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An investigation of Zika virus-associated microcephaly in Northeastern Brazil

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AN INVESTIGATION OF ZIKA VIRUS-ASSOCIATED MICROCEPHALY IN NORTHEASTERN BRAZIL

by

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ABSTRACT

Zika virus (ZIKV) had remained a relatively obscure flavivirus until an unexpected series of epidemics that began in Micronesia brought the virus into the forefront of global public health consciousness. Unlike its closely related flaviviruses, such as dengue, West Nile, and Japanese encephalitis viruses, that cause severe diseases, ZIKV causes asymptomatic or mild febrile infections that are dengue-like in infected individuals. However, ZIKV has exhibited teratogenic effects and infection of pregnant women can lead to microcephaly, a neurodevelopmental disorder in newborns. The teratogenic effects of ZIKV was most clearly highlighted during the epidemic in Brazil, due to the alarming 20-fold increase in microcephaly incidence experienced by the northeastern states where the first outbreak of the Western Hemisphere began, and quickly brought this virus into the spotlight. Although public health officials predicted that other Latin American countries would also experience an increase in microcephaly numbers of comparable scale, such an increase was not observed. This thesis seeks to understand the mechanisms underlying what appears to be a relatively out-of-proportion increase in microcephaly numbers in Brazil compared to other ZIKV-affected countries. The role that differences in vector survival and control, women’s behavior, interactions between
ZIKV and dengue virus, and timing of the outbreak may have played in causing the different magnitudes of microcephaly in Brazil, Colombia, and the U.S., in particular, is discussed. Understanding the factors that may have caused the abnormally large outbreak of ZIKV and microcephaly in Brazil versus other regions may assist countries that have not yet been affected by ZIKV develop effective programs to prevent future outbreaks and lessen the impact of a potential outbreak on microcephaly numbers. Experiments to elucidate the mechanism by which ZIKV infects the fetus to cause central nervous system damage will provide an understanding to develop safe and effective vaccines that may assist in efforts to prevent future rise in ZIKV-associated microcephaly cases.
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LIST OF ABBREVIATIONS

CDC .......................................................... Centers for Disease Control and Prevention
CNS ............................................................... Centers nervous system
CHIKV ............................................................ Chikungunya virus
cryo-EM ....................................................... cryo-electron microscopy
DENV .......................................................... Dengue virus
DENV-1 .......................................................... Dengue virus-1
PAHO ............................................................ Pan American Health Organization
RT-PCR ......................................................... Reverse transcription polymerase chain reaction
WHO ............................................................. World Health Organization
WNV ............................................................. West Nile virus
ZIKV ............................................................. Zika virus
INTRODUCTION

Zika virus (ZIKV) is a mosquito-transmitted flavivirus of the Flaviviridae family and is related to yellow fever, dengue, West Nile, and Japanese encephalitis viruses. For nearly half a century, ZIKV was only known to cause mild, sporadic human infections in Africa and Asia since the virus was first isolated in 1947 in Uganda. It was not until recently that ZIKV has become associated with more severe diseases in humans, such as microcephaly in newborns and Guillain-Barré syndrome in adults. The recent ZIKV outbreaks reported throughout the Americas and the Caribbean follow the emergence of other flaviviruses in these regions, including dengue, West Nile, and chikungunya.

The first part of this thesis will investigate the historical background of ZIKV and consider the global path that the virus has taken since its emergence in 2007 in Yap Island. The role of its primary vector, Aedes aegypti, in facilitating its global spread will also be discussed. Exploring the means by which ZIKV has been able to spread both rapidly and globally will provide key information about how to effectively prevent or control further spread of this and other similarly transmitted viruses.

Next, this thesis will describe the various methods by which ZIKV infection of humans can occur, including vector-borne transmission, blood-borne transmission, sexual transmission, and maternal-fetal transmission. This section will investigate the course of ZIKV pathogenesis and the molecular mechanisms of infection that have been proposed
thus far. Additionally, the mechanism through which ZIKV might reach the fetus to cause central nervous system damage in pregnant women will be discussed.

In the last chapter, this thesis will discuss the high number of microcephaly cases reported in the Americas in association with the recent ZIKV outbreaks. In particular, this section will investigate several factors related with ZIKV transmission and reporting that may account for the disproportionally large increase in the number of microcephaly cases in the northeastern region of Brazil. Factors including conditions for vector survival, vector control response, behavior of women, and previous exposure of different populations to dengue, will be discussed.
SPECIFIC AIMS

Specific aims of the following thesis include:

1. Comprehensive review of ZIKV literature to provide up-to-date information regarding ZIKV background and association with microcephaly.

2. Investigation of evidence for greater susceptibility of the population in Brazil, in particular the northeastern region, to central nervous system damage of fetuses by ZIKV through comparison with Colombia and the U.S.

3. Elucidate how the various factors that affect ZIKV transmission may have caused the disproportionately large microcephaly outbreak observed in Brazil.
HISTORY OF ZIKV

The Zika virus (ZIKV) was first isolated from a febrile sentinel monkey in the Zika forest in Entebbe, Uganda in April 1947 (Dick, 1952; Dick, Kitchen, & Haddow, 1952). The virus was isolated a second time from Aedes africanus mosquitoes in January 1948 from the same forest (Dick et al., 1952; A. J. Haddow, Williams, Woodall, Simpson, & Goma, 1964). These initial ZIKV studies found that primates act as the primary host for ZIKV; serum collected from small mammals residing in both the forest ground and trees of the Zika forest did not contain antibodies to ZIKV (A. J. Haddow et al., 1964).

In the ensuing decades, ZIKV was sporadically isolated from patients in Africa and Southeast Asia. In a study of arthropod-borne viral infections conducted between 1964 to 1970 in Nigeria, ZIKV was isolated from individuals of all age groups (D. Moore et al., 1975). Another virus isolation study carried out in 1975 isolated the yellow virus, dengue virus-1 (DENV-1), and ZIKV from blood samples taken from the Igbo-Ora community in southwest Nigeria (A. Fagbami, 1977). Seroepidemiological investigation of Oyo State in Nigeria, in which Igbo-Ora is located, revealed that ZIKV immunity was prevalent in Nigeria as nearly half of tested persons had neutralizing antibodies to ZIKV (A. H. Fagbami, 1979). This study also showed that many individuals with ZIKV-immunity had neutralizing antibodies to other flaviviruses as well, including DENV-1 (81%), yellow fever (55%), and West Nile (6%) (A. H. Fagbami, 1979)\(^1\).

\(^1\) Each percentage in parentheses indicates the percentage of ZIKV antisera samples that contained antibody to that particular flavivirus.
ZIKV AS AN EMERGENT PATHOGEN

Due to the mild nature of ZIKV infection, it was not until 2007 when an outbreak was reported in Yap Island (Micronesia), that ZIKV began to receive global attention (Duffy et al., 2009; Hayes, 2009). At this time, about 75% of the population of 7,391 residents was reported to have been infected during a short four-month period (Duffy et al., 2009; Weaver et al., 2016). The most commonly reported symptoms in that outbreak included rash, fever, arthralgia, and conjunctivis; no deaths or hospitalizations were reported (Duffy et al., 2009). The Yap Island outbreak was the first demonstration of ZIKV transmission outside Africa and Southeast Asia, and it is surmised that ZIKV was introduced to Micronesia by an infected mosquito or viremic person, especially considering that travel between Southeast Asia and Yap Island is not uncommon (Duffy et al., 2009). This hypothesis is further supported by the fact that no other nonhuman vertebrates and birds were found to be reservoirs and no nonhuman primates live in Yap Island (Duffy et al., 2009). Aedes hensilli was identified retrospectively as the primary vector during the 2007 epidemic (Duffy et al., 2009).

Although ZIKV infection was reported in 2010 in a 3-year-old Cambodian boy, the next regional outbreak did not occur until 2013 in French Polynesia (Cao-Lormeau et al., 2014; Heang et al., 2012). The increased number of patients, recorded by the French Polynesia Department of Health, who presented in clinics with rash and mild dengue-like symptoms led researchers to test samples from different archipelagoes for ZIKV; these samples tested positive for the virus by reverse transcription-polymerase chain reaction (RT-PCR) (Cao-Lormeau et al., 2014). Officials estimated that approximately 19,000
persons were infected with ZIKV in this epidemic; no infection related death was reported (Cao-Lormeau et al., 2014). This was the largest and first known outbreak caused by an arbovirus other than dengue virus (DENV) in these islands. Sequencing studies revealed that the ZIKV responsible for the French Polynesian outbreak was similar to the strains isolated from Cambodia and Yap Islands (Faye et al., 2014; A. D. Haddow et al., 2012). Phylogenetic analyses of these and previously isolated ZIKV strains showed that ZIKV strains can be categorized into either African or Asian lineage based on nucleotide and amino acid sequence variation, and that the outbreaks thus far have been due to ZIKV strains of the Asian lineage (A. D. Haddow et al., 2012).

Entomological studies proposed that *Aedes aegypti* and possibly *Aedes polynesiensis* were responsible for this outbreak since both are prevalent throughout these islands (Cao-Lormeau et al., 2014; Horwood et al., 2013).

During the year of 2014, ZIKV spread throughout Oceania with cases reported in New Caledonia, Cook Island, and Easter Island (Foy et al., 2011; Ioos et al., 2014; Tognarelli et al., 2016). ZIKV was likely transmitted to the Pacific islands when viremic travelers from French Polynesia were bitten by mosquitoes on the islands (Didier Musso, 2015). New Caldeonia reported autochthonous cases starting in January 2014 and patients presenting with ZIKV infection imported from French Polynesia were also reported in Japan, Norway, and continental France at this time (Ioos et al., 2014; Kutsuna et al., 2014). The general westward trend of the ZIKV spread is thought to have been facilitated primarily by *Aedes aegypti* and *Aedes albopictus* as both vectors are prevalent throughout
most of these areas (Horwood et al., 2013). Figure 1 shows the distribution of *Aedes* vectors in the Pacific Ocean.

**Figure 1. Distribution of *Aedes* vectors.** Figure reproduced from Horwood et al., 2016.

**ENTRY OF ZIKV INTO THE AMERICAS**

ZIKV was first detected in the Western Hemisphere in early 2015 when sera from patients presenting with “dengue-like symptoms” in the northeastern state of Rio Grande de Norte, Brazil tested positive for ZIKV by RT-PCR (Zanluca et al., 2015). An abundance of the *Aedes aegypti* vector in Brazil allowed the virus to spread rapidly throughout the region and researchers in Bahia pointed to ZIKV to explain the increased number of patients who presented in clinic with rash, fever, and arthralgia (Campos, Bandeira, & Sardi, 2015). By December 2015, Brazil’s Ministry of Health estimated 440,000 to 1,300,000 suspected ZIKV cases in the country (Zanluca et al., 2015).
Phylogenetic analysis using the ZIKV envelope gene sequence revealed that the ZIKV isolated from Salvador and Natal, two large cities in northeastern Brazil, were nearly identical to the French Polynesian isolate (Baronti et al., 2014; Campos et al., 2015). Currently, there are two main hypotheses that attempt to explain the route of ZIKV entry into Brazil. One hypothesis is that the virus entered the country during the World Cup soccer games that had been held between June and July of 2014 in Brazil (Zanluca et al., 2015). However, none of the countries that participated in these games had reports of ZIKV infection at that time. Thus, a second hypothesis proposes that ZIKV entered Brazil in August 2014 during the World Sprint Championships, a canoeing race, that had been held in Rio de Janeiro; athletes from the Pacific islands in which ZIKV had been reported in 2014 participated in this tournament (Didier Musso, 2015). However, the state of Rio de Janeiro where the race took place is in southern Brazil and the Brazilian outbreak began in the northeastern states. Additionally, a recent study employing phylogenetic and molecular clock analyses has proposed that ZIKV may, in fact, have been introduced into the Americas sometime between May and December of 2013, which is nearly a year before ZIKV was first detected in Brazil (Faria et al., 2016). Thus, the first location and route by which ZIKV entered the Western Hemisphere is still unclear.

Although the Brazilian outbreak was initially concentrated in the northeastern states, the virus spread quickly throughout the rest of the continent (Figure 2). Samples collected from patients during October and November of 2015 from Sincelejo, Colombia, located along the north coast, tested positive for ZIKV infection (Camacho, Paternina-
Gomez, Blanco, Osorio, & Aliota, 2016). At this time, researchers hypothesized that the virus entered the country from Brazil due to tourist activities along Colombia’s northern coast (Camacho et al., 2016). However, a recent study has reported that ZIKV was first isolated in the Caribbean in 2014, around the same time that the virus was introduced to Easter Island (Lednicky et al., 2016). This report, in conjunction with the fact that ZIKV was first isolated from the northern tip of Colombia, suggests that the strain that caused the Colombian outbreak originated from the Caribbean rather than Brazil (Lahon et al., 2016; Lednicky et al., 2016). These findings imply that ZIKV was circulating in the Western Hemisphere before the 2015 outbreak in Brazil and demonstrate the challenge of accurately pinpointing ZIKV disease due to shared symptoms with DENV.

Figure 2. Countries with ZIKV transmission as of December 2015. For countries shaded in link pink (serosurvey data only), ZIKV transmission was assumed based on detection of antibodies in healthy individuals. (Figure reproduced and adapted from Fauci et al., 2016)

The first case of ZIKV disease in Puerto Rico was reported in November 2015 and the territory reported its first locally acquired ZIKV case in late December of 2015
Patients most commonly cited symptoms of rash, myalgia, arthralgia, and fever (Thomas et al., 2016). Over 90% of the laboratory-confirmed cases from this report were from the San Juan metropolitan area of Puerto Rico (Thomas et al., 2016). As Aedes aegypti is prevalent throughout Puerto Rico, the virus easily spread across the island. By October 2016, the Puerto Rico Department of Health reported approximately 30,000 confirmed ZIKV cases (Lozier et al., 2016). A notable feature of this report was that unlike in previous arbovirus epidemics, ZIKV disease incidence was higher in women aged ≥20 years; for both the DENV and chikungunya virus (CHIKV) outbreaks of 2010 and 2014, respectively, cases were equally distributed among the sexes in Puerto Rico (Lozier et al., 2016; Sharp et al., 2013, 2014). Researchers have postulated that the higher disease incidence in women may be due to greater exposure to Aedes mosquitoes in the home, differences in health care-seeking behavior, and more effective male-to-female sexual transmission (Coelho et al., 2016; Lozier et al., 2016; Santos et al., 2016). Different rates of infection or susceptibility to disease between men and women may also account for the skewed distribution, but further research is required in order to confirm these possibilities.

As of January 2016, 26 countries in the Americas reported autochthonous transmission of ZIKV: Barbados, Bolivia, Brazil, Colombia, Curacao, Guadeloupe, Guatemala, Guyana, Haiti, Honduras, Martinique, Mexico, Nicaragua, Panama, Paraguay, Puerto Rico, Saint Martin Suriname, Venezuela, and Virgin Island (Petersen et al., 2016). The U.S. reported its first travel-associated ZIKV case in 2007 and from then through 2014, the Centers for Disease Control and Prevention (CDC) reported that 14
travelers who returned to the U.S. from Latin American countries tested positive for ZIKV (Centers for Disease Control and Prevention, 2016). During June to August of 2016, the U.S. reported its first case of local mosquito-borne transmission of ZIKV in the Miami-Dade and Broward counties of Florida; 4 persons were reported to have acquired the infection through local vector transmission and further investigations revealed that 29 persons from the surrounding neighborhoods were positive for ZIKV (Likos et al., 2016). Aggressive mosquito control efforts contributed to limiting the outbreak to a small geographic area and to ending this outbreak (Likos et al., 2016). As of March 2017, over 5,000 ZIKV disease cases have been reported to the CDC and although nearly 95% of these cases are attributable to travelers returning from ZIKV-endemic areas, 221 cases have been attributed to local mosquito-borne transmission in Florida and Texas (Centers for Disease Control and Prevention, 2017a). The *Aedes aegypti* and *Aedes albopictus* vectors are widely distributed throughout the southern U.S. and likely facilitated the recent outbreaks in Florida and Texas (Figure 3) (Diaz, 2016). Since the geographical distribution ranges of both vectors have continued to expand throughout the U.S. as well as other parts of the globe, developing effective methods of vector control may be an important way to prevent future ZIKV outbreaks. The details of vector transmission are discussed in the following chapter.
Figure 3. Geographical distribution ranges of *Aedes aegypti* and *Aedes albopictus* in the U.S. *Aedes aegypti* is distributed primarily throughout the southern tier of the U.S. and is the predominant vector of ZIKV in Latin America and the Caribbean. *Aedes albopictus* has a significantly broader distribution range in the continental U.S. and is also distributed throughout the Hawaiian Islands. (Figure reproduced and legend adapted from Diaz, 2016)
MOLECULAR BIOLOGY OF THE ZIKA VIRUS

VIROLOGY

Zika virus (ZIKV) is an enveloped, positive-sense, single-stranded RNA virus belonging to the Flavivirus genus in the family of Flaviviridae. The genome size is 10,794 nucleotides, which encode 3,419 amino acids, and is flanked by 5’ and 3’ untranslated regions. The genome also contains an open reading frame that encodes the capsid (C), precursor of membrane (prM), envelope (E), and seven non-structural (NS) proteins (Faye et al., 2014; Kuno & Chang, 2007). For flaviviruses, the prM and E proteins form heterodimers in the endoplasmic reticulum (ER) of the infected cell that then drive the budding of immature virions into the ER lumen (Barba-Spaeth et al., 2016). As the particle matures, prM is cleaved; cleavage of prM is necessary for particles to become infectious. The mature ZIKV particle has 90 dimerized E proteins on the surface in an icosahedral pattern (Figure 4) (R. J. Kuhn et al., 2002; Zhang et al., 2013).

Three-dimensional cryo-electron microscopy (cryo-EM) structures of mature ZIKV particles have shown that the organization of ZIKV is essentially the same as dengue virus (DENV) and West Nile virus (WNV) (Mukhopadhyay, Kim, Chipman, Rossmann, & Kuhn, 2003; Zhang et al., 2013). In addition, ZIKV strains have been found to contain either one or no N-linked glycosylation site in the E protein (Faye et al., 2014). The reverse transcription-polymerase chain reaction (RT-PCR) tool being used during the
current outbreak to diagnose ZIKV infection uses primers that target the E gene or one of the NS genes (Duffy et al., 2009; Faye et al., 2013; Lanciotti et al., 2008).

![Schematic representation of the ZIKV structure](image)

**Figure 4. Schematic representation of the ZIKV structure.** ZIKV is an enveloped, single-stranded RNA virus that is about 50 nanometers in diameter. Mature ZIKV particles have 90 E dimers organized with icosahedral symmetry in a herringbone pattern. (Figure reproduced from Viral Zone)

**MODES OF TRANSMISSION**

*Vector-borne Transmission*

ZIKV has been isolated from several species of mosquitoes belonging to the genus *Aedes*, but recently reported outbreaks have occurred primarily due to transmission by *Aedes aegypti* mosquitoes (Hayes, 2009; Kraemer et al., 2015). The widespread *Aedes aegypti* species is considered the preferred ZIKV vector worldwide and is also often described as ‘highly domesticated’ because of its urban abundance and anthropophilic nature (Diaz, 2016; Powell & Tabachnick, 2013). Studies hypothesize that this species was introduced into the New World from Africa with the onset of the slave trade (Brown et al., 2014). Introduction of the species to other continents is attributed to international trade and its
endophilic behavior (Hofhuis et al., 2009; Nawrocki & Hawley, 1987; Tatem, Hay, & Rogers, 2006). Today, the *Aedes aegypti* species can be found in many large Asian cities and the majority of the Americas; in the U.S., this species is distributed coast to coast throughout the southern tier and has been noted in 26 states (Diaz, 2016; Kraemer et al., 2015). *Aedes aegypti* mosquitoes prefer to feed during daylight hours and feed almost exclusively on humans (Scott & Takken, 2012).

The *Aedes albopictus* species, otherwise known as the Asian tiger mosquito, is considered a secondary ZIKV vector. Like the *Aedes aegypti* species, *Aedes albopictus* is capable of transmitting other flaviviruses, including DENV and chikungunya viruses (CHIKV), and recently surpassed the former species as a preferred vector for both of these two flaviviruses (Diaz, 2016). Considering this trend, it is plausible that the *Aedes albopictus* species may come to assume a larger role in the spread of ZIKV in the future as it has for other flaviviruses. Unlike the *Aedes aegypti* species, *Aedes albopictus* mosquitoes prefer underpopulated areas with significant vegetation and bite humans and animals opportunistically (Paupy, Delatte, Bagny, Corbel, & Fontenille, 2009). However, deforestation and expansion of human settlements into areas that were once relatively devoid of humans have increased the frequency of contact between humans and this species, which has, in turn, led to the adaptation of this species to urban settings (Kraemer et al., 2015; Y. Li et al., 2014). The geographical distribution of *Aedes albopictus* in the U.S. is broader than the primary ZIKV vector and extends into several Midwestern states and even the Hawaiian islands (Diaz, 2016). Based on *Aedes albopictus* species’ previous role as a ‘bridge vector’ for sylvatic DENV into urban
human settings in Asia, researchers recognize the possibility that this species may serve to establish an enzootic ZIKV transmission cycle in the Western Hemisphere (Hanley et al., 2013; Vasilakis, Cardosa, Hanley, Holmes, & Weaver, 2011).

Studies thus far identify monkeys as the primary reservoir host for ZIKV, although the primary species is yet unknown, and humans as the amplifying host (Figure 5) (Duffy et al., 2009; McCrae & Kirya, 1982). The combination of urban settings and favorable survival conditions for vectors lead to and sustain regional outbreaks. Thus, mosquito-borne transmission is the main cause of ZIKV outbreaks; however, other modes of transmission account for ZIKV import into regions outside of vector territory or areas that have not yet experienced an outbreak.

**Figure 5. ZIKV transmission cycles.** Monkeys serve as the primary reservoir for ZIKV and humans as the amplifying host. Figure illustrates the sylvatic ZIKV transmission cycles in Africa and patterns of emergence into the urban transmission cycle. (Figure reproduced and legend adapted from Weaver et al., 2016)

*Sexual Transmission*

The first report of ZIKV infection by sexual transmission dates back to 2011 when the wife of an American scientist who returned to Colorado from Senegal had confirmed ZIKV infection nine days after his return (Foy et al., 2011). This study reported that
sexual transmission in this case was highly probable since the wife had not left the U.S. since 2007 and the temperatures of Colorado did not match those typical of *Aedes* mosquito habitats. Since then, cases of patients developing symptoms of ZIKV infection after sexual contact with individuals returning from ZIKV-endemic areas have also been reported from other parts of the U.S., Italy, and Chile (Armstrong et al., 2016; Grischott, Puhan, Hatz, & Schlagenhauf, 2016; Hills et al., 2016; McCarthy, 2016; Venturi et al., 2016).

Although no other arboviruses have previously been isolated from human semen, these findings suggested that ZIKV must be present in seminal fluid; this hypothesis was confirmed by several studies that detected ZIKV RNA in several men who displayed symptoms of ZIKV infection (Atkinson et al., 2016; Foy et al., 2011; Hearn, Atkinson, Hewson, & Brooks, 2014; Mansuy et al., 2016; Didier Musso, Roche, Robin, Nhan, & Teissier, 2015). Patients presented with typical symptoms of ZIKV infection and hematospermia was noted in only two patients (Foy et al., 2011; Didier Musso et al., 2015). In three of these studies, the ZIKV RNA load in the seminal fluid was significantly higher than that in the urine or blood (Atkinson et al., 2016; Mansuy et al., 2016; Didier Musso et al., 2015). Furthermore, studies found that infectious ZIKV could be isolated from semen even after clearance of viremia (Didier Musso et al., 2015). Together, these findings suggest that sexual transmission, in addition to direct infection of a pregnant woman by a mosquito bite, can also be a route through which ZIKV can cause microcephaly.
Thus far, male-to-female, female-to-male, and male-to-male sexual transmissions have been documented (Davidson, Slavinski, Komoto, Rakeman, & Weiss, 2016; Deckard et al., 2016; Foy et al., 2011). Hence, case reports indicate that ZIKV can be transmitted through both vaginal and anal sex. All reported cases of sexual transmission have involved ZIKV carriers who exhibited typical clinical symptoms of the disease (Grischott et al., 2016). Due to the higher incidence of ZIKV infection in women than in men, some researchers hypothesize that ZIKV-positive seminal fluid presents a greater risk for transmission of the infection than ZIKV-positive vaginal fluid (Coelho et al., 2016).

Sexual transmission is unlikely to cause major ZIKV outbreaks. However, several reports thus far have demonstrated that this mode of transmission can play a significant role in importing ZIKV infection into areas, such as parts of Europe and the U.S., that have not yet experienced an outbreak and in which local mosquito transmission is less likely. As the geographical distributions of the Aedes mosquitoes continue to expand, it is plausible that introduction of ZIKV into yet uninfected areas by sexual transmission could eventually lead to establishment of autochthonous transmission and an outbreak. Additionally, findings that have demonstrated the nature of ZIKV to persist in the testes suggest that this immune-privileged tissue could act as a reservoir from which new transmission cycles may be initiated in the future as has been the case previously for the Ebola virus (Deen et al., 2015; Mate et al., 2015).
Blood-borne Transmission

Although fewer reports of ZIKV transmission by blood have been reported, this transmission route is still important to consider since many ZIKV-infected individuals are asymptomatic and a viremic donor could contaminate the blood supply. A serological study conducted in French Polynesia from November 2013 to February 2014 demonstrated this possibility as the study reported that 2.8% of asymptomatic blood donors had been found positive for ZIKV by RT-PCR (D. Musso, Nhan, et al., 2014). This was an increase from the 0.8% positivity that had been reported before the epidemic (2011-2013) in French Polynesia (Aubry et al., 2015). In Brazil, four cases of probable ZIKV transmission by blood transfusion have been reported (Barjas-Castro et al., 2016; Jimenez, Shaz, & Bloch, 2016).

Many areas, including the U.S., Canada, and Europe, already employ nucleic acid amplification tests to screen the blood supply for WNV (Centers for Disease Control and Prevention, 2013; Custer, Kamel, Kiely, Murphy, & Busch, 2009; O’Brien et al., 2010). A similar approach for ZIKV must be implemented in order to prevent future cases of ZIKV transmission by blood. Ensuring the availability of safe blood has become even more important as ZIKV-endemic areas, such as Puerto Rico, increase the amount of blood that is imported from yet unaffected areas (Vasquez, Sapiano, Basavaraju, Kuehnert, & Rivera-Garcia, 2016).

For countries without an approved diagnostic assay or where importation of blood products from foreign blood bank centers is not routinely possible, other strategies can be employed to prevent ZIKV transmission by blood. A recent in vitro study demonstrated
that a combination of amotosalen and ultraviolet A illumination can inactivate ZIKV in plasma (Aubry, Richard, Green, Broult, & Musso, 2016). This method has proved effective against other arboviruses, including WNV, CHIKV, and DENV, and implementing this proactive strategy in ZIKV endemic areas will be critical in preventing further ZIKV transmission by transfusion (Aubry et al., 2016; D. Musso, Richard, Broult, & Cao-Lormeau, 2014).

Maternal-Fetal Transmission

To date, case reports indicate that ZIKV may also be transmitted through breastmilk and in utero. One study from 2014 found that breast milk samples collected from two febrile mothers from French Polynesia with confirmed ZIKV infection tested positive for the virus (Besnard, Lastère, Teissier, Cao-Lormeau, & Musso, 2014). Since viral transmission by breastmilk has been previously documented for other flaviviruses, it is a possibility that these mothers passed on the virus to their newborns by breastfeeding (Barthel et al., 2013; Centers for Disease Control and Prevention, 2002; S. Kuhn, Twelle-Montecinos, MacDonald, Webster, & Law, 2011). The 2016 case report of a ZIKV-infected mother in New Caledonia is the only other study thus far that has provided any evidence to support the possibility of transmission by breastfeeding (Dupont-Rouzeyrol, Biron, OConnor, Huguon, & Descloux, 2016). ZIKV RNA load in breast milk samples was much higher than in sera for both these reports. Interestingly, the New Caledonian sample contained infectious ZIKV particles whereas the French Polynesian samples did not (Besnard et al., 2014; Dupont-Rouzeyrol et al., 2016). Due to this discrepancy, the limited number of case reports, and the fact that the newborns could have been infected
by blood-borne transmission during delivery or *in utero* transmission, support for the possibility of ZIKV transmission by breastmilk remains weak and infected mothers have not been discouraged to breastfeed their infants.

Due to the startling increase in the reported number of microcephaly cases in Brazil soon after its 2015 ZIKV outbreak, the possibility of infection by transplacental transmission received significant attention from researchers. Over 4,000 cases of microcephaly were reported in the span of four months in the northeastern states of Brazil where the ZIKV outbreak began; this number represented an approximate 20-fold increase from prior years (Butler, 2016; Schuler-Faccini et al., 2016; Soares de Araujo et al., 2016; Victora et al., 2016). The possibility that ZIKV could be crossing the placental barrier to cause central nervous system (CNS) damage in fetuses was not entirely foreign since other RNA viruses, such as the rubella virus, are capable of crossing the placenta to cause microcephaly; what was unusual, however, was that ZIKV would be the only flavivirus documented to have this capability if research did confirm that ZIKV infection in pregnant women was the reason for the surge in microcephaly cases (Calisher & Sever, 1995; Naing et al., 2016).

Although the association between ZIKV and microcephaly remained controversial, the continuing reports about ZIKV-infected mothers and their microcephalic newborns provided increasing support for the hypothesis that ZIKV was the causative agent in the recent surge in microcephaly. In one report, a fetal autopsy and RT-PCR assay of a microcephalic fetus revealed that ZIKV was present in the brain tissue; the mother had exhibited signs of ZIKV infection (febrile illness and rash) at the
end of her first trimester of pregnancy (Mlakar et al., 2016). In another case, ZIKV was detected in the amniotic fluid of two pregnant women whose fetuses had been diagnosed with microcephaly, which strongly suggested that ZIKV is able to cross the placental barrier (Calvet et al., 2016). Thus, in light of this growing body of evidence, health officials urged women to take precautions in avoiding mosquito bites and even to delay pregnancy.

By April 2016, officials at the Centers for Disease Control confirmed the association between ZIKV-infected pregnant women and the occurrence of microcephaly of newborns (Belluck & McNeil Jr., 2016). Furthermore, other adverse outcomes besides microcephaly have been attributed to ZIKV infection of the mother, including intracranial calcifications, intrauterine fetal demise, ocular abnormalities, hypertonia, and arthrogryposis in infected fetuses or children (Brasil et al., 2016; De Barros Miranda-Filho et al., 2016; Driggers et al., 2016; Faria et al., 2016; Kleber de Oliveira et al., 2016; Martines et al., 2016; Meaney-Delman et al., 2016; Mlakar et al., 2016; Villamil-Gomez et al., 2016). Details regarding the mechanism of ZIKV pathogenesis is discussed in a later section.

*Other Modes of Transmission*

Additional, although minor, possible modes of transmission include mucocutaneous exposure to infected blood from a monkey bite, hemodialysis, or organ transplantation; these transmission routes are those previously reported for other flaviviruses (Centers for Disease Control and Prevention, 2003, 2004; Chen & Wilson, 2004; Iwamoto et al., 2003; Leung, Baird, Druce, & Anstey, 2015; Rigau-Pérez, Vorndam, & Clark, 2001).
Since studies have indicated that ZIKV is shed in urine for over a month in some cases, patients receiving kidneys from donors in ZIKV-endemic areas may become infected through the donated organ (Fonseca et al., 2014; Gourinat, O’Connor, Calvez, Goarant, & Dupont-Rouzeyrol, 2015; Korhonen et al., 2016; Leung et al., 2015). ZIKV RNA has been detected in nasopharyngeal swabs and saliva samples as well, but studies have not yet demonstrated whether transmission is possible from contact with these substances (Fonseca et al., 2014; Leung et al., 2015).

PATHOGENESIS OF ZIKV INFECTION

As is the case for other flaviviruses, it is likely that ZIKV replicates in the epithelial and salivary gland cells of the mosquito after it ingests a blood meal from an infected individual (M. I. Li, Wong, Ng, & Tan, 2012; Wong, Li, Chong, Ng, & Tan, 2013). After a period of 5-10 days, ZIKV can be found in the mosquito’s saliva and transmitted to humans (M. I. Li et al., 2012; Wong et al., 2013). When the infected mosquito bites, the infected saliva deposits the virus into the epidermis and dermis of the host, which leads to ZIKV infection of both the host’s skin fibroblasts and keratinocytes (Hamel et al., 2015). Infection of the epidermal keratinocytes is indicated by the appearance of pyknotic nuclei in the stratum granulosum layer, which is similar to what has been observed with DENV infection of human epidermal cells (Hamel et al., 2015; Limon-Flores et al., 2005). ZIKV entry into these cells is mediated by the receptor proteins AXL, Tyro3, and TIM-1 (Hamel et al., 2015). ZIKV can also infect the antigen-presenting Langerhans cells by interacting with the receptor protein DC-SIGN; infection of these cells allows the virus to
disseminate to regional lymph nodes from which it can reach the blood stream and consequently other tissues, including the CNS (Hamel et al., 2015).

The possibility that ZIKV exhibits neurotropism was first demonstrated over 50 years ago when Dick (1952) demonstrated the ability of ZIKV to cause CNS disease in mice of various ages; younger mice (under 7 days of age) were susceptible to lethal infection, whereas adult mice were less so. Further examination of these mice revealed evidence of neuronal degeneration in the spinal cord and brain (Dick, 1952). Although these findings are limited by the fact that the study used the original ZIKV strain (MR766), which differs from the strains causing the current epidemic, recent studies have presented similar findings regarding ZIKV effect on CNS. A case involving a fetus from a terminated pregnancy that was infected with a recent ZIKV strain reported evidence of damage to the brain stem and spinal cord as well as astrogliosis and Wallerian degeneration of the descending corticospinal tracts (Mlakar et al., 2016).

ZIKV neurotropism has been confirmed by several additional studies. One study that used mice and both the contemporary human isolate of ZIKV from French Polynesia and the original strain (MR766) found that CNS tissues had higher viral loads and that ZIKV RNA persisted in those tissues even after clinical disease signs had resolved in surviving mice (Lazear et al., 2016). This study also showed that interferon-α/β signaling plays a key role in restricting ZIKV infection in mice since an increase in ZIKV viremia was observed in those mice that had been treated with MAR1-5A3, an antibody that blocks interferon-α/β signaling (Lazear et al., 2016). Although these findings cannot be translated directly into humans as mice lack key components of antiviral immunity, this
study was important in providing a mouse model for the pathogenesis of ZIKV and serves as a small animal model to evaluate vaccines and therapeutics.

Another study by Tang et al. (2016) has provided evidence to support ZIKV neurotropism. Using human induced pluripotent stem cells (hiPSCs) as an in vitro model, this study differentiated hiPSCs into forebrain-specific human neural progenitor cells (hNPCs), a constitutive population of an embryonic brain, and infected them with ZIKV; researchers found that these cells were readily infected by the virus and that the infection spread to 90% of cells within three days of inoculation (Tang et al., 2016). More importantly, this study found that ZIKV infection of this cell population leads to both dysregulation of the cell cycle and an increase in number of cell deaths (Tang et al., 2016). These findings provide more insight regarding the mechanism by which ZIKV infection in mothers leads to CNS disease in newborns. Despite the fact that this study used the MR766 strain instead of one of the contemporary strains isolated from humans, these results in conjunction with those provided by Lazear et al. (2016) provide convincing evidence to support the theory of ZIKV neurotropism.

Further support for the causal association between ZIKV and neural diseases is provided by Garcez et al. (2016). This group examined the effects of infection in human neural stem cells growing as neurospheres and brain organoids in vitro. Their results showed that ZIKV-infected neural stem cells exhibited morphological abnormalities and cell detachment as they developed into neurospheres; in these infected neurospheres, ZIKV was found both bound to the membranes and within mitochondria and vesicles (Garcez et al., 2016). In other words, Garcez et al. (2016) showed that ZIKV targets
human brain cells and interferes with neurogenesis during brain development. Thus, the
neurotropism and persistence of ZIKV demonstrated by these studies using both mouse
and human stem cell models help explain the rise in microcephaly and Guillain-Barré
cases in association with ZIKV outbreaks.

Regarding the teratogenic mechanism of ZIKV, Adibi et al. (2016) have proposed
two hypotheses (Figure 6). The direct transfer hypothesis proposes that ZIKV directly
accesses and damages the embryonic brain (Adibi, Marques, Cartus, & Beigi, 2016).
According to this hypothesis, the virus is carried through the placenta as part of an
immunocomplex with non-neutralizing antibodies or as part of a placental exosome,
similar to DENV, that then travel to the embryonic neuroepithelium (Chahar, Bao, &
Casola, 2015). Findings from the case report by Mlakar et al. (2016) support this
hypothesis since autopsy of the fetus showed high ZIKV load in the brain tissue; the
mother had been exposed to the virus late in the first trimester of pregnancy.
Additionally, Calvet et al. (2016) reported isolating ZIKV genome in the amniotic fluid
of two infected pregnant women whose fetuses had been diagnosed with microcephaly at
28 weeks. Although ZIKV was detected in the amniotic samples, the virus had not been
detected in the women’s serum and urine (Calvet et al., 2016).

The second hypothesis proposed by Adibi et al. (2016) is the placental mediation
hypothesis, which states that microcephaly and other CNS defects are caused by the
placental response to the virus. More specifically, ZIKV may cause an inflammatory
response of the placenta, which in turn disrupts embryonic brain development, or the
virus might disrupt the placenta from producing molecules important for proper brain
development. Previous studies have demonstrated that mutations in genes that cause disruption in synthesis of molecules within the placental chorionic villi can lead to microcephaly (Gilmore & Walsh, 2013; Homem, Repic, & Knoblich, 2015; Pulvers, Journiac, Arai, & Nardelli, 2015). CNS damage by ZIKV may also be due to both of these effects since these hypotheses are not mutually exclusive, but further study is required to elucidate these details. Further clarification of the mechanism through which fetuses become infected with ZIKV will aid in the development of pharmacological methods to block the teratogenic effect of the virus.
Figure 6. Gestational sac before onset of maternal-placental blood flow (1st trimester). The embryo is surrounded by the placenta, which consists of the chorion and chrionic villi. Molecules that enter the embryo are synthesized and secreted by the placenta. (Figure reproduced and legend adapted from Adibi et al., 2016)
COMPARISON OF CONDITIONS IN BRAZIL, COLOMBIA, AND THE U.S.

Zika virus (ZIKV) spread rapidly throughout the rest of Latin America and the Caribbean following the 2015 outbreak in Brazil. The Brazilian outbreak was particularly notable because of the rapid rise in reported number of microcephaly cases following the start of the epidemic. Since the Centers for Disease Control and Prevention (CDC) confirmed the association between ZIKV and microcephaly in 2016, researchers predicted that other countries experiencing outbreaks following Brazil’s outbreak would experience a similar surge in microcephaly cases. Although countries experiencing ZIKV outbreaks following the one in Brazil also reported increases in microcephaly and other central nervous system (CNS) malformations, the reported increases were not as drastic as those seen in Brazil. The following chapter will investigate several factors that may have had a role in causing this phenomenon in Brazil by comparing them between Brazil, Colombia and the U.S.

EPIDEMIC SEQUENCE

Differences in geographical location, socio-economic settings, and government policies may have caused the occurrence of microcephaly in Brazil to be proportionally greater than in other regions that also had a ZIKV outbreak. However, the primary reason that Brazil experienced the alarming 20-fold increase in microcephaly cases is most likely attributable to timing; Brazil was the first region in the Americas to experience such a
large outbreak. Research regarding the possible association between ZIKV infection and CNS disease intensified only after Brazil’s Ministry of Health reported an abnormally large increase in microcephaly cases from previous years. Furthermore, the CDC confirmed the association over a year after the outbreak began in Brazil. As a result, women in Brazil did not receive the forewarning that women in many other countries did about the teratogenic effects of ZIKV. Of course, differences in climate, socio-economic settings, and previous exposure to the closely related dengue virus (DENV) between regions may have contributed to Brazil’s abnormally large microcephalic outbreak as well and the following sections investigate these.

VECTOR ADAPTATION, SURVIVAL, AND CONTROL

The global spread of ZIKV infection since the outbreak in Yap Islands in 2007 has been primarily facilitated by vector-borne transmission. Since Aedes aegypti and Aedes albopictus mosquito species have been the primary and secondary vectors, respectively, for ZIKV thus far, this section will investigate the conditions for Aedes vector survival in Brazil, Colombia, and the U.S., as well as the adaptations that these species have undergone in recent years. In addition, the vector control responses of different countries and their effectiveness will be discussed. Comparing these characterizations between regions that have experienced the ZIKV outbreak will provide further insights regarding the particular role that the Aedes vector has in facilitating the spread of ZIKV and causing microcephaly.
Origin of Aedes Mosquitoes

The two Aedes species responsible for the ZIKV outbreaks in the Western Hemisphere differ in origin and conditions for survival. Aedes aegypti was introduced to the Americas from Africa between the 15th and 18th centuries (Tabachnick, 1991). In the U.S., the species established its territory across the southeastern regions. On the other hand, Aedes albopictus is of Asian origin and spread to the islands in the Indian and Pacific Oceans beginning in the early 20th century (Delatte, Gimonneau, Triboire, & Fontenille, 2009). This species rapidly expanded its range during the 1980s to include the U.S., Europe, and Brazil (Carvalho, Lourenco-De-Oliveira, & Braga, 2014; Medlock et al., 2012; Sprenger & Wuthiranyagool, 1986). In the U.S., the Asian tiger mosquito spread rapidly across the southeastern U.S. since its first establishment in Houston, Texas (Hawley, 1988; C. G. Moore, 1999). Studies have proposed that the recent rapid decline and extinctions of Aedes aegypti populations in southeastern U.S. is due, in part, to the expansion of Aedes albopictus range (Bargielowski, Lounibos, & Carrasquilla, 2013).

Survival and Adaptation

To date, the majority of studies that investigated survival conditions for Aedes mosquitoes primarily focused on the impacts of temperature on these species. Two studies that used the established territories of Aedes albopictus mosquitoes in Japan and Asia found that the distribution was limited by the minimum mean temperatures of -2°C and -5°C, respectively. Studies that examined the survival of both Aedes aegypti and Aedes albopictus mosquitoes under laboratory and field conditions reported higher survival rates for Asian tiger mosquitoes, but wider range of temperatures for Aedes
Aedes aegypti mosquitoes (Brady et al., 2013, 2014). Additionally, Aedes mosquitoes are limited by elevation and are typically not found in areas that are elevated more than 2,000 meters (Figure 7) (Centers for Disease Control and Prevention, 2017b).

Figure 7. Map of South America elevations. Dark purple regions are areas of low elevation (ideal for Aedes survival) and light purple regions indicate areas of high elevation. (Figure adapted from CDC Travelers’ Health)

Although climate change is an important factor in determining the range of mosquito vectors, studies of previous arboviruses, including malaria, yellow fever, and dengue, have shown that human activities play a more significant role. For example, studies have found that deforestation and expansion of human settlements into areas that were once relatively devoid of humans has caused the Aedes albopictus species, which
originally preferred underpopulated areas and fed opportunistically on both animals and humans, to adapt its life cycle to urban settings (P. Reiter, 2001). This adaptation of *Aedes albopictus* mosquitoes has led to increased interaction with the ‘urban’ *Aedes aegypti* species and this, in turn, has led to the decline of *Aedes aegypti* populations, possibly due to inter-species competition and non-reciprocal cross-species inseminations (Bargielowski et al., 2013; Daugherty, Alto, & Juliano, 2000; Juliano, Lounibos, & O’Meara, 2004; O’Meara, Evans, Gettman, & Cuda, 1995). Since the *Aedes albopictus* species is considered a highly invasive species and studies have demonstrated it has better survival rates than the *Aedes aegypti* species, the expansion of *Aedes albopictus* territory represents the growing potential for continued spread and establishment of autochthonous ZIKV transmission in many yet unaffected countries.

Other factors that affect *Aedes* mosquito distribution include socio-economic elements such as housing quality, use of air-conditioning, and rate of urbanization (Åström et al., 2012; Ramos et al., 2008). Findings from a previous DENV study comparing Nuevo Laredo and Laredo, two cities on the international border between Mexico and the U.S, respectively, illustrate the effect that use of air-conditioning and type of lifestyle can have on the role of *Aedes* mosquito as a vector. This study found that the lower DENV transmission rate in Laredo, Texas could be explained by the fact that most of its shops, restaurant, and public places have air-conditioning, closed windows, and automatic doors; such features decreased the opportunity for mosquito-human contact compared to Nuevo Laredo, Mexico (Paul Reiter et al., 2003). Thus, poor housing quality and inadequate sanitary facilities allow vector populations to multiply quickly.
because of the higher frequency of mosquito-human interaction and probability that water is stored in open containers that serve as breeding sites (Castellanos et al., 2016).

Vector Control Methods

Several methods for vector control exist. Some effective physical vector control methods include using curtains and air-conditioning indoors, removing containers that can serve as breeding sites outdoors, and wearing light-colored, long-sleeved shirts and pants when outside (Diaz, 2016). As previously discussed, use of air-conditioning and window closures are quite effective in limiting the amount of mosquito-human interaction (Paul Reiter et al., 2003). Removal of containers and excessive vegetation outdoors that may serve as breeding grounds or as a resting place for the mosquitoes can significantly decrease the size of the vector population, but can be difficult to achieve in impoverished peri-urban areas (Diaz, 2016). Previously, an epidemiologic survey conducted in two contiguous cities along the U.S.-Mexican border found that discarded waste tires made up the largest category of infested containers in both cities; the water-holding capacity of tires and the insulating quality makes discarded tires an ideal spot for Aedes mosquitoes to breed (Ramos et al., 2008). As a result, removing these ideal containers can effectively decrease the size of the Aedes population. Additionally, wearing light-colored clothing is effective against these mosquitoes because these species are attracted to dark colors (Diaz, 2016).

There are chemical control methods as well. These include widespread organophosphate spraying of mosquito habitats, using household insecticides, larvaciding with monomolecular films and oils, and applying topical mosquito repellents. Of these
general methods, use of household insecticides and application of topical repellents in conjunction with pyrethroid-impregnated clothing when outdoors are typically most effective against *Aedes* mosquitoes (Diaz, 2016). Widespread spraying of organophosphate is not as effective against *Aedes* mosquitoes since both *Aedes aegypti* and *Aedes albopictus* species prefer, or have adapted to prefer, peridomestic habitats. Using monomolecular films and oils on freshwater surfaces that usually suffocate larvae is not as effective for a similar reason; the *Aedes* mosquitoes responsible for ZIKV transmission lay eggs near homes in discarded containers rather than in fresh water.

Researchers have shown renewed interest in the potential of sterile insect technique (SIT), a genetic vector control method. For this technique, radiation-sterilized or genetically engineered male mosquitoes are released into different regions and female *Aedes* mosquitoes that mate with these males produce nonviable eggs or the offspring receive a fatal gene, respectively. Limitations of SIT include higher costs, the need to release these males repeatedly into regions, and the fact that its success is dependent on the ability of the sterilized or engineered males to compete for mates with wild ones (Lees et al., 2014; Lees, Gilles, Hendrichs, Vreysen, & Bourtzis, 2015; Oliva, Damiens, & Benedict, 2014; Oliva, Damiens, Vreysen, Lemperière, & Gilles, 2013). Some researchers have proposed that the safest solution for population suppression may be to use irradiation in conjunction with genetically engineered male mosquitoes that will pass a symbiotic bacterium (*Wolbachia* species) to females since this will essentially render female *Aedes* mosquitoes infertile (Lees et al., 2015).
Comparison of Environmental Conditions in Brazil, Colombia, and the U.S.

Many factors make Brazil an ideal habitat for *Aedes* mosquitoes. The majority of Brazil’s climate is tropical, most of its terrain lies between 200-800 meters in elevation, and even the mountain ranges in the southeastern region only reach an elevation of 1,200 meters at their highest points (Central Intelligence Agency, 2017a). *Aedes aegypti* and *Aedes albopictus* are tropical species and can survive in elevations up to 2,000 meters as described previously (Centers for Disease Control and Prevention, 2017b). Brazil also has a large urban population (85% of total population) and relatively higher rates of urban crowding (Central Intelligence Agency, 2017a). The northeastern region of Brazil, in which the outbreak began, is also economically poorer; consequently, this region has less efficient waste management and greater likelihood that there are large amounts of waste containers that can serve as breeding sites for mosquitoes. In addition, one of Brazil’s worst droughts in decades hit the northeastern region in 2013 and has continued to persist; this has led to water rationing and loss of water through pipes for many communities. As a result, communities had to increase the use of water storage containers, which can also serve as breeding sites for *Aedes* vectors. Therefore, northeastern Brazil likely hosted a relatively larger population of ZIKV vectors, which led to higher transmission rates and the large outbreak of 2015.

In Colombia, the climate and terrain is relatively less suitable for *Aedes* mosquitoes. Colombia’s tropical climate is restricted to the flat coastal lowlands and the eastern plains; the climate in the central highlands and Andes Mountains is cooler (Central Intelligence Agency, 2017b). As a result, the distribution of *Aedes* mosquitoes is
not as widespread in Colombia as it is in Brazil. More importantly, however, is that the majority of Colombia’s population resides in the Andes Mountains and less than 6% lives in the lowlands where *Aedes* mosquitoes are abundant; in addition, 76% of Colombia’s total population is considered urban and its large urban centers are all located at elevations above 2,000 meters (Central Intelligence Agency, 2017b). With these environmental conditions, Colombia cannot host as large of a vector population as Brazil and with nearly the entire population residing in areas that *Aedes* mosquitoes have yet to completely adapt to, it would have experienced relatively less frequent mosquito-human interaction throughout its ZIKV outbreak than Brazil.

For the U.S., this thesis will focus specifically on Florida where the first local mosquito-borne transmission for the continental U.S. was reported. Florida’s climate is tropical, and as previously described, *Aedes* mosquitoes can be found throughout the region as they inhabit the entire southern tier of the U.S. (Central Intelligence Agency, 2017c). The majority of Florida’s terrain is less than 30 meters above sea level and the highest elevation point is only 105 meters (Norrell & Fuson, 2014). Thus, in terms of climate and terrain, Florida is a suitable habitat for *Aedes* mosquitoes, similar to Brazil. However, unlike Brazil and Colombia, the U.S. has comparatively low rates of urban crowding and ubiquitous use of window screens and air-conditioning (Jimenez et al., 2016). As Reiter et al. (2003) demonstrated in their study of two contiguous cities along the U.S.-Mexico border, these conditions can significantly dampen the size of a potential outbreak and consequently, the scale of Florida’s ZIKV outbreak did not match those observed in Central and South America.
Lastly, the decreasing effectiveness of several chemical vector control methods, such as the use of insecticides of the organophosphate and pyrethroid classes in Brazil may have also had a role in its large ZIKV outbreak and microcephaly numbers (E. P. Lima et al., 2011; J. B. Lima et al., 2003). DENV has been endemic in Brazil for more than 30 years and Brazil accounts for more than half of all dengue cases in the American continent; the majority of these cases are reported in the northeastern states of Brazil (World Health Organization, 1992). Aggressive government efforts to control *Aedes* vectors for decades have caused vector populations to develop resistance to various widely-used insecticides; more specifically, *Aedes* populations in different regions demonstrate resistance to different combinations of chemicals depending on the primary substances that have been used in each region (Brasil et al., 2016; E. P. Lima et al., 2011; J. B. Lima et al., 2003). Additionally, the resistance status of these vectors in each state of Brazil is not systematically verified and consequently, it is not yet possible to customize the deployment of vector control chemicals for each part of Brazil to attain maximal reduction in vector population size.

ZIKV vectors in Colombia have demonstrated growing resistance to various chemicals as well (Fonseca-Gonzalez, Quinones, Lenhart, & Brogdon, 2011; Grisales et al., 2013). However, vector mortality rates have not yet fallen to the extent seen in Brazil, which is likely attributable to the longer history of *Aedes* mosquito eradication campaigns in Brazil (Camargo, 1967; E. P. Lima et al., 2011; J. B. Lima et al., 2003; Ocampo, Mina, Carabali, Alexander, & Osorio, 2014). Since DENV and ZIKV share both *Aedes aegypti* and *Aedes albopictus* vectors, the resistance developed by these two species due to public
health programs that have been in place in Brazil since farther back may have allowed ZIKV to spread more rapidly in Brazil than in Colombia and the U.S. In addition, Colombia had more time than Brazil to ready its tools for vector control and having just experienced a chikungunya epidemic the year before its ZIKV outbreak, Colombia may have been more prepared to quickly utilize these methods (OPS/OMS, 2016).

In summary, a comparison of climate, terrain, urban population size, economic conditions, and vector response to vector control programs between Brazil, Colombia, and the U.S. shows that Brazil is an ideal habitat for Aedes vectors in several aspects. These differences may have led to relatively larger vector populations in Brazil that, in turn, led to rapid establishment of autochthonous transmission and infection, especially in the poorer northeastern states; this, in conjunction with the fact that Brazil did not have forewarning regarding the association between ZIKV and microcephaly, may help explain the high occurrence of microcephaly cases in Brazil soon after the start of the outbreak.

**BEHAVIOR OF WOMEN**

Differences in women’s behavior between countries may also have contributed to Brazil’s high microcephaly numbers. Since a large proportion of abortions are performed illegally in Colombia and Brazil, it is not possible to find out through hospital databases whether there was an increase in the number of abortions performed from pre-ZIKV years. For example, only 320 legal abortions were officially reported in Colombia in 2011, but the actual estimated number is around 400,000 each year, according to
Guttmacher Institute, a New York-based research organization for abortion rights (McNeil Jr. & Symmes-Cobb, 2016). However, researchers have found that the number of live births in Colombia between 2015 and 2016 decreased by approximately 18,000 (Cuevas et al., 2016). This number represents a 3.5% decrease in the number of live births. Brazil did not experience this type of decrease between pre-ZIKV years and during the outbreak. We can infer from the decrease observed in Colombia that women sought abortions in greater numbers or followed the recommendations provided by the Ministry of Health in February 2016 for women to consider delaying pregnancy altogether for at least 6 months.

Legal abortion is difficult to obtain in both Latin American countries, but Colombia has relatively progressive laws and regulations. The 2006 Constitutional Court decision allows abortion to be carried out in cases in which the mother’s life or physical health is in danger, in cases of rape or incest, or in pregnancies involving fatal or life-threatening fetal abnormalities (Ralston & Podrebarac, 2008). In Brazil, abortion is only legal if the mother’s life is in danger or in the case of rape or incest, and in nearly all cases, abortion is a crime that is punishable by up to three years in prison (McDonald, 2016; Ralston & Podrebarac, 2008). Brazil’s strict policies regarding abortion may have deterred more women from seeking abortion even in cases of suspected microcephaly than in Colombia or the U.S. In addition, the heightened awareness of gynecologists in Colombia to the ZIKV threat and microcephaly from cases in Brazil meant more women had ultrasounds performed early enough in order to make decisions about their pregnancy. This difference may help explain the significant decrease in birth numbers
observed in Colombia between pre-ZIKV years and during the outbreak as well as why Colombia did not experience a similar 20-fold increase in microcephaly numbers like Brazil.

In the U.S., abortion is legal, although controversial, and compared to both Colombia and Brazil, women in the U.S. had the most time to learn about the association between ZIKV and fetal malformations. The smaller size and duration of the outbreak in the U.S. may be the primary reasons why the U.S. did not experience the alarming spike in microcephaly occurrence, but better access to health care and abortion services for women in the U.S. likely contributed as well.

ANTIBODY-DEPENDENT ENHANCEMENT

Another highly cited possibility for the alarming increase in microcephaly numbers that Brazil experienced is its history with DENV and the effect that antibody-dependent enhancement (ADE) mechanism may have had on ZIKV transmission during the outbreak. ADE describes a phenomenon in which the antibodies from a primary viral infection help facilitate viral entry in the case of a secondary infection by opsonizing, but not neutralizing, the viral particles into host cells. Until now, ADE was largely a mechanism understood in the context of DENV; patients who had been infected with or vaccinated against one DENV serotype experienced more severe disease symptoms when infected a second time by a different serotype. In other words, in the case of a secondary infection, the host’s own immune system enables DENV to infect cells more easily due to a prior infection, causing more severe disease symptoms in patients. Given the similarity
between DENV and ZIKV, exemplified by the cross-reactivity observed in serological assays, researchers propose that ADE influenced ZIKV pathogenesis in the recent epidemic. This is a possibility considering that the most recent ZIKV outbreaks occurred in regions with DENV hyperendemicity. Most of the population in these regions have been infected by one or more DENV serotypes and have antibodies for DENV that may have allowed easier entry of ZIKV into cells via ADE mechanism.

The circumstances of the 2013-2014 ZIKV outbreak in French Polynesia serve as evidence to support the hypothesis that ADE had a role in Brazil’s outbreak and microcephaly increase. French Polynesia experienced concomitant ZIKV, dengue virus-1 and dengue virus-3 outbreaks and seroprevalence studies demonstrated that a majority of the French Polynesian population had previously been infected by DENV; about 80% of blood donors were seropositive for at least one DENV serotype (Aubry et al., 2015). This outbreak was associated with a 20-fold increase in number of Guillain-Barré syndrome cases from prior years; this dramatic increase may have been because many patients with history of DENV disease became infected with ZIKV and through ADE of infection, the CNS-damaging effects of ZIKV infection were amplified (Cao-Lormeau et al., 2016; Oehler et al., 2014).

Two recent studies also suggest that ZIKV infection may be exacerbated by prior DENV infection. Priyamvada et al. (2016) found that pre-existing DENV antibodies may modulate the body’s immune response to ZIKV infection. Using a human monocytic cell line (U937) that is widely used to study ADE of DENV infection, this study tested the effect of DENV sera and plasmablast-derived monoclonal antibodies on ZIKV infection;
the sera were from five patients infected with dengue virus-2 and the antibodies were derived from plasmablasts from these patients. Researchers found that all five of the DENV-infected sera significantly enhanced ZIKV infection of U937 cells (Priyamvada et al., 2016).

Stettler et al. (2016) investigated the possibility of ADE of ZIKV and DENV infection using both in vitro and mouse models. Using a human erythroleukemic cell line (K562), the study demonstrated that cross-reactive antibodies elicited by either infection mediates heterologous ADE in vitro (Stettler et al., 2016). In other words, monoclonal antibodies from prior ZIKV or DENV infection led to enhanced infection of K562 cells by DENV or ZIKV, respectively. This study also tested the capacity of antibodies elicited by ZIKV or DENV to enhance infection in a mouse model; interestingly, results showed that only ZIKV-induced antibodies enhanced DENV infection and that no enhancement for ZIKV infection was observed with DENV-induced antibodies (Stettler et al., 2016). The reason for this effect is unclear. In order to corroborate these findings, further studies using prior DENV-infected sera collected from patients from various regions and mouse models are required. However, the results thus far strongly suggest that the extensive cross-reactivity between ZIKV and DENV antibodies has implications for disease virulence in populations with prior DENV exposure.

Brazil experienced an increase of over 200% in the number of DENV cases in 2015 compared to prior years and the percentage of the population that had been or were infected DENV would have been at an all-time high; introduction of ZIKV into Brazil at this time would have allowed the infection to spread all the more rapidly due to ADE.
mechanism throughout the vulnerable population (BBC News, 2015). DENV is endemic in Colombia as well, but unlike Brazil, Colombia had experienced its largest DENV epidemic in 2010, about five years before its ZIKV introduction (Schnirring, 2010). As a result, the percentage of the population with history of DENV disease may have been relatively smaller at the time of their ZIKV outbreak so that the effects of ADE mechanism on ZIKV transmission in Colombia were not as apparent as they had been in Brazil. DENV is not endemic in the continental U.S. and ZIKV transmission during the Florida outbreak could not have been mediated by the ADE mechanism. Hence, current evidence supports the possibility that the size of Brazil’s ZIKV outbreak and dramatic increase in microcephaly cases can be explained, at least in part, by the cross-reactivity of ZIKV and DENV antibodies and the ADE mechanism.

OTHER MINOR FACTORS

This section will discuss a few additional factors that may also have had a role in causing Brazil to report significantly increased microcephaly numbers following the start of its ZIKV outbreak.

Reporting Bias

Some researchers have expressed that the reported 20-fold increase in Brazil’s microcephaly cases may have been overdramatized. Soares et al. (2016) have stated that since reporting of microcephaly was not compulsory and lacked a clearly defined criteria prior to the outbreak, there is a possibility that the prevalence of microcephaly in Brazil
before the outbreak was higher than what has been reported. This study obtained information about the head circumference of about 100,000 neonates born between 2012 and 2015 and concluded that the microcephaly prevalence of 6.4 per 10,000 live births in Paraíba is an underrepresentation (Soares de Araujo et al., 2016). If this is the case, the underrepresentation would have made the increase in microcephaly numbers due to the ZIKV outbreak appear greater than what it actually is. Additionally, it is plausible that the surprising association between ZIKV and CNS malformations in newborns may have led to heightened awareness of clinicians to this possible association that could have, in turn, caused over-reporting of cases (Soares de Araujo et al., 2016). Thus, underrepresentation and over-reporting of microcephaly cases may have caused the difference between pre- and post-outbreak years appear much larger.

Change in Microcephaly Definition

Another possible source of error may be the change in recommended cutoff for head circumference that was issued by Brazil’s Ministry of Health during the outbreak. Before December 8, 2015, the Ministry of Health recommended a cutoff of less than or equal to 33 centimeters for term newborn babies for both sexes (Victora et al., 2016). However, the case definition for suspected microcephaly in newborns was revised to include term newborn babies with head circumferences equal to or less than 32 centimeters (Victora et al., 2016).

The Pan American Health Organization (PAHO) has proposed the use of fixed cutoffs for term boys (32 centimeters) and girls (31.6 centimeters) based on the 3rd percentile for term newborns according to World Health Organization (WHO) Growth
Standards (World Health Organization, 2016). According to Victora et al. (2016), establishing a fixed cutoff for all term infants may not be an appropriate system either since this standard would not account for the term newborns that are below 40 weeks’ gestational age; Brazil has the world’s highest caesarean section rate and many term newborn babies are born below the 40 weeks’ gestational age (Gibbons et al., 2012).

Hence, the lack of a clear head circumference guideline for microcephaly in pre-ZIKV outbreak years and the reduction in size criterion in Brazil during the outbreak may have led to increased reporting of suspected microcephaly cases. In addition, there is a possibility that this reduction in criterion may have led to the inclusion of a significant number of normal children with smaller heads and therefore, caused an overestimation of actual microcephaly cases in Brazil (Victora et al., 2016).
CONCLUSION AND FUTURE DIRECTIONS

Zika virus (ZIKV) remained in relative obscurity since its initial isolation in Uganda during the mid-1900s. Recent outbreaks in Micronesia, French Polynesia, and Latin America, and the virus’ association with severe central nervous system (CNS) malformation in both newborns and adults has brought this once-obscure virus to the forefront of global public health consciousness. The introduction of this vector-borne disease into a new ecological system and naïve host population has enabled the virus to spread rapidly with significant implications for human health. The story of ZIKV emergence and spread throughout the Western Hemisphere is, however, not completely surprising or unprecedented as it is similar to the script followed by both dengue virus (DENV) and chikungunya virus (CHIKV) over the past two decades.

One of the most notable aspects of the recent ZIKV emergence has been its association with microcephaly, and in particular, the large increase in microcephaly cases reported in northeastern Brazil that appears to be out of proportion in comparison to other countries that have also experienced the outbreak. The reported annual incidence rate of microcephaly in all of Brazil averaged between 139 to 175 during 2010-2014 (6 per 100,000 live births), but this rate increased to over 3,500 (117 per 100,000 live births) during 2015 (Malone et al., 2016; Ministério da Saúde (Brazil), 2016). Although Colombia was also hit with ZIKV and experienced a rise in microcephaly numbers, the annual incidence rate went from 21 per 100,000 live births to 96 per 100,000 live births
between 2015 and 2016 (Cuevas et al., 2016). This has been the case for the majority of other ZIKV-affected countries as well.

Literature has provided several possibilities that may help explain the possibly overestimated number of microcephaly cases in Brazil and this thesis explored the specific role that each factor may have played in causing this phenomenon. More specifically, differences in these conditions between three countries – Brazil, Colombia, and the U.S. – were investigated in order to understand why only Brazil experienced what appears to be an out-of-proportion increase in microcephaly. Was it due to overestimation or due to differences between Brazil and other countries in regards to various factors related with the transmission cycle of ZIKV?

The differences between these three countries in terms of conditions for vector survival and control, behavior of women, and prevalence of DENV were investigated. The role of reporting errors and lack of a standard criterion for microcephaly were considered as well. Evidence thus far suggests that the ideal conditions for vector survival, lack of adequate vector control methods, and longer history of DENV epidemics in Brazil may have allowed a larger vector population to rapidly transmit ZIKV throughout the population. We can conclude from these findings in conjunction with the fact that Brazil was the first to experience such a large ZIKV epidemic in the Western Hemisphere that Brazil was at a disadvantage in terms of preparing for or controlling the spread of the outbreak and warning its population of the disastrous effects of the virus on newborns relative to other countries. Also, studies suggest that ZIKV may disproportionately infect women and that male-to-female sexual transmission is more
efficient; thus, it is plausible that a larger percentage of women became infected in Brazil since the population was not warned beforehand of the effects of ZIKV and because a large majority of infected individuals are asymptomatic. In other words, asymptomatic, infected men may have contributed to spreading the infection to women who were not warned of the possibility of sexual transmission and the virus’ association with microcephaly. In comparison, both the U.S. and Colombia’s population had time to prepare for the outbreak and receive warning about the CNS-related effects of ZIKV and the possibility of sexual transmission. These findings may thus help explain how Brazil experienced its abnormally high microcephaly numbers following the outbreak.

Interestingly, a recent report that retrospectively assessed the prevalence of birth defects associated with ZIKV infection during 2013-2014 to those reported in 2016 found that the incidence was 20 times higher after ZIKV was introduced into the U.S. (Cragan et al., 2017). However, further investigation is needed as this study only looked at Massachusetts, North Carolina, and Atlanta, Georgia, and the findings may not be generalizable to the rest of the U.S.

According to the World Health Organization (WHO), ZIKV is no longer considered an international medical emergency; similar to DENV and CHIKV, the course of this arbovirus’ spread throughout the globe has demonstrated that the virus is here to stay. As the territories of Aedes vectors continue to expand into the northern regions of the U.S., Europe, and even Canada due to temperature changes, urbanization, and high ecological plasticity of especially the Aedes albopictus species, it is becoming increasingly important to understand the molecular mechanism behind maternal-fetal
transmission, discover effective and efficient vector control methods, and develop safe vaccines. Further investigation of the reasons behind the higher ZIKV disease incidence in women versus men is important as well since this is a unique feature of ZIKV epidemics and it is not yet clear if this phenomenon has a biological basis or if it is attributable to sociological factors.
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