Cropley, I. (2016). Zika virus. *BMJ (Online)*, *352*(February), 1–7. https://doi.org/10.1136/bmj.i1049

Beaver, J. T., Lelutiu, N., Habib, R., & Skountzou, I. (2018). Evolution of two major Zika virus lineages: Implications for pathology, immune response, and vaccine development. *Frontiers in Immunology*, *9*(JUL). https://doi.org/10.3389/fimmu.2018.01640

Black, W. C., Bennett, K. E., Gorrochótegui-Escalante, N., Barillas-Mury, C. V, Fernández-Salas, I., De, M., Farfán-Alé, J., Olson, K., Beaty, B. J. (2002). *Flavivirus Susceptibility in Aedes aegypti*. *Archives of Medical Research* (Vol. 33).

Brady, O. J., Johansson, M. A., Guerra, C. A., Bhatt, S., Golding, N., Pigott, D. M., Delatte, H., Grech, M.G., Leisnham, P.T., Maciel-de-Freitas, R., Styer, L.M., Smith, D.L., Scott, T.W., Gething, P.W., Hay, S. I. (2013). Modelling adult Aedes aegypti and Aedes albopictus survival at different temperatures in laboratory and field settings. *Parasites & Vectors*, *6*(1). doi:10.1186/1756-3305-6-351

Carrington, L. B., Armijos, M. V., Lambrechts, L., Barker, C. M., & Scott, T. W. (2013a). Effects of Fluctuating Daily Temperatures at Critical Thermal Extremes on Aedes aegypti Life-History Traits. *PLoS ONE*, *8*(3). https://doi.org/10.1371/journal.pone.0058824

Carrington, L. B., Armijos, M. V., Lambrechts, L., & Scott, T. W. (2013b). Fluctuations at a Low Mean Temperature Accelerate Dengue Virus Transmission by Aedes aegypti. *PLoS Neglected Tropical Diseases*, *7*(4). https://doi.org/10.1371/journal.pntd.0002190

Carrington, L. B., Seifert, S. N., Armijos, M. V., Lambrechts, L., & Scott, T. W. (2013c). Reduction of Aedes aegypti vector competence for dengue virus under large temperature fluctuations. *American Journal of Tropical Medicine and Hygiene*, *88*(4), 689–697. https://doi.org/10.4269/ajtmh.12-0488

Carrington, L. B., Seifert, S. N., Willits, N. H., Lambrechts, L., & Scott, T. W. (2013d). Large Diurnal Temperature Fluctuations Negatively Influence Aedes aegypti (Diptera: Culicidae) Life-History Traits . *Journal of Medical Entomology*, *50*(1), 43–51. https://doi.org/10.1603/me11242

Carrington, L. B., & Simmons, C. P. (2014). Human to mosquito transmission of dengue viruses. *Frontiers in Immunology*, *5*(JUN), 1–8. https://doi.org/10.3389/fimmu.2014.00290

CDC Newsroom. CDC Issues Zika Virus Guidance for Brownsville, Texas. (2016, December 14). Retrieved from https://www.cdc.gov/media/releases/2016/s1214-brownsville-texas-zika-guidance.html

Chahad-Ehlers, S., Gentile, C., Lima, J. B. P., Peixoto, A. A., & Bruno, R. V. (2013). Analysis of cycle Gene Expression in Aedes aegypti Brains by In Situ Hybridization. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0052559

Champion, S. R., & Vitek, C. J. (2014). Aedes aegypti and Aedes albopictus Habitat Preferences in South Texas, USA. *Environmental Health Insights*, *8s2*, EHI.S16004. https://doi.org/10.4137/ehi.s16004

Chang, L. H., Hsu, E. L., Teng, H. J., & Ho, C. M. (2007). Differential survival of Aedes aegypti and Aedes albopictus (Diptera: Culicidae) larvae exposed to low temperatures in Taiwan. *Journal of medical entomology*, *44*(2), 205-210.

Christofferson, R. C., & Mores, C. N. (2016). Potential for Extrinsic Incubation Temperature to Alter Interplay between Transmission Potential and Mortality of Dengue-Infected Aedes aegypti. *Environmental Health Insights*, *10*, 119–123. https://doi.org/10.4137/EHI.S38345

Ciota, A. T., Bialosuknia, S. M., Ehrbar, D. J., & Kramer, L. D. (2017). Vertical transmission of zika virus by Aedes aegypti and Ae. Albopictus mosquitoes. *Emerging Infectious Diseases*, *23*(5), 880–882. https://doi.org/10.3201/eid2305.162041

Colón-González, F. J., Peres, C. A., Steiner São Bernardo, C., Hunter, P. R., & Lake, I. R. (2017). After the epidemic: Zika virus projections for Latin America and the Caribbean. *PLoS Neglected Tropical Diseases*, *11*(11), 1–19. https://doi.org/10.1371/journal.pntd.0006007

Cui, Y., Grant, D. G., Lin, J., Yu, X., & Franz, A. W. E. (2019). Zika virus dissemination from the midgut of aedes aegypti is facilitated by bloodmeal-mediated structural modification of the midgut basal lamina. *Viruses*. https://doi.org/10.3390/v11111056

Danforth, M. E., Reisen, W. K., & Barker, C. M. (2016). The impact of cycling temperature on the transmission of West Nile virus. *Journal of Medical Entomology*, *53*(3), 681–686.

https://doi.org/10.1093/jme/tjw013

Diaz, J. H. (2016). Preparing the United States for Zika Virus: Pre-emptive Vector Control and Personal Protection. *Wilderness and Environmental Medicine*, *27*(4), 450–457. https://doi.org/10.1016/j.wem.2016.07.006

Dinglasan, R. R., Kalume, D. E., Kanzok, S. M., Ghosh, A. K., Muratova, O., Pandey, A., & Jacobs-Lorena, M. (2007). Disruption of Plasmodium falciparum development by antibodies against a conserved mosquito midgut antigen. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.0702239104

Eisen, L., & Moore, C. G. (2013). Aedes (Stegomyia) aegypti in the Continental United States: A Vector at the Cool Margin of Its Geographic Range . *Journal of Medical Entomology*, *50*(3), 467–478. https://doi.org/10.1603/me12245

Epelboin, Y., Talaga, S., & Dusfour, I. (2017). Zika virus: An updated review of competent or naturally infected mosquitoes. *PLoS Neglected Tropical Diseases*, *11*(11), e0005933. https://doi.org/http://dx.doi.org/10.1371/journal.pntd.0005933

Fontaine, A., Jiolle, D., Moltini-Conclois, I., Lequime, S., & Lambrechts, L. (2016). Excretion of dengue virus RNA by Aedes aegypti allows non-destructive monitoring of viral dissemination in individual mosquitoes. *Scientific Reports*, *6*(February), 1–10. https://doi.org/10.1038/srep24885

Ganguly, S., Para, P. A., & Rajasthan, S. (2016). An overview on the latest pandemic outbreak of Zika virus in 2016 and its implications to human health An Overview on the Latest Pandemic Outbreak of Zika Virus in 2016 and its Implications to Human Health, (February), 7–9.

Gloria-Soria, A., Armstrong, P. M., Turner, P. E., & Turner, P. E. (2017). Infection rate of aedes aegypti mosquitoes with dengue virus depends on the interaction between temperature and mosquito genotype. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1864). https://doi.org/10.1098/rspb.2017.1506

Gorshkov, K., Shiryaev, S. A., Fertel, S., Lin, Y. W., Huang, C. T., Pinto, A., Farhy, C Strongin, A., Zheng, W., Terskikh, A. V. (2019). Zika virus: Origins, pathological action, and treatment strategies. *Frontiers in Microbiology*, *10*(JAN), 1–17. https://doi.org/10.3389/fmicb.2018.03252

Hernández-Ávila, J. E., Palacio-Mejía, L. S., López-Gatell, H., Alpuche-Aranda, C. M., Molina-Vélez, D., González-González, L., & Hernández-Ávila, M. (2018). Zika virus infection estimates, Mexico. *Bulletin of the World Health Organization*, *96*(5), 306–313. https://doi.org/10.2471/BLT.17.201004

Hills, S. L., Fischer, M., & Petersen, L. R. (2017). Epidemiology of Zika Virus Infection. *Journal of Infectious Diseases*, *216*, S868–S874. https://doi.org/10.1093/infdis/jix434

Huber, J. H., Childs, M. L., Caldwell, J. M., & Mordecai, E. A. (2018). Seasonal temperature variation influences climate suitability for dengue, chikungunya, and Zika transmission. *PLoS Neglected Tropical Diseases*, *12*(5). https://doi.org/10.1371/journal.pntd.0006451

Jamali Moghadam, S. R., Bayrami, S., Jamali Moghadam, S., Golrokhi, R., Golsoorat Pahlaviani, F., & SeyedAlinaghi, S. A. (2016). Zika virus: A review of literature. *Asian Pacific Journal of Tropical Biomedicine*, *6*(12), 989–994. https://doi.org/10.1016/j.apjtb.2016.09.007

Jansen, C. C., & Beebe, N. W. (2010). The dengue vector Aedes aegypti: what comes next. *Microbes and Infection*, *12*(4), 272–279. https://doi.org/10.1016/j.micinf.2009.12.011

Lambrechts, L., Paaijmans, K. P., Fansiri, T., Carrington, L. B., Kramer, L. D., Thomas, M. B., & Scott, T. W. (2011). Impact of daily temperature fluctuations on dengue virus transmission by Aedes aegypti. *Proceedings of the National Academy of Sciences*, *108*(18), 7460–7465. https://doi.org/10.1073/pnas.1101377108

Lim, E. X. Y., Lee, W. S., Madzokere, E. T., & Herrero, L. J. (2018). Mosquitoes as suitable vectors for alphaviruses. *Viruses*, *10*(2), 1–17. https://doi.org/10.3390/v10020084

Marinho, R. A., Beserra, E. B., Bezerra-gusmão, M. A., Porto, V. D. S., Olinda, R. A., & Santos, C. A. C. (2015). <2016 - Effects of temperature on the life cycle, expansion, and dispersion of Aedes aegypti (Diptera Culic.pdf>, *41*(1), 1–10.

Mercado-Curiel, R. F., Black IV, W. C., & Mũoz, M. D. L. (2008). A dengue receptor as possible genetic marker of vector competence in Aedes aegypti. *BMC Microbiology*, *8*(6), 1–15. https://doi.org/10.1186/1471-2180-8-118

Mordecai, E. A., Cohen, J. M., Evans, M. V., Gudapati, P., Johnson, L. R., Lippi, C. A., Miazgowicz, K.,Murdock, C., Rohr, J., Ryan, S., Savage, V., Shocket, M., Stewart Ibarra, A., Thomas, M., Weikel, D. P. (2017). Detecting the impact of temperature on transmission of Zika, dengue, and chikungunya using mechanistic models. *PLoS Neglected Tropical Diseases*, *11*(4), 1–18. https://doi.org/10.1371/journal.pntd.0005568

Muktar, Y., Tamerat, N., & Shewafera, A. (2016). Aedes aegypti as a Vector of Flavivirus.*Journal of Tropical Diseases*, *04*(05). https://doi.org/10.4172/2329-891x.1000223

Murdock, A. C. C., Paaijmans, K. P., Bell, A. S., King, J. G., Julián, F., Read, A. F., Thomas, M., Mur, C., Paaijmans, K., Bell, A., King, J.,Hillyer, J., Read, A., Thomas, M. B. (2012). Complex effects of temperature on mosquito immune function Linked references are available on JSTOR for this article : THE o ROYAL immune function, *279*(1741), 3357–3366.

Musso, D., Ko, A. I., & Baud, D. (2019). Zika virus infection — After the pandemic. *New England Journal of Medicine*, *381*(15), 1444–1457. https://doi.org/10.1056/NEJMra1808246

Onyango, M. G., Bialosuknia, S. M., Payne, A. F., Mathias, N., Kuo, L., Vigneron, A., De Gennaro, M., Ciota, A., Kramer, L. D. (2020). Increased temperatures reduce the vectorial capacity of Aedes mosquitoes for Zika virus. *Emerging Microbes and Infections*, *9*(1), 67–77. https://doi.org/10.1080/22221751.2019.1707125

Paixão, E. S., Teixeira, M. G., & Rodrigues, L. C. (2018). Zika, chikungunya and dengue: The causes and threats of new and reemerging arboviral diseases. *BMJ Global Health*, *3*, 1–6. https://doi.org/10.1136/bmjgh-2017-000530

Powell, J. R., & Tabachnick, W. J. (2013). History of domestication and spread of Aedes aegypti--a review. *Memórias Do Instituto Oswaldo Cruz*, *108*(October), 11–17. https://doi.org/10.1590/0074-0276130395

Rabaan, A. A., Bazzi, A. M., Al-Ahmed, S. H., Al-Ghaith, M. H., & Al-Tawfiq, J. A. (2017). Overview of Zika infection, epidemiology, transmission and control measures. *Journal of Infection and Public Health*, *10*(2), 141–149. https://doi.org/10.1016/j.jiph.2016.05.007

Ramos, M. M., Mohammed, H., Zielinski-Gutierrez, E., Hayden, M. H., Lopez, J. L. R., Fournier, M., Trujillo, A., Burton, R., Brunkard, J., Anaya-Lopez, L., Banicki, A., Morales, P., Smith, B., Muñoz, J., Waterman, S., Moya-Rabelly, C., Álvarez-Lucas, C., Mancha, C., Frausto, L., Hernandez, E., Reyes, N., Fuentes, V., Contreras, O., Ramirez, J., Beatty, M., Moreno-Sanchez, R., Sosa, I., Escobedo, M. (2008). Epidemic dengue and dengue hemorrhagic fever at the Texas-Mexico border: Results of a household-based seroepidemiologic survey, December 2005. *American Journal of Tropical Medicine and Hygiene*, *78*(3), 364–369. https://doi.org/10.4269/ajtmh.2008.78.364

Reinhold, J. M., Lazzari, C. R., & Lahondère, C. (2018). Effects of the environmental temperature on Aedes aegypti and Aedes albopictus mosquitoes: A review. *Insects*, *9*(4). https://doi.org/10.3390/insects9040158

Reiter, P. (2001). Climate change and mosquito-borne disease. *Environmental Health Perspectives*, *109*(SUPPL. 1), 141–161. https://doi.org/10.2307/3434853

Rohani, A., Wong, Y. C., Zamre, I., Lee, H. L., & Zurainee, M. N. (2009). The effect of extrinsic incubation temperature on development of dengue serotype 2 and 4 viruses in Aedes aegypti (L.). *Southeast Asian Journal of Tropical Medicine and Public Health*, *40*(5), 942–950.

Rossi, P., Ricci, I., Cappelli, A., Damiani, C., Ulissi, U., Mancini, M. V., Valzano, M., Capone, A., Epis, S., Crotti, E., Chouaia, B., Scuppa, P., Joshi, D., Xi, Z., Mandrioli, M., Sacchi, L., O'Neill, S., Favia, G. (2015). Mutual exclusion of Asaia and Wolbachia in the reproductive organs of mosquito vectors. *Parasites and Vectors*. https://doi.org/10.1186/s13071-015-0888-0

Samuel, G. H., Adelman, Z. N., & Myles, K. M. (2016). Temperature-dependent effects on the replication and transmission of arthropod-borne viruses in their insect hosts. *Current Opinion in Insect Science*. https://doi.org/10.1016/j.cois.2016.06.005

Simonin, Y., van Riel, D., Van de Perre, P., Rockx, B., & Salinas, S. (2017). Differential virulence between Asian and African lineages of Zika virus. *PLoS Neglected Tropical Diseases*, *11*(9), 1–8. https://doi.org/10.1371/journal.pntd.0005821

Tesla, B., Demakovsky, L. R., Mordecai, E. A., Ryan, S. J., Bonds, M. H., Ngonghala, C. N. Brindley, M., Murdock, C. C. (2018). Temperature drives Zika virus transmission: Evidence from empirical and mathematical models. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1884). https://doi.org/10.1098/rspb.2018.0795

Theel, E. S., & Jane Hata, D. (2018). Diagnostic testing for Zika virus: A postoutbreak update. *Journal of Clinical Microbiology*, *56*(4), 1–12. https://doi.org/10.1128/JCM.01972-17

U.S. Climate Data McAllen, TX. (n.d.). Retrieved 2017, from https://www.usclimatedata.com/climate/mcallen/texas/united-states/ustx0854

Vasilakis, N., & Weaver, S. C. (2017, February 1). Flavivirus transmission focusing on Zika. *Current Opinion in Virology*. Elsevier B.V. https://doi.org/10.1016/j.coviro.2016.11.007

Wang, A., Thurmond, S., Islas, L., Hui, K., & Hai, R. (2017). Zika virus genome biology and molecular pathogenesis. *Emerging Microbes and Infections*. https://doi.org/10.1038/emi.2016.141

Walker, W. L., Lindsey, N. P., Lehman, J. A., Krow-Lucal, E. R., Rabe, I. B., Hills, S. L., ... & Staples, J. E. (2016). Zika virus disease cases—50 states and the District of Columbia, January 1–July 31, 2016. *Morbidity and Mortality Weekly Report*, *65*(36), 983-986.

Watts, D. M., Burke, D. S., Harrison, B. A., Whitmire, R. E., Nisalak, A. (1987). Effect of temperature on the vector efficiency of Aedes aegypti for dengue 2 virus. *American Journal of Tropical Medicine and Hygiene*, *3*(1), 1–5. https://doi.org/10.1139/b89-069

Xiao, F. Z., Zhang, Y., Deng, Y. Q., He, S., Xie, H. G., Zhou, X. N., & Yan, Y. S. (2014). The effect of temperature on the extrinsic incubation period and infection rate of dengue virus serotype 2 infection in Aedes albopictus. *Archives of Virology*, *159*(11), 3053–3057. https://doi.org/10.1007/s00705-014-2051-1

Yang, H. M., Macoris, M. L. G., Galavani, K. C., Andrighetti, M. T. M., & Wanderley, D. M. V. (2009). Assessing the effects of temperature on the population of Aedes aegypti, the vector of dengue. *Epidemiology and Infection*, *137*(08), 1188. https://doi.org/10.1017/S0950268809002040

Ziegler, S. A., Nuckols, J., McGee, C. E., Huang, Y. J. S., Vanlandingham, D. L., Tesh, R. B., & Higgs, S. (2011). In vivo imaging of Chikungunya virus in mice and Aedes mosquitoes using a Renilla luciferase clone. *Vector-Borne and Zoonotic Diseases*, *11*(11), 1471–1477. https://doi.org/10.1089/vbz.2011.0648

BIOGRAPHICAL SKETCH

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