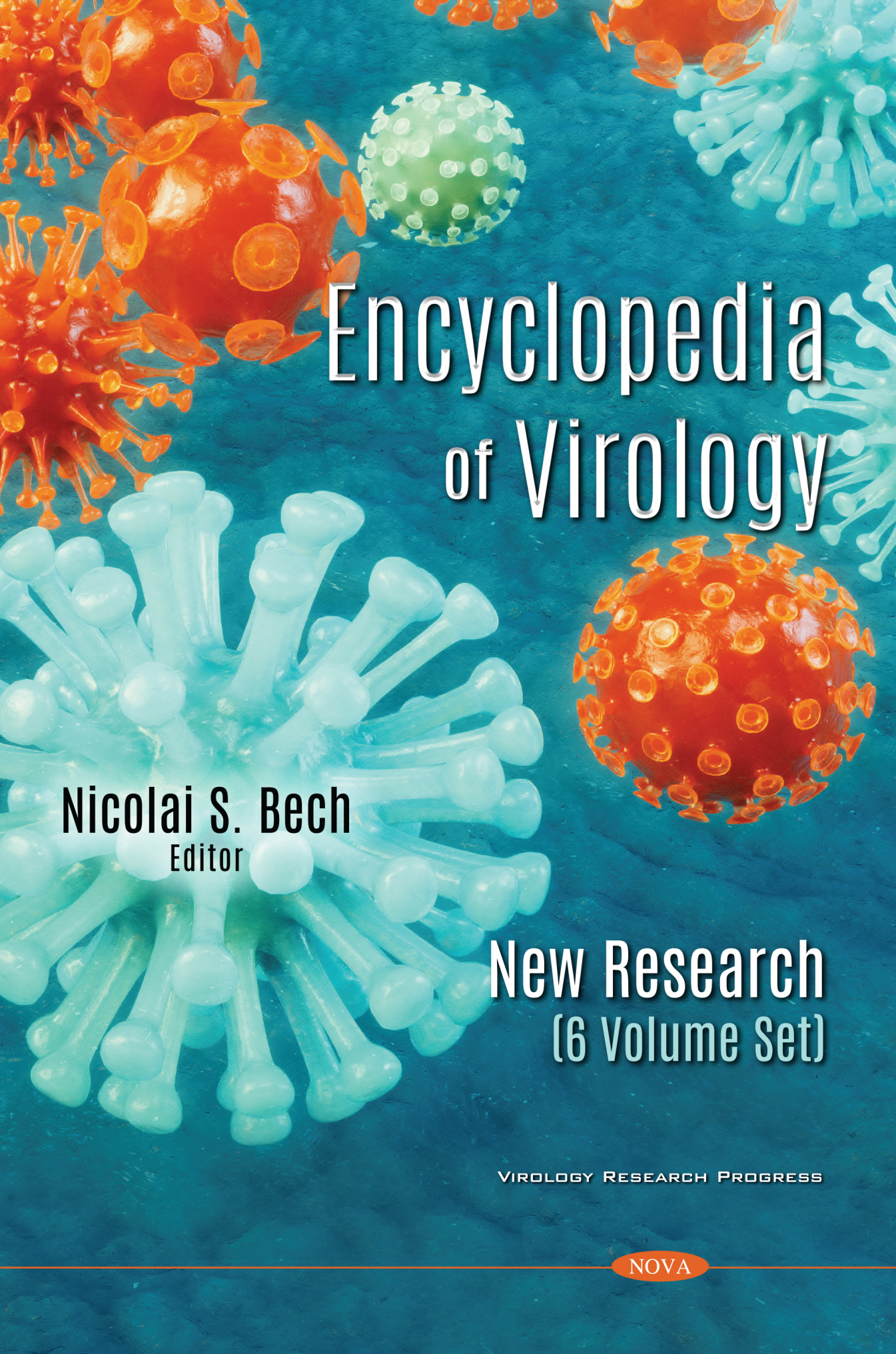




Encyclopedia of Virology

Nicolai S. Bech
Editor

New Research
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NICOLAI S. BECH
EDITOR



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PREFACE

This six volume set presents important research on virology. Some of the topics addressed include:

- the fundamentals of Zika virus
- preparation for pandemic diseases
- West Nile virus
- the management of the spread of Ebola virus disease
- human papillomaviruses
- viral hemorrhagic fevers
- the treatment of avian influenza virus
- bronchiolitis

Chapter 1

ZIKA VIRUS: AN INTRODUCTION

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ABSTRACT

This first chapter by the three editors of the book provides an overview of the status of the Zika virus (ZIKV) with emphasis on: a) Discovery and brief history of Zika virus, b) Status of global Zika infection and transmission, c) Virology of the Zika virus, d) Clinical complications of the virus, e) Diagnosis of ZIKV, f) Biochemistry of ZIKV interaction with permissive cell types, and (g) Efforts in the discovery and design for anti-ZIKV drugs and vaccines. It specifically deals with a four-pronged approach, viz., data-driven epidemiological characterization of emerging pathogens, mathematical sequence descriptor based fast surveillance approaches, computer-assisted vaccine design, and computer-aided anti-Zika drug discovery to demonstrate how together the work can be an effective approach for the prevention, mitigation, and treatment of the Zika virus infection.

Keywords: Zika Virus (ZIKV), Viral Epidemics, Time to the most recent common ancestors (tMRCAs), The battle of Imphal, Antonine Plague or Plague of Galen, Untranslated Regions (UTRs), NS₅ RNA-dependent RNA polymerase, Exocytosis, Endocytosis, Phylogenetic tree, 2D graphical method, Guillain-Barré syndrome, Microcephaly, Reverse transcription-polymerase chain reaction (RT-PCR), Enzyme-linked immunosorbent assay (ELISA), Biomarker of Zika.

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1. DISCOVERY AND BRIEF HISTORY

Viral epidemics often arise without warning and take humanity by surprise, sometimes with devastating consequences. The bubonic plague of Justinian (540-750 AD) killing 25-50% of the population of the known world, the Black Death plague of the fourteenth century that killed 20 to 30 million Europeans, the ravages of small pox in the Americas from the sixteenth century introduced by European soldiers and settlers that decimated many native populations, the Spanish Flu of 1918 that killed between 20-50 million people worldwide, the human immunodeficiency virus (HIV) that became a virulent pandemic leading to millions of incidences, among others, are noteworthy instances. The Zika virus (ZIKV) rampage in the Americas in 2015-17 is no exception [1].

The Zika virus came to occupy heightened attention following huge incidence of microcephaly in neo-natal cases in the Latin Americas in 2015. It was first isolated from a febrile rhesus monkey in the Zika forest (Figures 1.1 & 1.2) of Uganda in 1947 [2]. In 1952, the virus was isolated from humans. It then migrated through Africa and Asia and the first major outbreak of Zika infection was reported from the Island of Yap in the Federated States of Micronesia in 2007. The World Health Organization (WHO) report gives a brief history of the origin and spread of ZIKV. Serosurvey of humans detected spreads of ZIKV throughout Africa, Asia, and Oceania. In 1954, the first three cases of ZIKV infection in humans were reported in Oyo state, Nigeria [3]. Fagbami [4] noted in his 1979 study that 40% of Nigerian adults and 25% of Nigerian children had antibodies of the virus.



Figure 1.1. Zika Forest of Uganda.



Figure 1.2. Picture of a rhesus monkey in the Zika Forest of Uganda. (Ref: https://images.search.yahoo.com/yhs/search?p=Zika+forest+of+Uganda+pictures&fr=yhs-adk-adk_sbnt&hspart=adk&hsimp=yhs-adk_sbnt&imgurl=http%3A%2F%2Fmarkacadeey.com%2Fwp-content%2Fuploads%2F2016%2F02%2FZIKA-FOREST-UGANDA.jpg#id=8&iurl=http%3A%2F%2Fi.dailymail.co.uk%2Fi%2Fpix%2F2016%2F02%2F02%2F13%2F30CF2AEA00000578-3428099-image-a-7_1454418507460.jpg&action=click).

Table 1. Zika virus detected from 1947, the pre-epidemic period [1]

Year	Country	Remarks
1947	Uganda	First isolation and identification of ZIKV. Found in rhesus monkey, R766, caged in Zika forest.
1948	Uganda	Detected in <i>A. africanus</i> mosquitoes. Found in Zika forest.
1951	Nigeria	First instance of ZIKV antibodies in human blood, found in children.
1952	Uganda, Tanganyika	First human cases of ZIKV infection detected.
	India	ZIKV antibodies found in human blood.
1953	Malaya, North Borneo, Philippines	ZIKV antibodies found in residents.
	Nigeria	ZIKV infection detected in three persons.
1954	Egypt, Vietnam	ZIKV antibodies found in few residents.
1955	Nigeria	ZIKV antibodies found in human blood.
1957	Mozambique	ZIKV antibodies found in human blood.
1958	Uganda	Two strains of ZIKV found in <i>A. aegypti</i> mosquitoes in Zika forest.
1960	Angola	ZIKV antibodies found in indigenous residents.
1961-62	Central African Republic	ZIKV antibodies found in human blood.
1961-64	Ethiopia	ZIKV antibodies found in human blood.
1962	Senegal	ZIKV antibodies found in human blood.
1963-64	Central African Republic, Burkina-Faso	ZIKV antibodies found in human blood.
1963-65	Ivory Coast	ZIKV antibodies found in human blood.
1964	Uganda	First confirmation that ZIKV causes human disease. Clinical features reported.
1964-65	Guinea-Bissau	ZIKV antibodies found in human blood.
1964-66	Togo, Cameroon	ZIKV antibodies found in human blood.
1965	Niger	ZIKV antibodies found in human blood.
1965-67	Nigeria	ZIKV antibodies found in human blood.
1967	Benin, Gabon, Liberia	ZIKV antibodies found in human blood.
1966-67	Uganda, Kenya, Somalia, Morocco	ZIKV antibodies found in human blood.
1967-69	Uganda	ZIKV antibodies found in human blood.
1968	Kenya	ZIKV antibodies found in human blood.
1969-72	Nigeria	ZIKV antibodies found in human blood.
1969	Malaysia	ZIKV found in <i>A. aegypti</i> mosquitoes.
1969-83	Indonesia, Malaysia, Pakistan	ZIKV found in mosquitoes. Sporadic human infections.
1970	Nigeria	ZIKV antibodies found in human blood.
1971-72	Angola	ZIKV antibodies found in human blood.
1972,1975, 1988,1990	Senegal	ZIKV antibodies found in human blood.
1979	Central African Republic	ZIKV antibodies found in human blood.
1980	Nigeria	ZIKV antibodies found in human blood.
1984	Uganda	ZIKV antibodies found in human blood.
1996-97	Malaysia	ZIKV antibodies found in human blood.
1999	Ivory Coast	ZIKV antibodies found in human blood.

A. africanus, *Aedes africanus*; ZIKV, Zika virus.

Table 2. ZIKV epidemics, 2007 to present

Year	Country	Remarks
2007	Yap Island, Micronesia	First outbreak reported in humans.
2008	Senegal	First reported case of traveler infected in Senegal returning to home country and passing infection through sexual contact.
2010	Cameroon	ZIKV antibodies found in human blood.
2010-2015	Cambodia, Indonesia, Malaysia, Philippines, Thailand, Maldives	Mosquito transmission of ZIKV in these countries to travelers who then carried the infection to their home countries.
2011-2014	French Polynesia	Second reported outbreak of ZIKV infections. Connection with microcephaly and neurological disorders established later.
2013-2014	Chile, Cook Islands, New Caledonia	ZIKV outbreak.
2013	Tahiti	ZIKV isolated from patient's semen showing sexual transmissibility.
2014	Zambia	ZIKV antibodies found in human blood.
2015 April/May	Brazil, Bahia state	National Reference Laboratory, Brazil confirmed, by PCR, ZIKV infections, for the first time in the Americas
2015 July	Brazil	Zika cases confirmed by laboratory tests in 12 states. Neurological disorders associated with prior viral infections detected primarily in the Bahia region.
2015 October	South America	Colombia, Republic of Cabo Verde report confirmed cases of ZIKV infections. Brazil reported unusual increase in the number of cases of neonatal microcephaly.
2015 November	Central and South America	Brazil reported 141 suspected cases of microcephaly and declared a national public health emergency. Brazil reported detection of ZIKV in amniotic fluid of fetuses with confirmed microcephaly. Suriname, Panama, El Salvador, Guatemala and Paraguay confirmed cases of ZIKV infection. The Pan American Health Organization and WHO issued an epidemiological alert.
2015 November	Mexico	Three cases of ZIKV infection confirmed by PCR.
2015 November	French Polynesia	Retrospective analysis reveals unusually large number of central nervous system malformations in fetuses and infants in 2014-2015.
2015 December	Central and South America	Honduras, French Guiana and Martinique reported confirmed cases of ZIKV infections.
2015 December	Puerto Rico	First confirmed case of Zika infection reported.
2016	Maldives	Finnish national working in Maldives tested positive for Zika after return to Finland.
2016 January	Americas	Guyana reported the first PCR-confirmed case of locally acquired Zika infection. Ecuador, Bolivia, Barbados, Haiti, Dominican Republic, Nicaragua, Curacao and Jamaica reported the first confirmed cases of Zika infections. First case of Zika in St. Martin reported.
2016 January	US Virgin Islands	First confirmed case of Zika in St. Croix reported.
2016 February	Americas	First confirmed case of ZIKV infection in Chile reported. First case of sexually transmitted Zika infection in Texas, USA reported.
2016	Various countries	Angola, Antigua, British Virgin Islands, Trinidad and Tobago, Guadelope, Fiji, Marshall Islands, Papua New Guinea, and other countries report first cases of ZIKV infections.
2016	Singapore	ZIKV infection reported.
2016/2017	India	Three cases of ZIKV infection reported in Ahmedabad.
2017	Singapore	Several cases of locally transmitted ZIKV confirmed.

Data summarized from source: (1): Kindhauser, M. K., Allen T., Frank, V., Santhana, R. S. and Dye, C. Zika: the origin and spread of a mosquito-borne virus. Bull World Health Organ.

2016;94(9):675–686C. doi:10.2471/BLT.16.171082; 2) WHO Situation Report on Zika Virus, 9th March 2017 <http://apps.who.int/iris/bitstream/10665/254714/1/zikasitrepre10Mar17-eng.pdf?ua=1>.

**Table 3. Cumulative cases of ZIKV infection in the Americas
2015-2017 as of 11 May 2017**

Region*	Confirmed autochthonous cases	Imported cases	Deaths from ZIKV infection	Confirmed congenital syndrome associated with ZIKV infection
North America	225	5478	0	67
Latin America (Mexico)	8731	15	0	5
Central American Isthmus	6309	77	0	81
Latin Caribbean	41823	205	5	143
Andean area	15136	41	0	167
Brazil	133527	0	11	2653
Southern cone	100	75	0	4
Non-Latin Caribbean	5649	29	4	10
TOTAL	211500	5920	20	3130

*Regions defined as follows: North America: Bermuda, Canada, United States of America; Central American Isthmus: Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama; Latin Caribbean: Cuba, Dominican Republic, French Guiana, Guadeloupe, Haiti, Martinique, Puerto Rico, St Barthelemy, St Martin; Andean Region: Bolivia, Colombia, Ecuador, Peru; Southern cone: Argentina, Chile, Paraguay, Uruguay; Non-Latin Caribbean: Anguilla, Antigua and Barbuda, Aruba, Bahamas, Barbados, Bonaire, St Eustatius and Saba, Cayman Islands, Curacao, Dominica, Grenada, Guyana, Jamaica, Montserrat, St Kitts and Nevis, St Lucia, St Vincent and the Grenadines, St Maarten, Suriname, Trinidad and Tobago, Turks and Caicos Islands, Virgin Islands (UK), Virgin Islands (USA).

Data summarized from source: Pan American Health Organization/World Health Organization. Zika suspected and confirmed cases reported by countries and territories in the Americas Cumulative cases, 2015–2017. Updated as of 11 May 2017. Washington, D.C.: PAHO/WHO; 2017; Pan American Health Organization • www.paho.org • © PAHO/WHO, 2017.

Tables 1 and 2 provide data on: a) the cases of ZIKV detected during the pre-epidemic period (1947 to 1999) and b) the case history of ZIKV during 2007 to 2017, respectively.

As the epidemic progressed the number of cases in the regions of the Western hemisphere increased. Table 3 below summarizes the number of cases of ZIKV infection in the regions of the Americas detected up to 11 May 2017.

On 2 March 2015 Brazil notified WHO of reports of an illness characterized by skin rash in northeastern states. From February 2015 to 29 April 2015, nearly 7000 cases of illness with skin rash were reported in these states. All cases were mild, with no reported deaths. Zika was not suspected at that stage, and no tests for Zika were carried out. On 1 February 2016, WHO [2] declared that the recent association of Zika infection with clusters of microcephaly and other neurological disorders constituted a Public Health Emergency of International Concern. It should be mentioned that WHO later relaxed this warning subsequently in November of the same year.

Liang et al. [5] carried out computational analysis of ZIKV evolution, nucleotide substitution rate and the time to the most recent common ancestors (tMRCA). These authors proposed that a “global dissemination of ZIKV spread was likely to have originated from Africa, followed by eastward transmission to South-eastern Asia, Oceania, South America, Caribbean and Central America.” Regarding the spread of the virus from Africa to Asia, the authors had the following interesting observations:

“Relatively few studies on the origin of the South Pacific Rim lineage had been reported in the literature. The tMRCA for South Pacific Rim was estimated to be 1947 (95% HPD interval.35 to 1966) in our study. Coincidentally, during the Second World War in South-eastern Asia, around 100,000 East and West African soldiers were brought into combat in the Burma Campaign from January 1942 to July 1945. Specifically, the British Empire colonial unit 11th (East Africa) Infantry Division comprised troops from East and

West African countries such as Kenya, Uganda, Nyasaland, Tanganyika and Rhodesia (Burma Star Association--The 11th East African Division). During those three-years' conflict, both sides suffered heavy casualties, including at least 20,000 Japanese soldiers who died because of disease in the *battle of Imphal*. It is also noteworthy that Thai army was also involved in this campaign and that after the Japanese surrendered, troops were continued to be deployed to the then Malaya. The whole campaign could serve as a possible portal of entry for the transmission of ZIKV from Africa to South-eastern Asia during that wartime period.”

This scenario of infectious disease transmission is not without precedents. The first incidences of possibly smallpox in Europe was the Antonine Plague of 165 to 180 AD, also known as the Plague of Galen (from the name of the Greek physician living in the Roman Empire who described it), was an ancient pandemic brought back to the Roman Empire by troops returning from the Near East campaigns [6]. The Plague of Justinian (541–542 AD) afflicted the Eastern Roman (Byzantine) Empire, especially its capital Constantinople, the Sasanian Empire, and port cities around the entire Mediterranean. It probably originated in Ethiopia and came through rodents in ships carrying grain to Rome. Smallpox infections that decimated the native populations of the New World was carried there by the Spanish conquistadores [7]; in turn syphilis was carried eastwards from the New World to Europe and thence the whole world. Currently, pandemic viruses gain increasingly rapid circulation through globalization and worldwide fast travels in our inter-connected world.

2. STATUS OF GLOBAL ZIKA INFECTION AND TRANSMISSION

Viral epidemics and pandemics at the initial stage grow very fast, then after a period of a few months fall into quiescence as the populations get herd immunity or the virus itself mutates away from its destructive form. Often as in the case of the bubonic plague, or the H1N1 influenza, the virus may start another round of epidemic and the process goes on and on.

Following a similar trend, of late, the Zika epidemic has lost its severity. Governments that had issued travel advisories at the height of the ZIKV pandemic have revised the advisories, but still caution against travel to Zika infested areas. Here is the current CDC Zika Travel Advisory and Information [8].

2.1. Areas with Risk of Zika

Because Zika infection during pregnancy can cause severe birth defects, pregnant women should not travel to the areas below. Partners of pregnant women and couples considering pregnancy should know the risks to pregnancy and take prevention steps. All travelers should strictly follow steps to prevent mosquito bites and prevent sexual transmission during and after the trip.

Africa: Angola, Benin, Burkina-Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, Congo (Congo-Brazzaville), Côte d’Ivoire, Democratic Republic of the Congo (Congo-Kinshasa), Equatorial Guinea, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Kenya,

Liberia, Mali, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, South Sudan, Sudan, Tanzania, Togo, Uganda.

Asia: Bangladesh, Burma (Myanmar), Cambodia, India, Indonesia, Laos, Malaysia, Maldives, Pakistan, Philippines, Singapore, Thailand, Timor-Leste (East Timor), Vietnam

The Caribbean: Anguilla; Antigua and Barbuda; Aruba; Barbados; Bonaire; British Virgin Islands; Cuba; Curaçao; Dominica; Dominican Republic; Grenada; Haiti; Jamaica; Montserrat; the Commonwealth of Puerto Rico, a US territory; Saba; Saint Kitts and Nevis; Saint Lucia; Saint Martin; Saint Vincent and the Grenadines; Sint Eustatius; Sint Maarten; Trinidad and Tobago; Turks and Caicos Islands; US Virgin Islands

Central America: Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama

North America: Mexico

The Pacific Islands: Fiji, Papua New Guinea, Samoa, Solomon Islands, Tonga

South America: Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Suriname, Venezuela

2.2. Areas with Interrupted Transmission

Zika was previously found in the locations on this list, but scientists have determined the virus is no longer present. This means all travelers, including pregnant women, can visit these destinations with no known risk of getting Zika from mosquitoes. If Zika returns to a country or territory on this list, CDC will remove it from the list and post updated information. Check this page for the most up-to-date information before making travel plans.

We give below the information regarding the geographical area and the date of Interruption [8].

Area	Date of interruption
American Samoa	13 April 2017
The Bahamas	2 February 2018
Cayman Islands	20 July 2017
Cook Islands	10 March 2017
Guadeloupe	29 June 2017
French Polynesia	10 March 2017
Isla de Pascua, Chile	10 March 2017
Marshall Islands	9 January 2018
Martinique	29 June 2017
Micronesia	23 November 2017
New Caledonia	10 March 2017
Palau	23 November 2017
Saint Barthélemy	24 May 2017
Vanuatu	10 March 2017

3. VIROLOGY OF THE ZIKA VIRUS

The Zika virus belongs to the Flavivirus genus that is part of the family Flaviviridae. Some important disease-causing viruses of this genus are Dengue fever virus, Tick-borne encephalitis virus, West Nile virus, Japanese encephalitis virus, and Zika virus [9]. The viruses of this group contain a single stranded positive sense RNA genome containing about 11,000 nucleotides [10]. Consequently, the RNAs of such viruses can be directly translated to a large polyprotein that is subsequently converted by viral and host cell proteases into two sets of proteins: Structural and nonstructural. The three structural proteins are: Envelope, E; membrane precursor, PrM; and capsid, C (Figure 1.3). These are critical for the formation of the capsid and envelop of the mature virus. The seven non-structural (NS) proteins, on the other hand, have important roles in the replication of the virus. The NS group of proteins include: NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5 as shown in Figure 1.3 [11].

Table 4 summarizes the structure of Flavivirus genome including the untranslated regions, 5'-UTR and 3'-UTR regions [1, 11].

The 3' terminus has a sub-genomic Flavivirus RNA (sfRNA) structure that is essential for causing disease in humans [12, 13]. The envelope protein comprises most of the virion surface and participates in viral-host cell binding and membrane fusion during replication.

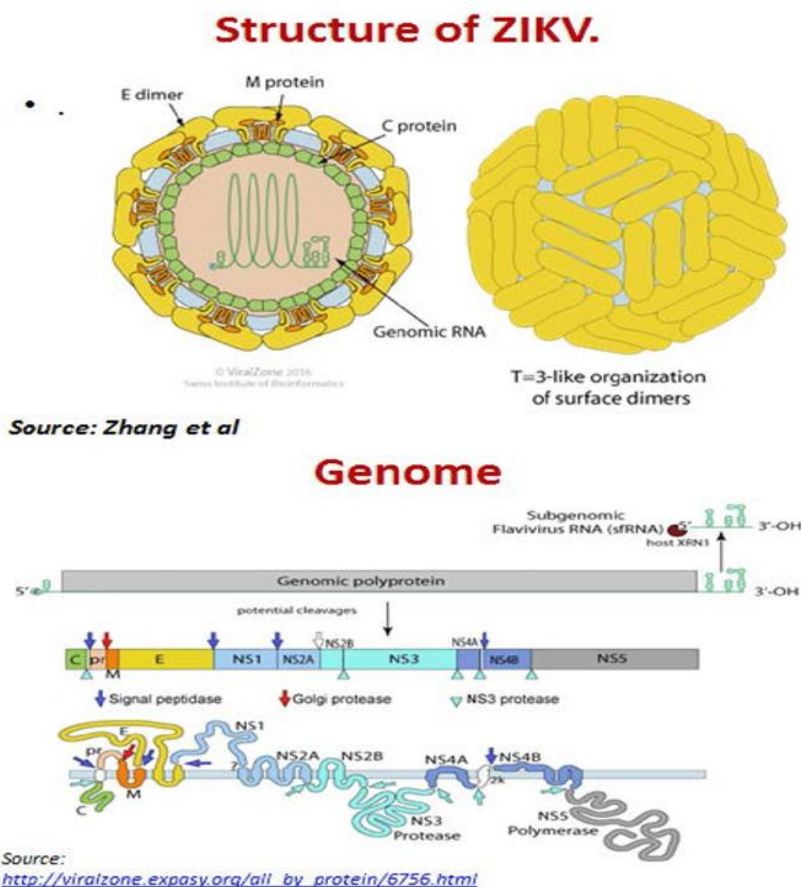


Figure 1.3. The three structural proteins and seven non-structural (NS) proteins of Flaviviruses.

Replication of ZIKV, like other Flaviviruses, occurs in the cytoplasm of infected cells [14, 15]. After endocytosis mediated by a still unknown cellular receptor, viral fusion occurs with endosomal membranes. This results in the uncoating of the viral particle and release of the viral genome. The genome is subsequently translated into a single polyprotein at the endoplasmic reticulum (ER). The polyprotein is processed by viral NS2B–NS3 protease and other cellular proteases generating mature viral proteins. The positive-sense viral RNA genome is then copied to produce a negative-sense RNA molecule chain, which is used as the template for synthesis of the full length positive-sense viral genomic RNA. Synthesis of viral genome occurs in association with the ER and is catalyzed by a replication complex consisting of NS5 RNA-dependent RNA polymerase and other NS proteins. The new genomes are packaged by the C protein; they acquire envelopes while budding from the ER. These immature virions thus produced are translocated through the cellular secretory pathways, where E glycosylation and cleavage of prM by host furin protease occurs producing the mature virions that are released by exocytosis.

Table 4. Structure of a typical flavivirus genome including UTRs

Srl No	Gene	Sequence span, nt	Sequence length*	Protein/Biological function
1	5'-UTR	1-107	107	Encodes regions essential for genome cyclization/replication.
2	Capsid	108-473	366	Virion structure.
3	prM/M	474-977	504	prM forms heterodimers with E to form immature virion. prM then cleaved and mature virions formed with M.
4	E	978-2489	1512	Viral entry into host cell.
5	NS1	2490-3545	1056	Viral replication, immune evasion, genome synthesis.
6	NS2A	3546-4223	678	Transmembrane protein, part of replication complex; assembly/secretion of virus particles.
7	NS2B	4224-4613	390	Cofactor for proteinase domain of NS3; proteolytic processing.
8	NS3	4614-6464	1851	Protease/helicase.
9	NS4A	6465-6914	381	Viral RNA replication and amplification.
10	2K	6846-6914	69	Peptide generated by cleavage at the N terminus of the NS4B signal sequence.
11	NS4B	6915-7667	753	Facilitates viral replication complexes; counteracts innate immune responses.
12	NS5	7668-10376	2709	Methyltransferase; RNA-dependent RNA polymerase.
13	3'-UTR	10380-10807	427	Facilitates viral replication and translation.

* Typical length data taken from ZIKV isolate ZIKV/*H.sapiens*/Brazil/PE243/2015 (GenBank Locus ID KX197192), complete genome. E, envelope; NS, non-structural; nt, nucleotide; prM/M, pre-membrane/membrane; UTR, untranslated region.

From its initial stage in Uganda, the ZIKV has undergone quite a few changes. Figure 1.4 gives a phylogenetic tree of 22 ZIKV sequences [16].

Although the typical nucleotide sequences provide a complete description of the linear arrangement of the bases in the gene, graphical representation methods [17] may lead to visualization of the differences among sequences. Figure 1.5 shows such a representation of ZIKV genomes from Central African Republic 1976 to Brazil 2015 by the 2D graphical method

developed by one of the authors of this chapter (Nandy) for compact representation and visualization of nucleic acid sequences [17].

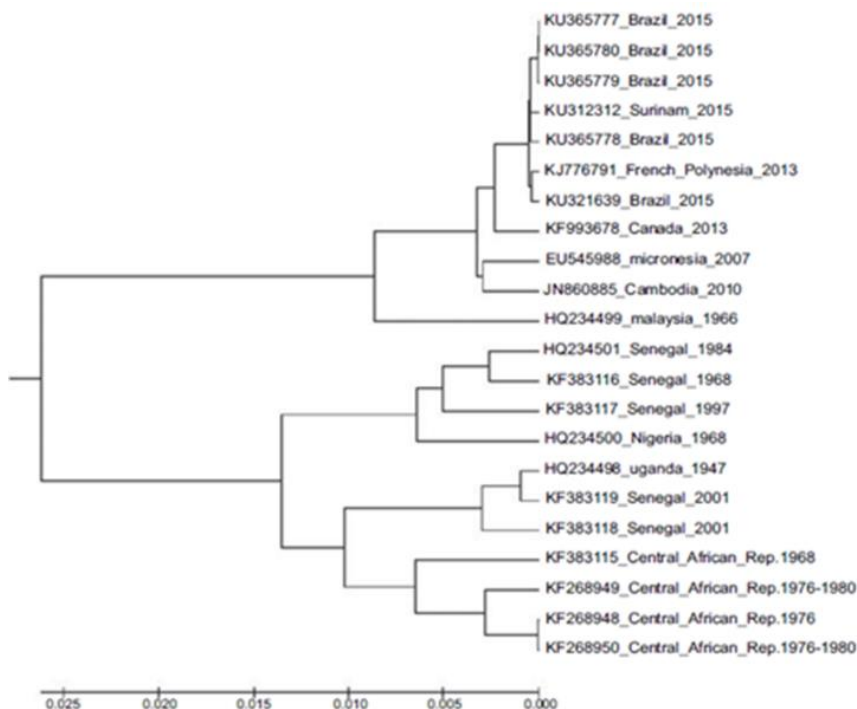


Figure 1.4. Phylogenetic tree of 22 ZIKV genome sequences. Nandy et al.16. Reproduced with permission from Bentham Science Publishers, www.benthamscience.com. ZIKV, Zika virus.

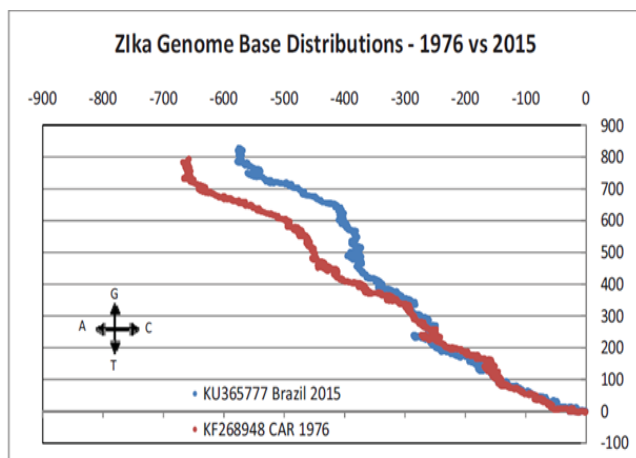


Figure 1.5. Comparison of ZIKV genomes from Central African Republic 1976 to Brazil 2015 in 2D graphical representation¹⁶. Axes AGCT as shown in the inset.

The algorithm involved starting at the origin and plotting a point for each base in the nucleotide sequence by a step in the xy-plane defined by A(-1,0), C(+1,0), G(0,+1), T(0,-1) until the end of the sequence, thus plotting a graph that reflects the base distribution and composition of the sequence. Figure reproduced with permission from Bentham Science Publishers, www.benthamscience.com. 2D, two-dimensional; ZIKV, Zika virus.

4. CLINICAL COMPLICATIONS AND ZIKA VIRUS

4.1. Clinical Signs and Symptoms

The Centers for Disease Control and Prevention [18] gives a detailed list of information about the clinical aspects of the Zika virus infection.

Many people infected with Zika virus do not develop many overt symptoms. The symptoms where they do develop last for several days to a week. However, there have been cases of Guillain-Barré syndrome reported in patients after Zika virus infection. It has been concluded by CDC that Zika virus infection during pregnancy is associated with microcephaly and other severe fetal brain defects. Due to this concern for microcephaly arising out of maternal Zika virus infection, fetuses and infants of women infected with Zika virus during pregnancy should be evaluated for possible congenital infection and neurologic abnormalities.

4.2. Zika and Guillain-Barre Syndrome

Another complication of ZIKV infection is its association with the Guillain-Barré syndrome (GBS), a neurological disorder [19-21].

The exact cause of Guillain-Barré syndrome is unknown. It can occur a few days or weeks after the person has had symptoms of a respiratory or gastrointestinal viral infection. Occasionally, surgery will trigger the syndrome. In rare instances, vaccinations may increase the risk of GBS. Recently, some countries around the world have reported an increased incidence of GBS following infection with the Zika virus.

As is well known, currently there is no available cure for GBS [22]. Some therapeutic interventions can only decrease the severity of disease and shorten recovery time.

4.3. Risk factors for Zika Virus [18]

Factors that put a person at greater risk of developing Zika virus disease include:

Living in or traveling to areas where there have been ZIKV outbreaks. Being in tropical and subtropical areas increases the risk of exposure to the Zika virus disease.

Having unprotected sex. Isolated cases of sexually transmitted Zika virus have been reported. The CDC advises abstinence from sexual activity during pregnancy or condom use during all sexual contact for men with a pregnant sex partner if the man has traveled to an area of active Zika virus transmission.

5. MODES OF TRANSMISSION OF ZIKA VIRUS

Current CDC website gives the following information regarding the transmission of the ZIKV transmission [23].

Zika virus can spread in various ways described below [23, 24]:

Through mosquito bites: Zika virus is transmitted to people primarily through the bite of infected mosquitoes (*Ae. aegypti* or *Ae. albopictus*). Mosquitoes become infected when they feed on a person already infected with the virus. Infected mosquitoes can then spread the virus to other people through bites.

From mother to child: A pregnant woman can pass Zika virus to her fetus during pregnancy.

Through sex: Zika can be passed through sex from a person who has Zika to his or her partners. Zika can be passed through sex, even if the infected person does not have symptoms at the time. It can be passed from a person with Zika before their symptoms start, while they have symptoms, and after their symptoms end.

Through blood transfusion: There have been multiple reports of blood transfusion transmission cases in Brazil. These reports are currently being investigated.

During the French Polynesian outbreak, 2.8% of blood donors tested positive for Zika and in previous outbreaks, the virus has been found in blood donors.

Through laboratory and healthcare setting exposure:

Prior to the current outbreak, there were four reports of laboratory acquired Zika virus infections, although the route of transmission was not clearly established in all cases.

As of June 15, 2016, there has been one reported case of laboratory-acquired Zika virus disease in the United States [23].

To date, no cases of confirmed Zika virus transmission in healthcare settings have been reported in the United States. Recommendations are available for healthcare providers to help prevent exposure to Zika virus in healthcare settings.

6. DIAGNOSIS OF ZIKA VIRUS

The diagnosis and isolation of ZIKV is a complex process which include assays such as reverse transcription-polymerase chain reaction (RT-PCR), real-time qRT-PCR, loop-mediated isothermal amplification (LAMP), lateral flow assays (LFAs), biosensors, nucleic acid sequence-based amplification (NASBA) tests, strand invasion-based amplification tests, and immune assays like enzyme-linked immunosorbent assay (ELISA). The readers are advised to consult the relevant literature on the subject [25, 26].

7. ZIKV INTERACTION WITH PERMISSIVE CELL TYPES

ZIKV is a versatile infective agents and attacks different types of permissive cells in the human body using multiple mechanisms. In Table 5 we give a few examples of how ZIKV interacts with various permissive cell types.

As is clear from data [27-29] in Table 5 above and other studies [30-32], ZIKV uses a diverse set of biochemical mechanisms to infect the target cells.

Table 5. Examples of ZIKV infection and entry into different types of cells

Cell/Tissue	Virus source/strain	Mechanism of entry	Literature source
Human dermal fibroblasts, epidermal keratinocytes and immature dendritic cells	ZIKV isolate, from French Polynesia	Entry and/or adhesion factors included DC-SIGN1, AXL, Tyro3, and, to a lesser extent, TIM-1, permitted ZIKV entry, with a major role for the TAM receptor AXL.	Ref [27]
Human microglia and astrocytes	ZIKV ^{BR}	Activates AXL kinase activity, which down modulates interferon signaling	Ref [28]
Vero cells, human brain microvascular endothelial cells, human neural stem cells, and mouse neurospheres.	ZIKV strain MR 766 (Uganda/Africa, accession no.: NC012532.1	Entry of ZIKV inhibited by Chloroquine	Ref [29]

CONCLUSION

From its initial reservoir in the Zika forest of Uganda ZIKV has spread into large parts of the globe which includes Asia, other parts of Africa, the Caribbean, Central America, The Pacific Islands, and South America. Currently, the virus has two dominant subtypes: a) Southern-Eastern Asian type and b) African type. It can attack different organs of the human body and cause multiple short-term and long-term pathologies. ZIKV enters human cells and interacts with them using a multiplicity of mechanisms. Understanding the mechanisms of ZIKV- human cell interactions at the ultrastructural and biochemical levels may lead not only to more thorough understanding of Zika virus virology, but also to intervention strategies through rational vaccine design as well as novel drug discovery.

One interesting area of research in ZIKV biochemistry is the discovery of biomarkers [33] associated with infection by the virus and subsequent pathological conditions. This may help in an in-depth understanding of ZIKV mediated pathological processes.

In view of the frequent occurrence of emerging pathogens in recent times, we need to focus research on the characterization of reservoirs of next possible pathogens. This aspect is covered in chapter 5 of this book. Once the emerging outbreak of virus has been identified, it must be sequenced quickly to understand what type it is and by which molecular mechanisms it spreads through human populations and how it affects the infected individuals. Fast computational sequence comparison methods are needed for this purpose and this aspect is discussed in chapter 4 of the book.

Currently, no drug or vaccine is known for treatment of ZIKV. For emerging pathogens like the ZIKV, we need both vaccine and new drugs that can prevent the infection and treat the clinical symptoms produced by the virus. Chapter 2 and 3 cover these two aspects. Chapter 2 covers computer-assisted discovery and design of potential anti-ZIKV drugs and Chapter 3 discusses computer-assisted design of peptide vaccines for prevention against Zika virus.

Discovery of a new drug takes about 10 to 15 years and costs over two billion US dollars [34, 35]. Therefore, any technology that can improve the efficiency of the process is highly valuable to this endeavor. Computer aided drug discovery thus plays a pivotal role in bringing

new potential anti-ZIKV drug to the forefront for this effort. Since no antiviral drug is currently available for tackling ZIKV, a fast track approach is necessary to counter the menacing movement of the virus. One quick approach could be “repurposing” of existing or abandoned drugs and shortlisting them using computational methods to circumvent the urgent need for anti-ZIKV drugs. Novel uses of already existing drugs will cost much less as compared to the pursuit of new drug discovery process. A successful recent attempt was reported through anti-ZIKV testing of 774 Food and Drug Administration (FDA) approved drugs [36]. The knowledge contained in these compounds can further be utilized in combination with chemoinformatic tools to develop models for harnessing the advantage toward more effective discovery of anti-ZIKV drugs. Pharmacophore based screening is one such tool that can be effective since the target structure of proteins involved in ZIKV virus is still uncertain. Since pharmacophore transcends the chemical structural class and captures only the features responsible for activity, use of pharmacophore has the advantage for identification of potentially new biologically active compounds of novel chemical scaffolds to open new chapters for chemotherapy of ZIKV [37, 38].

This book describes a holistic approach to the Zika virus problem involving characterization of reservoirs, surveillance of pathogens using genomics well as sequence comparison methodologies, vaccine design for the prevention of infection, and computer-assisted drug discovery.

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Chapter 2

ZIKA VIRUS INFECTION: A NEW THREAD FOR PREGNANCY

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ABSTRACT

Zika Virus (ZIKV) is a ribonucleic acid (RNA) virus of the *Flaviviridae* family, genus *Flavivirus*, *Spondweni* group, *Zika* species, isolated for the first time in 1947 from a primate in Uganda, in the Zika Forest, a natural reserve near Entebbe. In the years 2010, there were occurrences of the disease in all continents and isolated cases were also recorded in Europe, on subjects who were returning from travel in tropical areas and without any form of transmission of the virus in Europe. In humans, it causes a disease known as “zika” or zika fever. The virus is closely related to those that cause dengue, yellow fever, West Nile encephalitis, and Japanese encephalitis, all transmitted mainly by insect bites and therefore called “arthropode-borne viruses” (arboviruses). The virus is transmitted by numerous mosquitoes of the genus *Aedes*, in equatorial environments especially by *Aedes aegypti*. Studies on the vectors involved in the transmission of the virus have allowed to isolate ZIKV also in some populations of *Aedes albopictus* (tiger mosquito) coming from Gabon. As this mosquito has become very common even in the temperate climates of the northern hemisphere, such as in North America, the Italian peninsula, and other areas of the Mediterranean basin, it could in the future pose a serious risk for the expansion of the virus even in these areas of the planet. In this regard, however, there are no documented cases of transmission of the virus in Europe by this mosquito. Like the other viruses in this family, ZIKV is enveloped by an icosahedral capsid and the genome consists of a single positive-sense RNA helix, 10,794 nucleotides long. From a phylogenetic point of view, it is closely related to the Spondweni Virus, a virus that forms a group of its own (clade) within the *Flavivirus* genus with ZIKV. It shares the same structural characteristics and replication cycle with the other members of the genre, as well as the genetic organization itself, except for the 3' untranslated region (UTR). Other members belonging to the same genus are the

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Ilheus Virus (ILHV), the virus that causes Rocio encephalitis or Rocio Virus (ROCV), and Saint-Louis Encephalitis Virus (SLEV). There are cases of possible viral co-infection such as dengue + chikungunya + zika. Zika fever is predominantly contracted by arthropod (mosquitoes) bites, although direct infection through blood products or by sexual means is possible, given the presence of virions in the semen of affected men. Main animal reservoirs of infection are primates, some large mammals (hippopotamuses, impalas, elephants, goats, sheep, hartebeests, lions, wildebeests, and zebras) and rodents. During the first week of infection, ZIKV is present in the bloodstream and can be isolated by the subject's blood. Many mosquito species have been identified as vectors of the virus, belonging to the genera *Aedes*, *Anopheles*, *Mansonia*, and *Eretmapodites*. In hot climates, the main vector is *A. africanus* and *A. aegypti* and, to a lesser extent, *A. polynesiensis*, *A. unilineatus*, *A. vittatus*, and *A. hensilli*. During the zika fever epidemic in Yap Island in 2007, *Aedes hensilli* was identified as the main vector. In the other more temperate climates, a potential vector could become the tiger mosquito (*Aedes albopictus*), abundantly distributed throughout the world. However, no cases of transmission of the virus in European areas have been documented although this mosquito is now quite common in some areas such as the Italian peninsula or other areas of the Mediterranean basin and central Europe.

The known transmission routes are: perinatal, sexual transmission, saliva, urine, semen, and breast milk. The possibility of maternal-fetal contagion is also likely, since the virus was found inside the placenta and in the amniotic fluid of pregnant patients, with consequent teratogenic effects reported on the embryo, especially in the first trimester of pregnancy. It is reported the possibility of virus transmission with the saliva/kiss, but this way is very remote, due to the low viral load in the saliva. The possibility of sperm transmission, on the contrary, is instead documented, having been officially confirmed in February 2016 the second case of transmission of the virus by sexual means, in Texas, in a woman infected by the partner returned from a trip to South America. A Brazilian study published by *The Lancet* on February 17th, 2016 suggested the possible confirmation of maternal-fetal transmission with amniotic fluid (vertical transmission). The strain affecting Brazil in 2016 is genetically related to that found in French Polynesia in 2013; in addition, researchers wrote that this strain is responsible for the cases of microcephaly found in Brazil to an abnormally high extent compared to before ZIKV epidemic, even though there is still no definitive evidence of the tropism of the virus on human nerve cells. The virus was isolated in 1947 from a specimen of mulatto macaque present in the Zika Forest of Uganda, and later in 1968 it was isolated for the first time in humans in Nigeria. From 1951 to 1981, similar human specimens were also positive in Uganda, Tanzania, Egypt, Central African Republic, Sierra Leone, Senegal, and Gabon. In Asia, it was found in India, Malaysia, Philippines, Thailand, Vietnam, and Indonesia where it was found in 2006. The first known zika outbreak from Africa occurred in 2007 in Yap Island, Micronesia. ZIKV was found in 2015 also in Colombia, Suriname, El Salvador, Guatemala, Cape Verde, Fiji, Vanuatu, Samoa, New Caledonia, Solomon Islands, Indonesia, Mexico, and Brazil. The World Health Organization (WHO) declared in February 2016 that ZIKV epidemic is a Public Health Emergency of International Concern (PHEIC) or "an international public health urgency"; just a few months before the start of the 2016 Olympics that would take place in Brazil in August. Still, the WHO, waiting for a vaccine to be prepared, suggests resorting to therapeutic abortion to the governments of the Countries involved in the epidemic, in order to avoid possible explosive growth of cases of microcephaly in the offspring of infected pregnant women. Studies that have analyzed the whole RNA of the infectious agent have demonstrated high genomic homology between the Latin-American stock and the one that circulated in the Pacific between 2013 and 2014. In 2015, in Brazil, the prevalence of new cases was estimated between 440,000 and 1,300,000, while native cases were recorded in Colombia, El Salvador, Guatemala, Mexico, Paraguay, Puerto Rico, and Venezuela. Furthermore, again in Brazil, in 2015 there were as many as 3,500 cases of

microcephaly in infants, compared to the previous 150 cases/year. In healthy United States (US) travelers, returning from Countries where the virus is endemic, serological positivity to specific antibodies has been reported. These imported cases are destined to increase and may lead to the local spread of the virus in some areas of the US. *The New York Times* of January 17th, 2016 reported the first US case of microcephaly in a newborn, born in Ohau in Hawaii, whose mother had stayed in Brazil for several months during pregnancy. An additional case has been reported in Texas. On January 27th, 2016, the first case of zika in Argentina was confirmed. In Spain, the first case in Europe of pregnant woman infected by ZIKV was reported on February 4th, 2016. As of February 6th, 2016, 9 cases of ZIKV infection were reported in Italy. The first case of ZIKV disease in China was reported on February 9th, 2016.

It is hypothesized that the infection starts from dendritic cells close to the inoculum site, followed by development in the satellite lymph nodes and then through the bloodstream. Typically, Flaviviruses replicate in the cytoplasm, but ZIKV antigen has also been found in the nuclei of infected cells. The incubation period of the disease after the puncture with the vector is about 10 days. The disease caused by this virus is asymptomatic in 25% of cases (according to the Centers for Disease Control and Prevention [CDC], 80% of people infected with the virus are asymptomatic), or causes mild symptomatology, as in dengue, with which it can be confused. In this case, symptoms may be mild fever, rash (exanthema), conjunctivitis, and headache. ZIKV infection is usually mild, with symptoms lasting only a few days. Since there is no etiological therapy that reduces infection, treatment focuses essentially on alleviating symptoms and preventing further infection. Prevention and control are based on reducing the development of mosquitoes of the *Aedes* genus and minimizing the contact between vector mosquitoes and people, by means of barriers (repellents, mosquito nets), reducing water stagnations that support development mosquito pups and larvae, and breaking down, with disinfection, the population of adult mosquitoes in communities at risk. The differential diagnosis should be made with the following infections: dengue, leptospirosis, malaria, rickettsiosis, group A streptococcus, rubella, measles, parvovirus, enterovirus, adenovirus, alphavirus (Chikungunya Virus [CHIKV], Mayaro Virus [MAYV], Ross River Virus [RRV], Barmah Forest Virus [BFV], O'Nyong-Nyong Virus [ONNV], and Sindbis Virus [SINV]). The diagnostic tests for ZIKV are: deoxy-ribonucleic acid (DNA) reverse transcription polymerase chain reaction (RT-PCR) on serum. Immunoglobulins M (IgM), neutralizing antibodies that develop, generally, towards the end of the first week of specific virus disease, are in cross-reaction with the related Flaviviruses (e.g., Dengue Virus [DENV] and Yellow Fever Virus [YFV]); the virus is common and can be difficult to distinguish. In order to refine the diagnosis, specific tests have been introduced to distinguish zika from other Flaviviruses. The mechanisms with which the clinical complications of zika infection are expressed seem to be due to a duplication/amplification of the centrosomes of the nerve cells. This causes in the mice neural delayed mitosis, increased apoptosis, with stem cell disorientation, premature neuronal differentiation, and reduction of the number of progenitor cells, all mechanisms that well explain microcephaly cases. Infection with this virus in pregnant women is believed to result in microcephaly and fetal malformations in the baby. In 2015, in the North of Brazil there was a very important increase in cases of microcephaly, whose cause seems to be closely related to ZIKV infection in pregnant women, even asymptomatic. The CDC in Atlanta, US, in January 2016 issued level two alert for pregnant or at risk to be pregnant women so that, if possible, they avoid going to Countries at risk (Brazil, Colombia, El Salvador, French Guiana, Guatemala, Haiti, Honduras, Martinique, Mexico, Panama, Paraguay, Puerto Rico, Dominican Republic, Suriname, and Venezuela) or observe adequate precautions. On autopsy examination of a 29-week fetus, aborted by a woman with zika fever in the first trimester of pregnancy, it was observed that she presented: "almost complete agyria (lack of brain convolutions), hydrocephalus, and multifocal dystrophic calcifications in the cortex and subcortical white matter, with

associated cortical displacement and mild focal inflammation.” ZIKV was found in fetal brain tissue and it was possible to study and have its complete genome. With this work, the relationship between ZIKV and microcephaly in pregnancy was confirmed. There is a correlation between ZIKV infection and Guillain-Barré syndrome (GBS): 74 cases of this disease were diagnosed in individuals already affected by the infection in French Polynesia. On February 6th, 2016, three deaths were confirmed due to this severe syndrome in subjects with previous ZIKV infection. Research has shown that 2.4 patients every 10,000 affected by ZIKV get GBS after a few days of infection, with a frequency about 20 times greater than expected cases; of these, 3-5% undergo mortal complications. This causes serious concern for the health systems and populations of Countries affected by the zika outbreak. A report from the European CDC (ECDC) indicates that in addition to an increase in the number of cases of GBS, there may also be a significant increase in cases of encephalitis, meningoencephalitis, facial paralysis, and myelitis. In February 2016, a Brazilian case-report correlated hypertensive uveitis with zika infection. Other Brazilian researchers have found important malformations of the optic nerve and of the macula in neonates affected by post-zika microcephaly.

Since no specific antiviral treatment exists, therapy is essentially based on rest, fluids, and the use of antipyretics if needed. Because of the symptomatic similarity, and up to the precise identification of the infectious agent, it is advisable to consider patients with suspected zika in the same way as suspected dengue or chikungunya. For this reason, the intake of aspirin or other non-steroidal anti-inflammatory drugs (NSAID) may be inadvisable to reduce the risk of bleeding, as in dengue. The Authorities believe that this virus can have some impact also in transfusion medicine. In fact, the ECDC requires 28-day postponement of blood donation in subjects who have had possible symptoms of the disease, and 14-day one in those who returned from Countries at risk. All zika patients, or suspected ones, must also be immediately adequately isolated from the possible contact with mosquitoes, in order to reduce the risk of local transmission. First, avoid, where possible, travel in the epidemic areas, above all for pregnant women or women at risk of pregnancy during stay abroad. Aggressive methods of prevention against vectors (mosquitoes), including repellents based on N,N-diethyl-meta-toluamide (DEET), picaridin, and Insect Repellent 3535 (IR3535) are advisable; these are considered safe even during pregnancy. Serological tests, including RT-PCR, should be made on women who have returned from stays in areas endemic to zika fever.

Keywords: Zika Virus, pregnancy, microcephaly, Guillain-Barré syndrome

INTRODUCTION

At present, one of greatest concerns of medical caregivers is the arthropod-borne virus (arbovirus) Zika Virus (ZIKV). ZIKV is a mosquito-borne single-stranded ribonucleic acid (RNA) virus, member of *Flaviviridae* family in the genus *Flavivirus*, closely related to Japanese Encephalitis Virus (JEV), West Nile Virus (WNV), Dengue Virus (DENV), Chikungunya Virus (CHIKV), and Yellow Fever Virus (YFV). ZIKV spread across Africa and Asia to the Americas, in part owing to unique genomic evolutionary conditions and pressures resulting in specific human disease manifestations, complications, and pathogenesis. The virus is transmitted primarily through the bite of infected blood-sucking *Aedes aegypti* and *Aedes albopictus* (*Stegomyia*) mosquitoes, but other routes of transmission include sexual, mother-to-fetus during pregnancy, mother-to-infant at delivery, laboratory exposure, and, possibly, transfusion of blood products. Historically, ZIKV infection was considered a sporadic,

relatively mild disease. Although ZIKV infection generally causes only mild symptoms in some infected individuals, it is associated with a range of neuro-immunological disorders, including meningoencephalitis and myelitis, due to a reduction of proliferative zones and disrupted cortical layers and synaptogenesis. An increased incidence of Guillain-Barré syndrome (GBS), an immune-mediated neuropathy of the peripheral nervous system (PNS), has also been reported in ZIKV-infected patients. Moreover, the amniotic fluids, blood cord, and miscarriage tissues of mothers have been detected with ZIKV, which indicates that the virus either gets transferred from mother to fetus or seeks direct entry in the fetus, causing microcephaly and other brain anomalies in the newborn babies and even abortion [1]. Reports have highlighted the sexual transmission of ZIKV, as it has been detected in the semen and saliva of affected persons [2]. With the increased number of cases, new complications such as ocular involvement and severe thrombocytopenia have been reported. Head and neck manifestations include conjunctivitis, retro-orbital pain, cephalgia, and odynophagia [3, 4].

Though ZIKV has been reported for quite a long time, its rapid emergence, new modes of transmission, and, more importantly, the congenital anomalies associated with it have made the situation worse. Since its first discovery in the Zika Forest in Uganda in 1947, incidentally isolated from a sentinel Rhesus monkey and from mosquitoes in the same forest the following year, and its first human infection in 1952, ZIKV has continued to expand its geographic range, from equatorial Africa and Asia to the Pacific Islands, then further afield to South and Central America and the Caribbean. Although entomological and virologic surveillance have reported ZIKV enzootic activity in diverse Countries of Africa and Asia, few human cases were reported until 2007, when a Zika fever epidemic took place in Micronesia. The rapid expansion of ZIKV in the South and Central America has recently gained medical attention, emphasizing the capacity of ZIKV to spread to non-endemic regions. In the context of West Africa, the World Health Organization (WHO) Collaborating Center for Arboviruses and Hemorrhagic Fever at Institute Pasteur of Dakar reported the periodic circulation of ZIKV since 1968. Currently, ZIKV is actively circulating not only in much of Latin America and its neighbors but also in parts of the Pacific Islands and Southeast Asia. Since local mosquito-borne transmission of ZIKV was first reported in Brazil in early 2015, the virus has spread rapidly to several surrounding Countries, with active transmission reported in at least 61 Countries and territories worldwide, including the United States (US) and there is concern about the spread of the virus in areas where competent vectors are present. Though it is asymptomatic in maximum number of cases, the serious complications of the infection should be prevented at the earliest. Despite its emergence as an important public health problem, however, little is known about ZIKV biology, and neither vaccine nor effective therapies currently exist for treating patients infected with ZIKV, thus treatment is supportive and prevention is based on individual measures and on vectors control. But research continues and hopefully, success is not far off. The right information about this infection should reach patients as well as physicians. It will prevent unnecessary panic [5, 6]. A large number of studies have been published in the last five years, revealing the structure of the virus, how it is transmitted, and how it affects human cells. Many different animal models have been developed, which recapitulate several features of ZIKV disease and its neurological consequences. Moreover, several vaccine candidates are now in active pre-clinical development, and three of them have already entered phase I clinical trials. Likewise, many different compounds targeting viral and cellular components are being tested in *in vitro* and in experimental animal models [7, 8].

ZIKV has emerged as a global public health threat over the last decade, with the accelerated geographic spread of the virus noted during the last 10 years. The WHO predicted that millions of cases of ZIKV were likely to occur in the Americas during the last five years. These projections, in conjunction with suspected ZIKV-associated increase in newborn microcephaly cases, resulted in activation of the Center for Disease Control and Prevention (CDC)'s Emergency Operations Center (EOC) on January 22nd, 2016, to ensure a coordinated response and timely dissemination of information, and led the WHO to declare a Public Health Emergency of International Concern (PHEIC) on February 1st, 2016. ZIKV-associated illness is characterized by an incubation period of three to 12 days. Most patients (i.e., ~80%) remain asymptomatic after contracting the virus. When symptomatic, clinical presentation is usually mild and consists of a self-limiting febrile illness that lasts approximately two to seven days. Among common clinical manifestations are fever, arthralgia, non-purulent conjunctivitis, myalgia, headache, maculopapular rash, and weakness. Hospitalization and complication rates are low, with fatalities being extremely rare. Newborn microcephaly, the most devastating and insidious complication associated with ZIKV, has been described in the offspring of women who became infected while pregnant. Much remains to be elucidated about the timing of ZIKV infection in the context of the temporal progression of pregnancy, the corresponding *in utero* fetal development stages, and the risk of microcephaly. Without further knowledge of the pathophysiology involved, the true risk of ZIKV to the unborn remains difficult to quantify. Accurate, portable, and inexpensive point-of-care testing is required to better identify cases and manage the current and future outbreaks of ZIKV, including optimization of preventive approaches and the identification of more effective risk-reduction strategies. ZIKV testing is recommended for persons with possible exposure (those who have traveled to or live in an area with active transmission, or persons who had sex without a condom with a person with possible exposure) if they have symptoms consistent with ZIKV disease. Testing is also recommended for pregnant women with possible exposure, regardless of whether symptoms are present. Clinicians should understand the infection, its diagnosis, and testing, and monitor pregnant women for travel history to outbreak regions and for the presence of clinical symptoms. Patient education on preventive measures offers the best option to avoid ZIKV infection. Given the rapid geographic spread of ZIKV in recent years, a coordinated local, regional, and global effort is needed to generate sufficient resources and political traction to effectively halt and contain further expansion of the current outbreak [9, 10]. During the past years, public health agencies and researchers worldwide have collaborated to protect pregnant women, inform clinicians and the public, and advance knowledge about ZIKV. The primary methods of prevention include avoiding bites of infected *Aedes* mosquitoes and reducing the risk of sexual transmission. To protect pregnant women and their fetuses and infants from the effects of ZIKV infection during pregnancy, public health activities must focus on preventing mosquito-borne transmission through vector control and personal protective practices, preventing sexual transmission by advising abstinence from sex or consistent and correct use of condoms, and preventing unintended pregnancies by reducing barriers to access to highly effective reversible contraception [11]. Pregnant women should not travel to areas with active ZIKV transmission, and men and women who are planning to conceive in the near future should consider avoiding non-essential travel to these areas [12-15].

MICROBIOLOGY

Many members of the genus *Flavivirus* are the agents of important diseases of humans, livestock, and wildlife. Until the last 10 years, no complete genome sequence was available for the three African viruses, Bagaza Virus (BAGV), Kedougou Virus (KEDV), and ZIKV, each representing a distinct virus subgroup according to the latest virus classification. It has been obtained a complete genome sequence of each of those three viruses and characterized the open reading frames (ORF) with respect to gene sizes, cleavage sites, potential glycosylation sites, distribution of cysteine residues, and unique motifs [16]. Whereas much is known about the biology of related Flaviviruses, the unique features of ZIKV pathogenesis, including infection of the fetus, persistence in immune-privileged sites, and sexual transmission, have presented new challenges. The rapid development of cell culture and animal models has facilitated a new appreciation of ZIKV biology. This knowledge has created opportunities for the development of countermeasures, including multiple ZIKV vaccine candidates, which are advancing through clinical trials [17]. There are three genetic lineages of ZIKV: the East African, West African, and Asian lineages. Despite several reports on ZIKV, the genetic relationships among viral strains from West Africa remain poorly understood. ZIKV has possibly undergone recombination in nature and a loss of the N-linked glycosylation site (N154) in the envelope protein was a possible adaptive response to the *Aedes dalzieli* vector [18]. Despite the relatively short time interval, an intensive effort by the global scientific community has resulted in development of animal models to study multiple aspects of ZIKV biology. Several animal models have been established to investigate pathogenesis of ZIKV in adults, pregnant mothers, and developing fetuses [19, 20].

A mosquito-borne virus resulting in congenital anomalies is unique, and zika's ability to cause neurological defects on a large scale was a grim reminder of the Rubella Virus (RuV) epidemic in the 1950s. Over the past six years, studies have provided insight on how ZIKV infects cells and causes disease, but much remains unknown about the long-term risks of zika exposure on infant growth and development. The impact of ZIKV on pregnancy extends beyond microcephaly and may only first be identified in infancy. The virus has a long latency in semen and can be transmitted sexually. Transplacental passage occurs through infection of primary human placental macrophages, commonly referred to as Hofbauer cells, in the trophoblast. This has led to questions of how a Flavivirus is capable of crossing the placental barrier to reach the fetal brain. Permissive ZIKV infection of Hofbauer cells and placental villous fibroblasts has been demonstrated. It has been also demonstrated ZIKV infection of Hofbauer cells within the context of the tissue ex vivo using term placental villous explants. In addition to amplifying infectious virus within a usually inaccessible area, the putative migratory activities of Hofbauer cells may aid in dissemination of ZIKV to the fetal brain [21]. Hofbauer cells are placental villous macrophages of fetal origin that are present throughout pregnancy. Although Hofbauer cell populations are antigenically and morphologically heterogeneous, their epigenetic, antigenic, and functional profiles most closely resemble alternatively activated macrophages or what are referred to as M2a, M2b, M2c, and M2d polarity subtypes. Consistent with an M2-like profile, these cells play an important role in placental development including vasculo-genesis and angiogenesis. During placental inflammation, Hofbauer cells may produce pro-inflammatory cytokines or mediators that damage the villous cell barrier, and induce fibrotic responses within the villi as a continuum of chronic inflammation. However, to date,

there is no evidence that Hofbauer cells become classically activated or adopt an M1 polarity phenotype that is able to kill microbes. To the contrary, their predominant M2-like qualities may be why these cells are ineffective in controlling most “TORCH” (*Toxoplasma gondii*, other, RuV, Cytomegalovirus [CMV], and Herpes Simplex Virus [HSV]) infections. Moreover, Hofbauer cells may contribute to vertical transmission of various pathogens to the fetus since they can harbor live virus and serve as reservoirs within the placenta [22]. Yet, placentas from fetuses with congenital ZIKV infection are concordant in not having viral-induced placental inflammation. Special stains reveal proliferation and prominent hyperplasia of Hofbauer cells in the chorionic villi of infected placentas. ZIKV infection is present in Hofbauer cells from second and third trimester placentas. Experimental studies and placentae from infected fetuses reveal that the spectrum of placental cell types infected with ZIKV is broader during the first trimester than later in gestation. Inflammatory abnormalities of the placenta are not a component of vertical transmission of ZIKV. The major placental response in second and third trimester transplacental ZIKV infection is proliferation and hyperplasia of Hofbauer cells, which also demonstrate viral infection [23].

Nevertheless, it is uncertain when during pregnancy the human conceptus is most vulnerable to the virus. The cells from term placentas, which resist infection, do not express genes encoding most attachment factors implicated in ZIKV entry but do express many genes associated with antiviral defense. By contrast, the embryonic stem cells (ESC)-derived trophoblasts possess a wide range of attachment factors for ZIKV entry and lack components of a robust antiviral response system. These cells, particularly areas of syncytiotrophoblast within the colonies, quickly become infected, produce infectious virus and undergo lysis within 48 h after exposure to low titers (multiplicity of infection >0.07) of an African lineage strain (MR766 Uganda [ZIKVU]) considered to be benign with regards to effects on fetal development. These data suggest that the developing fetus might be most vulnerable to ZIKV early in the first trimester before a protective zone of mature villous trophoblast has been established. Additionally, ZIKVU is highly trophic toward primitive trophoblast, which may put the early conceptus of an infected mother at high risk for destruction [24]. However, the cellular tropism and the mechanisms of ZIKV transmission to the fetus during early pregnancy are still largely unknown. ZIKV infects and damages tissue architecture of the maternal decidua basalis, the fetal placenta, and umbilical cord. ZIKV replicates differentially in a wide range of maternal and fetal cells, including decidual fibroblasts and macrophages, trophoblasts, Hofbauer cells, as well as umbilical cord mesenchymal stem cells (MSC). The striking cellular tropism of ZIKV and its cytopathic-induced tissue injury during the first trimester of pregnancy could provide an explanation for the irreversible congenital damages [25].

Secretory autophagy or exosome pathways may participate in this virus transfer. Autophagy modulators regulate autophagosome formation or membrane fusion with lysosomal vesicles and therefore inhibit viral nucleocapsid releasing or virus transfer to the fetus hypothetically. However, some autophagy modulators may enhance virus replication. Autophagy inhibitors may arrest placental development, while exaggeration of autophagy in human placenta may be associated with fetal intrauterine growth restriction (IUGR). Therefore, autophagy modulators should be used carefully due to their complex clinical effects. Alternatively, exosome-specific inhibitors might be also considered, although their safety of both maternal and fetal conditions must be carefully assessed before any advancement to human clinical trials [26]. A recent study investigated the key aspects of the ZIKV infectious life cycle. It has been demonstrated how inflammatory-activated stimulator of interferon (IFN) genes

(STING)-dependent antiviral macroautophagy/autophagy is sufficient to restrict ZIKV infection in the *Drosophila melanogaster* brain. Additionally, this study provides further evidence for the ancestral function of autophagy in protecting host cells from viral invaders [27]. Circumstantial evidence has been presented in support of the hypothesis that ZIKV effects are due to an endogenous form of hypervitaminosis A resulting from ZIKV infection-induced damage to the liver and the spillage of stored vitamin A compounds (“retinoids”) into the maternal and fetal circulation in toxic concentrations. Retinoids are mainly stored in the liver (about 80%) and are essential for numerous biological functions. In higher concentrations, retinoids are potentially cytotoxic, pro-oxidant, mutagenic, and teratogenic, especially if sudden shifts occur in their bodily distribution. Although liver involvement has not been mentioned specifically in recent reports, conventional liver enzyme testing underestimates the true extent of liver dysfunction. The proposed model could be tested by comparing retinoid concentration and expression profiles in microcephalic newborns of ZIKV-infected mothers and non-microcephalic newborn controls, and by correlating these profiles with measures of clinical severity [28].

Emerging evidence, mainly from *in vitro* studies, suggests that ZIKV may have direct effects on neuronal cells [29, 30]. ZIKV preferentially infects human neural progenitor cells (hNPC) and triggers cell apoptosis. ZIKV RNA has been identified in fetal brain tissue and brains of microcephalic infants who died and cerebrospinal fluid (CSF) and meninges of newborns. The increase in the number of GBS cases during the ZIKV outbreak in the Americas provides epidemiological evidence for the link between ZIKV infection and GBS. Less frequently reported ZIKV neurological complications include encephalitis/meningoencephalitis, acute disseminated encephalomyelitis, myelitis, cerebrovascular complications (ischemic infarction and vasculopathy), seizures and encephalopathy, sensory polyneuropathy, and sensory neuronopathy. Analysis of GBS incidence could serve as an epidemiological “marker” or sentinel for ZIKV disease and other neurological complications associated to ZIKV. An expanding spectrum of neurological complications associated with ZIKV infection is being recognized [31]. Recently, it has been demonstrated that in the brain, ZIKV preferentially infects neural stem cells (NSC), astrocytes, oligodendrocyte precursor cells, and microglia, whereas neurons are less susceptible to infection. Infection of induced pluripotent stem cell (iPSC)-derived hNPC results in cell cycle abnormalities and apoptosis. Many cellular pathways correlate with clinical pathologies following ZIKV infection such as microcephaly, congenital nervous system disorders, and epilepsy. Furthermore, ZIKV activates several inflammatory signals within infected hNPC that are implicated in innate and acquired immune responses, while CMV-infected hNPC show limited representation of these pathways. Moreover, several genes related to pathogen responses are significantly up-regulated upon ZIKV infection, but not perturbed in CMV-infected hNPC. hNPC are capable of signaling through canonic pro-inflammatory pathways following viral infection. By defining gene expression profiles, new factors in the pathogenesis of ZIKV have been identified which could help develop new therapeutic strategies [32]. hNPC are responsible for populating the developing central nervous system (CNS) with neurons and glia. Neural progenitor dysfunction can lead to severe birth defects, namely, lissencephaly, microcephaly, and cognitive deficits. ZIKV isolates from Asian and African lineages displayed lineage-specific replication kinetics, cytopathic effects, and impacts on hNPC function and neuronal differentiation. The currently circulating ZIKV isolates exhibit a unique profile of virulence, cytopathic effect, and impaired cellular functions that likely contribute to the

pathological mechanism of congenital zika syndrome (CZS). Infection with Asian-lineage ZIKV isolates impaired the proliferation and migration of hNPC, and neuron maturation. In contrast, the African-lineage infections resulted in abrupt and extensive cell death [33].

These findings indeed suggest mechanisms for microcephaly and other pathological features of infants with congenital ZIKV infection that are not explained by NSC infection alone, such as calcifications in the cortical plate. Furthermore, it has been found that blocking the glia-enriched putative viral entry receptor AXL reduces *in vitro* ZIKV infection of astrocytes, and genetic knockdown of AXL in a glial cell line nearly abolishes infection. Finally, it has been showed that the macrolide antibiotic azithromycin reduces viral proliferation and virus-induced cytopathic effects in glial cell lines and human astrocytes. Characterization of infection in the developing human brain clarifies the pathogenesis of congenital ZIKV infection and provides the basis for investigating possible therapeutic strategies to safely alleviate or prevent the most severe consequences of the epidemic [34]. In addition to the developing CNS, ZIKV has been recently shown to infect cells of the PNS, highlighting its potential to cause acute peripheral neuropathies in adults, such as GBS, in a pure motor axonal variant. Myelinating dorsal root ganglia (DRG) explants obtained from IFN- α/β receptor knock-out mice are productively infected by ZIKV. Virus replication is cytopathic in both peripheral neurons and myelinating Schwann cells, leading to myelin disruption. These results confirm and extend previous observations suggesting that the PNS is indeed a potential site of ZIKV infection, replication, and cytopathicity [35]. Moreover, vertical ZIKV infection can cause a wide spectrum of neurological manifestations that go beyond microcephaly, and even the non-microcephalic child should be followed during the first years of life, because infection may be asymptomatic or lead to neuropsychomotor delay, epilepsy, and visual abnormalities. The appropriate prospective multidisciplinary follow-up of these patients aims to understand the natural history of this new agent and to provide a better development and quality of life for them and their families [36]. But whether it can induce neurological sequelae that appear later in life remains unclear. The virus unexpectedly alters the DNA methylome of neural progenitors, astrocytes, and differentiated neurons at genes that have been implicated in the pathogenesis of a number of brain disorders, most prominently mental retardation and schizophrenia. These results suggest that ZIKV infection during fetal development could lead to a spectrum of delayed-onset neuropsychiatric complications. Scientific research on human NSC and cerebral organoids has confirmed the congenital neurotropic and neuro-destructive nature of ZIKV. However, the extent to which prenatal ZIKV infection is associated with more subtle brain alterations, such as epigenetic changes, remains ill-defined [37].

In the last few years, genomic and molecular investigations have established a remarkable progress on the pathogenic mechanisms of ZIKV infection using *in vitro* and *in vivo* models [38]. Numerous experimental approaches have been taken to address how the virus can cross the placenta, alter normal fetal development, and disrupt specific cellular functions. Many areas concerning the mechanisms of transmission, especially from mother to fetus, are largely unknown but demand further research. Several promising new studies provide insight into possible mechanisms of transmission, different cell types affected, and immune responses towards the virus. By aiming to better understand the processes behind altered fetal neuronal development due to ZIKV infection, the hope is to find ways to increase protection of the fetus and prevent congenital abnormalities such as microcephaly. As ZIKV infection is spreading to increasingly more areas and bringing harmful outcomes and birth defects with it, it is imperative

to identify the mechanisms of transmitting this infectious agent, consider different genetic backgrounds of hosts and strain types, and navigate methods to protect those affected from the detrimental effects of this newly emerging virus [39]. A major difficulty in management of ZIKV disease is that most infections are asymptomatic and the diagnostic methods are not ideal, making both diagnosis and ascertainment of timing of infection problematic. Understanding of the mechanisms of ZIKV-host interaction at the cellular level will contribute crucial insights into the development of ZIKV therapeutics and vaccines. Several different types of vaccines are in development. Large studies are ongoing to determine the risk and total spectrum of anomalies based on the timing of infection and other environmental exposures [40-43].

EPIDEMIOLOGY

Since many persons with mild ZIKV disease are asymptomatic and might not seek care, it is difficult to estimate the actual incidence of ZIKV infection. Arbovirus infections were at first of public health interest in East Africa, where a very widespread epidemic of o'nyong-nyong fever was reported in 1959-60 and where the threat of yellow fever, present in neighboring areas such as Ethiopia, remains. The prevalence of arbovirus infection varies markedly from area to area in Africa. Yellow fever antibodies in the coastal area must be due either to vaccination or to cross-reactions with other group-B arboviruses [44]. Serological studies of persons infected with yellow fever during the 1970 epidemic in Okwoga District, Nigeria, indicated that epidemic yellow fever occurred despite a high prevalence of pre-existing group-B arbovirus immunity, which increased with age. The viruses involved were primarily DENV, Wesselsbron Virus (WESSV), and ZIKV [45]. Mosquito and sentinel animal surveillance studies have demonstrated that ZIKV is endemic to Africa and Southeast Asia. In June 2007, an epidemic of relatively mild disease characterized by fever, rash, arthralgia, and conjunctivitis associated with ZIKV was detected in Yap State, Federated States of Micronesia, in the southwestern Pacific Ocean, where hundreds of dengue-like syndromes were reported [46]. This was the first time that ZIKV was detected outside of Africa and Asia. Then, in 2013-2014, it was reported in New Caledonia and French Polynesia, where the first neurological effects were observed. The emergence of ZIKV outside of its previously known geographic range prompted awareness of the potential for ZIKV to spread to other Pacific Islands and the Americas [47-49].

A recent study indicated the existence of three geographically distinct viral lineages. Although ZIKV has been known to circulate in both Africa and Asia since at least the 1950s, little is known about the genetic relationships between geographically distinct virus strains. Moreover, the geographic origin of the strains responsible for the epidemic that occurred on Yap Island in 2007, and a 2010 pediatric case in Cambodia, has not been determined. The basal position of the ZIKV strain isolated in Malaysia in 1966 suggests that the recent outbreak in Micronesia was initiated by a strain from Southeast Asia. Because ZIKV infection in humans produces an acute febrile illness that clinically resembles dengue fever and many other tropical infectious diseases, it is likely greatly misdiagnosed and under-reported [50, 51]. Yellow fever, zika fever, and dengue, all of which are major human diseases found in tropical regions of the globe, are zoonoses with a transmission cycle that involves primates as reservoirs and mosquitoes of the genus *Aedes* as vectors. The recent upsurge of urban epidemics of yellow

fever, zika fever, and dengue has involved human-to-human transmission with mosquitoes as the vector [52]. Clinical descriptions of chikungunya fever have been performed in the American outbreak observing that fever, polyarthralgia, myalgia, and rash are the most common symptoms in the acute phase, while chronic arthralgia has persisted in 37-90% of small cohorts. The Asian origin of American strains of CHIKV and ZIKV evidences a dissemination route in common and both are being transmitted by *Aedes aegypti*. Regarding zika fever, the association of congenital malformations with previous ZIKV exposure of pregnant women and potential sexual transmission of ZIKV are the most important discoveries in the New World. Massive outbreaks of chikungunya fever in 2014 and then followed by zika fever epidemics of lower magnitude in the next year throughout the American continent have their origins in Asia but may have used Pacific Islands as a path of dissemination. Reports of chronic arthralgia have been little described in the continent and more research is needed to measure the economic and health impact in patients who contracted CHIKV before. On the contrary, zika is menacing newborns' health because of its link with congenital microcephaly and sexual health by prolonged presence of viral particles in semen and urine [53].

As we have seen, before 2007, only sporadic human disease cases were reported from Countries in Africa and Asia. In 2007, the first documented outbreak of ZIKV disease was reported in Yap State; 73% of the population aged at least three years is estimated to have been infected. Subsequent outbreaks occurred in Southeast Asia and the Western Pacific, on the heels of a CHIKV epidemic in the Americas that began in 2013. In 2014, the WHO reported the first local transmission of ZIKV in the Region of the Americas on Easter Island, Chile, and in Northeast Brazil in May 2015. The virus was likely introduced in Brazil from competing athletes from French Polynesia and other Countries that participated in a competition there, from where it has rapidly expanded in the continent; the first case in Mexico was detected in October 2015, the first cases of local transmission have just been reported in Florida, and in the following months has spread also across southern states of US, including the Commonwealth of Puerto Rico and the US Virgin Islands (USVI). In May 2015, the Pan American Health Organization (PAHO), which serves as the Regional Office for the Americas (RAM) for the WHO, issued an alert regarding the first confirmed ZIKV infection in Brazil. In October 2015, in Pernambuco, Brazil, an increase in the number of newborns with microcephaly was reported. Women delivering in October would have been in the first trimester of pregnancy during the peak of a zika epidemic in March. By the end of 2015, 4,180 cases of suspected microcephaly had been reported [54]. In Salvador, Brazil, outbreaks of acute exanthematous illness (AEI) attributed to ZIKV, GBS, and microcephaly occurred in 2015. Temporal correlations and time lags between these outbreaks have been investigated to identify a common link between them by using epidemic curves and time series cross-correlations. Number of GBS cases peaked after a lag of five to nine weeks from the AEI peak. Number of suspected cases of microcephaly peaked after a lag of 30-33 weeks from the AEI peak, which corresponded to time of potential infections of pregnant mothers during the first trimester. These findings support the association of GBS and microcephaly with ZIKV infection and provide evidence for a temporal relationship between timing of arboviral infection of pregnant women during the first trimester and birth outcome [55].

Neurological disorders among zika patients were similar in Brazil and French Polynesia, but a causal link is not established. Incidence of zika infection in pregnant women is not known. In Brazil, during the zika outbreak the incidence of microcephaly increased more than 20 times. Among 35 infants with microcephaly, born from women suspected to have zika infection

during pregnancy in Northeast Brazil, 74% of the mothers reported rash during the first and second trimester and no exposure to other potentially teratogenic agents [56-58]. During October 12th, 2015-September 10th, 2016, the Haitian Ministry of Public Health and Population (MOPHP) detected 3,036 suspected cases of ZIKV infection in the general population, 22 suspected cases of ZIKV disease among pregnant women, 13 suspected cases of GBS, and 29 suspected cases of zika-associated congenital microcephaly. Nineteen (0.6%) patients with suspected ZIKV disease, residing in Ouest (10 patients), Artibonite (six), and Centre (three) administrative departments, have been confirmed by laboratory testing, including two among pregnant women and 17 in the general population [59]. Air, land, and sea transportation can facilitate rapid spread of infectious diseases. New York City (NYC) is home to a large number of persons who travel frequently to areas with active ZIKV transmission, including immigrants from these areas. In November 2015, the NYC Department of Health and Mental Hygiene (DOHMH) began developing and implementing plans for managing ZIKV and on February 1st, 2016, activated its Incident Command System (ICS). During January 1st-June 17th, 2016, DOHMH coordinated diagnostic laboratory testing for 3,605 persons with travel-associated exposure, 182 (5.0%) of whom had confirmed ZIKV infection. Twenty (11.0%) confirmed patients were pregnant at the time of diagnosis. In addition, two cases of ZIKV-associated GBS were diagnosed. DOHMH's response has focused on:

- 1) identifying and diagnosing suspected cases;
- 2) educating the public and medical providers about ZIKV risks, transmission, and prevention strategies, particularly in areas with large populations of immigrants from areas with ongoing ZIKV transmission;
- 3) monitoring pregnant women with ZIKV infection and their fetuses and infants;
- 4) detecting local mosquito-borne transmission through both human and mosquito surveillance; and
- 5) modifying existing *Culex* mosquito control measures by targeting *Aedes* species of mosquitoes through the use of larvicides and adulticides [60].

Recent outbreaks of ZIKV disease in the Pacific Islands and the Region of the Americas and the Caribbean, including Haiti, have identified new modes of transmission and clinical manifestations. During December 2015-January 2016, the American Samoa Department of Health (ASDoH) detected through surveillance an increase in the number of cases of acute febrile rash illness. Concurrently, a case of laboratory-confirmed ZIKV infection was reported in a traveler returning to New Zealand from American Samoa. In the absence of local laboratory capacity to test for ZIKV, ASDoH initiated arboviral disease control measures, including public education and vector source reduction campaigns. On February 1st, CDC staff members were deployed to American Samoa to assist ASDoH with testing and surveillance efforts [61]. In December 2015, the Ministry of Health (MoH) estimated that 440,000-1,300,000 suspected cases of ZIKV disease had occurred in Brazil in 2015. On December 31st, 2015, Puerto Rico Department of Health (PRDH) reported the first locally acquired case of ZIKV disease in a jurisdiction of the US in a patient from southeastern Puerto Rico, with the index patient reporting symptom onset on November 23rd, 2015. During November 23rd, 2015-January 28th, 2016, passive and enhanced surveillance for ZIKV disease identified 30 laboratory-confirmed cases. Most (93%) patients resided in eastern Puerto Rico or the San Juan metropolitan area. The most frequently reported signs and symptoms were rash (77%), myalgia (77%), arthralgia

(73%), and fever (73%). Three (10%) patients were hospitalized. One case occurred in a patient hospitalized for GBS, and one occurred in a pregnant woman. Because the most common mosquito vector of ZIKV, *Aedes aegypti*, is present throughout Puerto Rico, ZIKV was expected to continue to spread across the island. The public health response in Puerto Rico is being coordinated by PRDH with assistance from CDC. By January 20th, 2016, locally-transmitted cases had been reported to the PAHO from Puerto Rico and 19 other Countries or territories in the Americas. Further spread to other Countries in the region has been monitored closely [62]. During November 1st, 2015-April 14th, 2016, a total of 6,157 specimens from suspected ZIKV-infected patients were evaluated by the PRDH and CDC Dengue Branch (DB), which is located in San Juan, Puerto Rico, and 683 (11%) had laboratory evidence of current or recent ZIKV infection by one or more tests: reverse transcription-polymerase chain reaction (RT-PCR) or immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA). ZIKV-infected patients resided in 50 (64%) of 78 municipalities in Puerto Rico. Median age was 34 years (range = 35-89 years). The most frequently reported signs and symptoms were rash (74%), myalgia (68%), headache (63%), fever (63%), and arthralgia (63%). There were 65 (10%) symptomatic pregnant women who tested positive by RT-PCR or IgM ELISA. A total of 17 (2%) patients required hospitalization, including five (1%) patients with suspected GBS. One (<1%) patient died after developing severe thrombocytopenia. The public health response to the outbreak has included increased laboratory capacity to test for ZIKV infection (including blood donor screening), implementation of enhanced surveillance systems, and prevention activities focused on pregnant women. Vector control activities include indoor and outdoor residual spraying and reduction of mosquito breeding environments focused around pregnant women's homes [63]. During November 1st, 2015-July 7th, 2016, a total of 23,487 persons were evaluated by PRDH and CDC DB for ZIKV infection, including asymptomatic pregnant women and persons with signs or symptoms consistent with ZIKV disease or suspected GBS; 5,582 (24%) confirmed and presumptive ZIKV cases were identified. Persons with ZIKV infection were residents of 77 (99%) of Puerto Rico's 78 municipalities. During 2016, the percentage of positive ZIKV infection cases among symptomatic males and non-pregnant females who were tested increased from 14% in February to 64% in June. Among 9,343 pregnant women tested, 672 had confirmed or presumptive ZIKV infection, including 441 (66%) symptomatic women and 231 (34%) asymptomatic women. One patient died after developing severe thrombocytopenia. Evidence of ZIKV infection or recent unspecified Flavivirus infection was detected in 21 patients with confirmed GBS [64]. During November 1st, 2015-October 20th, 2016, 62,500 suspected ZIKV disease cases were reported to the PRDH; 29,345 (47%) were confirmed by RT-PCR testing, or were presumptively diagnosed based on serological testing. The highest incidence among confirmed or presumptive cases occurred among persons aged 20-29 years (1,150 cases per 100,000 residents). Among 28,219 (96.2%) non-pregnant patients with confirmed or presumptive ZIKV disease, incidence was higher among women (936 per 100,000 population) than men (576 per 100,000) for all age groups ≥ 20 years, and the majority (61%) of reported ZIKV disease cases occurred in females. Among suspected ZIKV disease cases in non-pregnant adults aged ≥ 40 years, the percentage that tested positive among females (52%) was higher than that among males (47%) ($p < 0.01$). Reasons for the higher incidence of ZIKV disease among women aged ≥ 20 years are not known; serosurveys of persons living near confirmed ZIKV disease cases might help to elucidate these findings. Residents of and travelers to Puerto Rico should remove or cover standing water, practice mosquito abatement, employ mosquito bite avoidance behaviors, take precautions to

reduce the risk for sexual transmission, and seek medical care for any acute illness with rash or fever [65]. Clinicians should report all cases of microcephaly, GBS, and suspected ZIKV disease. Other adverse reproductive outcomes, including fetal demise associated with ZIKV infection, should be reported. To avoid infection with ZIKV, residents of and visitors to these regions, particularly pregnant women, should strictly follow steps to avoid mosquito bites, including wearing pants and long-sleeved shirts, using permethrin-treated clothing and gear, using an Environmental Protection Agency (EPA)-registered insect repellent, and ensuring that windows and doors have intact screens [66].

During January 1st, 2015–February 26th, 2016, a total of 116 residents of 33 US and the District of Columbia (DC) had laboratory evidence of recent ZIKV infection based on testing performed at CDC. Cases include one congenital infection and 115 persons who reported recent travel to areas with active ZIKV transmission (n = 110) or sexual contact with such a traveler (n = 5). All 115 patients had clinical illness, with the most common signs and symptoms being rash (98%; n = 113), fever (82%; 94), and arthralgia (66%; 76) [67, 68]. As of September 3rd, 2016, a total of 2,382 confirmed and probable cases of ZIKV disease with symptom onset during January 1st–July 31st, 2016, had been reported from 48 of 50 US and the DC. Most cases (2,354; 99%) were travel-associated, with either direct travel or an epidemiological link to a traveler to a ZIKV-affected area. Twenty-eight (1%) cases were reported as locally acquired, including 26 associated with mosquito-borne transmission, one acquired in a laboratory, and one with an unknown mode of transmission. In 2016, a total of 5,168 non-congenital ZIKV disease cases were reported from US and the DC. Most cases (4,897, 95%) were in travelers returning from ZIKV-affected areas. A total of 224 (4%) cases were acquired through presumed local mosquito-borne transmission, and 47 (1%) were acquired by other routes. It is important that providers in the US continue to test symptomatic patients who live in or recently traveled to areas with ongoing ZIKV transmission or had unprotected sex with someone who lives in or traveled to those areas. All pregnant women and their partners should take measures to prevent ZIKV infection during pregnancy. ZIKV disease should be considered in patients with compatible clinical signs or symptoms who traveled to or reside in areas with ongoing ZIKV transmission or who had unprotected sex with someone who traveled to those areas. Health care providers should continue to educate patients, especially pregnant women, about the importance of avoiding infection with ZIKV, and all pregnant women should be assessed for possible ZIKV exposure at each prenatal visit [69–71].

On February 1st, 2016, the WHO declared the occurrence of ZIKV-related microcephaly clusters and other neurological disorders to be a PHEIC and that, although not yet scientifically proven, the link between the virus and growing numbers of microcephaly cases was “strongly suspected,” and now talks about a ZIKV pandemic. However, the causal relationship between zika and microcephaly is not universally accepted. Although ZIKV infections may explain many cases of brain damage in newborns, it may not be the only cause. We need a clear association between confirmed cases of zika infections in pregnant women and microcephaly in newborns. Certainly, the ZIKV, as many other viruses, has the capacity to induce severe cerebral lesions in fetuses of women infected during the first months of pregnancy. Moreover, more than 80% of cases of craniostenosys that verisimilarly took place in Brazil from September 2015 up to now were registered in few states of northeastern Brazil, where peaks of this malformation were registered several years before the appearance of ZIKV. In addition, in none of the 60 Countries where ZIKV is epidemiologically active an increase of the incidence of microcephaly had been reported. Despite initial skepticism regarding the causal association

of the ZIKV epidemic and birth defects, extensive basic and clinical research evidence has now confirmed this relationship [72]. However, data on the epidemiology and clinical findings of laboratory-confirmed ZIKV disease remain limited. Because ZIKV is a relatively new infection with several transmission routes that include mosquito bites, sexual, and possibly blood-related, a parallel between the zika epidemic and the acquired immunodeficiency syndrome (AIDS) epidemic for issues that relate particularly to pregnancy and epidemic response can be made. However, the viruses are different and the pathophysiology and disease manifestation are also different. Nevertheless, parallels between the zika and the Human Immunodeficiency Virus (HIV)/AIDS epidemics, particularly in relation to pregnancy, can be established. Lessons and successes from the HIV/AIDS epidemic control efforts can guide us towards comprehensive approaches to improve the health of women and infants at risk for zika [73].

The association between maternal ZIKV infection and adverse fetal and reproductive outcomes, including microcephaly, prompted CDC to issue a level two alert travel notice for the 37 Countries and three US territories (at the national and territorial level) that have reported recent ZIKV transmission as of March 11th, 2016. An estimated 216.3 million passenger journeys by air, land, and sea are made annually to the US from areas with local ZIKV transmission (as of March 8th). The destination states with the largest numbers of arrivals were Texas (by land) and Florida (by air and sea). An estimated 51.7 million passenger journeys were made by women of childbearing age and an estimated 2.3 million were made by pregnant women. Travel volume analyses provide important information that can be used to effectively target public health interventions as well as direct public health resources and efforts at local, regional, and Country-specific levels [74]. The vector is widely distributed in South America and control measures are the most effective means for prevention, not only of ZIKV, but also DENV and CHIKV [75]. In addition to mosquito bite precautions for all travelers, CDC advises that pregnant women postpone travel to affected Countries and US territories. Within a nation's borders, ecological characteristics, which determine the distribution of mosquito vectors, can vary considerably. CDC conducted a spatial analysis, focusing on the probability of occurrence of *Aedes aegypti*, to support the demarcation for sub-national travel alerts. Based on results of this analysis, travel that is limited to elevations higher than 2,000 m (6,562 ft) above sea level is considered to have minimal (approximately 1%) likelihood for mosquito-borne ZIKV transmission, even within countries reporting active transmission. Women who are pregnant should avoid travel to elevations <2,000 m in countries with active ZIKV transmission [76-82].

ZIKV originated and continues to circulate in a sylvatic transmission cycle between non-human primate hosts and arboreal mosquitoes in tropical Africa. Recently, ZIKV invaded the Americas, where it poses a threat to human health, especially to pregnant women and their infants. Brazil is home to multiple species of primates and mosquitoes potentially capable of ZIKV transmission, though direct assessment of host competence (ability to mount viremia sufficient to infect a feeding mosquito) and vector competence (ability to become infected with ZIKV and disseminate and transmit upon subsequent feedings) of New World species is lacking. Modeling reveals a high probability of establishment of sylvatic ZIKV across a large range of biologically plausible parameters. Probability of establishment is dependent on host and vector population sizes, host birthrates, and ZIKV force of infection. Research on the host competence of New World monkeys or other small mammals to ZIKV, on vector competence of New World *Aedes*, *Sabethes*, and *Hemagogus* mosquitoes for ZIKV, and on the geographic range of potential New World hosts and vectors is urgently needed. A sylvatic cycle of ZIKV would make future elimination efforts in the Americas practically impossible, and paints a dire

picture for the epidemiology of ZIKV and our ability to end the ongoing outbreak of CZS [83]. Mechanistic models of disease transmission can be used to forecast trajectories and likely disease burden but are currently hampered by substantial uncertainty on the epidemiology of the disease (e.g., the role of asymptomatic transmission, generation interval, incubation period, and key drivers). When insight is limited, phenomenological models provide a starting point for estimation of key transmission parameters, such as the reproduction number, and forecasts of epidemic impact. Phenomenological models represent promising tools to generate early forecasts of epidemic impact particularly in the context of substantial uncertainty in epidemiological parameters. Findings underscore the need to treat the reproduction number as a dynamic quantity even during the early growth phase, and emphasize the sensitivity of reproduction number estimates to assumptions on the generation interval distribution [84-86].

Mosquito-borne diseases pose a threat to individual health and population health on both a local and a global level. The threat is even more exaggerated during disasters, whether man-made or environmental. Education and training are instrumental in controlling the outbreak, and early detection can be life-saving [87]. This epidemic currently continues unabated. The explosive nature of recent outbreaks and concerning links to GBS and microcephaly are incompletely understood. Also unknown is the relative importance of sexual transmission of ZIKV and asymptomatic ZIKV infections to the overall burden of transmission. The limited understanding of ZIKV presents an enormous challenge for responses to this rapidly emerging threat to human health [88-94].

OBSTETRIC AND PEDIATRIC ISSUES

It has been recognized for centuries that pregnant women have unique susceptibilities to many infectious diseases that predispose them to untoward outcomes compared with the general adult population. It is thought a combination of adaptive alterations in immunity to allow for the fetal allograft combined with changes in anatomy and physiology accompanying pregnancy underlie these susceptibilities. Emerging infectious diseases are defined as those whose incidence in humans has increased in the past two decades or threaten to increase in the near future. The past decade alone has witnessed many such outbreaks, each with its own unique implications for pregnant women and their unborn fetuses as well as lessons for the health care community regarding response and mitigation. Examples of such outbreaks include, but are not limited to, severe acute respiratory syndrome (SARS), the 2009 A Hemagglutinin 1 and Neuraminidase 1 Virus (H1N1) pandemic influenza, Ebola Virus (EBOV), and, most recently, ZIKV. Although each emerging pathogen has unique features requiring specific considerations, there are many underlying principles that are shared in the recognition, communication, and mitigation of such infectious outbreaks. Some of these key principles include disease-specific delineation of transmission dynamics, understanding of pathogen-specific effects on both mothers and fetuses, and advance planning and contemporaneous management that prioritize communication among public health experts, clinicians, and patients. The productive and effective working collaboration among the CDC, the American College of Obstetricians and Gynecologists (ACOG), and the Society for Maternal-Fetal Medicine (SMFM) has been a key partnership in the successful communication and management of such outbreaks for women's health care providers and patients alike. Going

forward, the knowledge gained over the past decade will undoubtedly continue to inform future responses and will serve to optimize the education and care given to pregnant women in the face of current and future emerging infectious disease outbreaks [95]. Congenital infectious diseases, transmitted during the course of pregnancy, are estimated to affect nearly one in every 100 births worldwide. These infections may be associated with fetal and infant adverse health outcomes, due to congenital malformations caused by *in utero* transmission of the infectious organism itself (as is the case with CMV, toxoplasmosis, syphilis, and ZIKV), or due to chronic infection in the infant (as is the case with HIV and Hepatitis B Virus [HBV] and Hepatitis C Virus [HCV]). In addition, children who are exposed, yet uninfected, may still suffer from the consequences of exposure to infectious pathogens or to the drugs given to treat pregnant women and prevent *in utero* transmission (as may be the case with HIV infection) [96]. The recent association between ZIKV infection during pregnancy and fetal microcephaly has led to a renewed interest in the mechanisms by which vertically transmitted micro-organisms reach the fetus and cause congenital disease. Based on our current understanding of ZIKV pathogenesis and the developmental defects that are caused by fetal ZIKV infection, ZIKV should be considered a TORCH pathogen and future research and public health measures should be planned and implemented accordingly [97]. The acronym TORCH is used to refer to congenital infections, such as toxoplasmosis, other infections (such as syphilis, Varicella-Zoster Virus [VZV], and Parvovirus B19), CMV, and HSV. Three of the original TORCH agents are associated with an appreciable incidence of congenital microcephaly: CMV, RuV, and *Toxoplasma gondii*. The pathology of congenital microcephaly is characterized by neurotropic infectious agents that involve the fetal nervous system, leading to brain destruction with calcifications, microcephaly, sensorineural hearing loss, and ophthalmological abnormalities. The inflammatory reaction induced by these four agents has an important role in pathogenesis. Specific epidemiological factors, such as first and early second trimester maternal infection, and the manifestations of congenital infection in the infant, shed some light on the pathogenesis [98]. The classic findings in patients with TORCH infections include rash in the mother during pregnancy and ocular findings in the newborn. ZIKV has emerged as an important worldwide congenital infection. It fits well with other congenital TORCH infections since there is a rash in the mother and there are commonly ocular abnormalities in the newborn. TORCH infections are recognized to have neurological effects, such as ventriculomegaly, intraventricular adhesions, subependymal cysts, intracerebral calcifications, and microcephaly; however, ZIKV is intensely neurotropic. In fact, it targets hNPC, leading to a more severe spectrum of CNS abnormalities than is typically seen in other TORCH infections, while relatively sparing the other organ systems. Awareness of imaging features of common congenital infections may facilitate early diagnosis and may, at times, lead to prompt initiation of therapy [99].

By September 2015, reports of an increase in the number of infants born with microcephaly in ZIKV-affected areas began to emerge, and ZIKV RNA was identified in the amniotic fluid of two women whose fetuses had been found to have microcephaly by prenatal ultrasound. More than 4,700 suspected cases of microcephaly were reported from mid-2015 through January 2016, many of which occurred in infants born to women who lived in or had visited areas where ZIKV transmission was occurring [100]. Microcephaly surveillance was enhanced in late 2015 by implementing a more sensitive case definition. Based on the peak number of reported cases of microcephaly, and assuming an average estimated pregnancy duration of 38 weeks in Brazil, the first trimester of pregnancy coincided with reports of cases of febrile rash illness compatible with ZIKV disease in pregnant women in Bahia, Paraíba, and Pernambuco

states, supporting an association between ZIKV infection during early pregnancy and the occurrence of microcephaly [101]. The Brazil MoH established a task force to investigate the possible association of microcephaly with ZIKV infection during pregnancy and a registry for incident microcephaly cases (head circumference at least two standard deviations [SD] below the mean for sex and gestational age at birth) and pregnancy outcomes among women suspected to have had ZIKV infection during pregnancy. Among a cohort of 35 infants with microcephaly born during August-October 2015 in eight of Brazil's 26 states and reported to the registry, the mothers of all 35 had lived in or visited ZIKV-affected areas during pregnancy, 25 (71%) infants had severe microcephaly (head circumference more than three SD below the mean for sex and gestational age), 17 (49%) had at least one neurological abnormality, and among 27 infants who had neuroimaging studies, all had abnormalities. Tests for other congenital infections were negative. All infants had a lumbar puncture as part of the evaluation and CSF samples were sent to a reference laboratory in Brazil for ZIKV testing [102]. The link with brain defect, which has been detected mainly in some areas of Brazil, is supported by the following evidence:

- 1) ZIKV transmission from infected pregnant women to their fetuses; and
- 2) the potential of ZIKV to determine a specific congenital fetal syndrome characterized by abnormalities involving primarily the developing brain and eye.

In particular, the risk of transmission and congenital disease appears to be restricted to mother's infection during the first trimester of pregnancy. Among brain defects, microcephaly, brain calcifications, and ventriculomegaly are the most frequent abnormalities of the CNS detected so far [103]. About 60% to 70% of mothers report rash during pregnancy, mainly in the first trimester. Principal features are microcephaly, facial disproportionality, *cutis girata*, hypertonia/spasticity, hyperreflexia, and irritability; abnormal neuroimages include calcifications, ventriculomegaly, and lissencephaly. Hearing and visual abnormalities may be present. Preliminary data suggest that severe congenital abnormalities are linked to ZIKV infection. Cases have severe abnormalities, and although sharing many characteristics with congenital abnormalities associated with other viral infections, abnormalities presumably linked to ZIKV may have distinguishing characteristics. These severe neurological abnormalities may result in marked mental retardation and motor disabilities for many surviving offspring [104]. In most studies, ZIKV seems related to congenital ocular lesions and most mothers reported mild symptoms during the first trimester of pregnancy. Five fundus patterns were seen most often: macular chorioretinal atrophy, chorioretinal atrophy elsewhere, focal pigmentary changes in the macular region, optic nerve abnormalities, and combined types. A few studies have suggested that ZIKV might damage the anterior segment of these babies' eyes. Few reports have described the ocular complications seen in adults during the acute infection, including conjunctivitis, iridocyclitis, and chorioretinitis. Infants with CZS might have vision-threatening fundus abnormalities. Although the full spectrum of ocular lesions caused by ZIKV infection is not yet determined, a distinctive new disease has been observed. Recognition of these lesions can help ensure appropriate etiological evaluation and clinical investigation to define the range of anomalies in an affected infant and determine essential follow-up and ongoing care [105-107]. Hearing loss associated with other congenital viral infections is well described; however, little is known about hearing loss in infants with congenital ZIKV infection. A retrospective assessment of a series of 70 infants aged from zero

to 10 months with microcephaly and laboratory evidence of ZIKV infection was conducted by the Hospital Agamenon Magalhães in Brazil and partners. The infants were enrolled during November 2015-May 2016 and had screening and diagnostic hearing tests. Five (7%) infants had sensorineural hearing loss, all of whom had severe microcephaly; however, one child was tested after receiving treatment with an ototoxic antibiotic. If this child is excluded, the prevalence of sensorineural hearing loss was 5.8% (four of 69), which is similar to that seen in association with other congenital viral infections. Additional information is needed to understand the prevalence and spectrum of hearing loss in children with congenital ZIKV infection; all infants born to women with evidence of ZIKV infection during pregnancy should have their hearing tested, including infants who appear normal at birth [108].

Population-based birth defects surveillance systems are critical to monitor all infants and fetuses with birth defects potentially related to ZIKV infection, regardless of known exposure or laboratory evidence of ZIKV infection during pregnancy. To inform microcephaly surveillance efforts and assess ascertainment sources, the New York State Department of Health (NYSDOH) and the NYC Department of Health and Mental Hygiene (DOHMH) sought to determine the prevalence of microcephaly in New York during 2013-2015, before known importation of ZIKV infections. Suspected newborn microcephaly diagnoses were identified from:

- 1) reports submitted by birth hospitals in response to a request; and
- 2) queries of a hospital administrative discharge database for newborn microcephaly diagnoses.

Anthropometric measurements, maternal demographics, and pregnancy characteristics were abstracted from newborn records from both sources. Diagnoses were classified using microcephaly case definitions developed by CDC and the National Birth Defects Prevention Network (NBDPN). During 2013-2015, 284 newborns in New York met the case definition for severe congenital microcephaly (prevalence = 4.2 per 10,000 live births). Most newborns with severe congenital microcephaly were identified by both sources; 263 (93%) were identified through hospital requests and 256 (90%) were identified through administrative discharge data. The proportions of newborns with severe congenital microcephaly who were black (30%) or Hispanic (31%) were higher than the observed proportions of black (15%) or Hispanic (23%) infants among New York live births. Fifty-eight percent of newborns with severe congenital microcephaly were born to mothers with pregnancy complications or who had *in utero* or perinatal infections or teratogenic exposures, genetic disorders, or family histories of birth defects [109]. In November 2015, the Brazilian MoH declared the ZIKV outbreak a public health emergency after an increase in microcephaly cases was reported in the Northeast Region of the Country. During 2015-2016, 15 states in Brazil with laboratory-confirmed ZIKV transmission reported an increase in birth prevalence of microcephaly (2.8 cases per 10,000 live births), significantly exceeding prevalence in four states without confirmed transmission (0.6 per 10,000). Although children with microcephaly and laboratory evidence of ZIKV infection have been described in early infancy, their subsequent health and development have not been well characterized, constraining planning for the care and support of these children and their families. The Brazilian MoH, the State Health Secretariat of Paraíba, and CDC collaborated on a follow-up investigation of the health and development of children in northeastern Brazil who were reported to national surveillance with microcephaly at birth.

Nineteen children with microcephaly at birth and laboratory evidence of ZIKV infection were assessed through clinical evaluations, caregiver interviews, and review of medical records. At follow-up (ages 19-24 months), most of these children had severe motor impairment, seizure disorders, hearing and vision abnormalities, and sleep difficulties. Children with microcephaly and laboratory evidence of ZIKV infection have severe functional limitations and will require specialized care from clinicians and caregivers as they age [110]. Initial descriptions of the effects of *in utero* ZIKV infection centered prominently on the finding of congenital microcephaly. To assess the possibility of clinical presentations that do not include congenital microcephaly, a retrospective assessment of 13 infants from the Brazilian states of Pernambuco and Ceará with normal head size at birth and laboratory evidence of congenital ZIKV infection was conducted. All infants had brain abnormalities on neuroimaging consistent with CZS, including decreased brain volume, ventriculomegaly, subcortical calcifications, and cortical malformations. The earliest evaluation occurred on the second day of life. Among all infants, head growth was documented to have decelerated as early as five months of age, and 11 infants had microcephaly. These findings provide evidence that among infants with prenatal exposure to ZIKV, the absence of microcephaly at birth does not exclude congenital ZIKV infection or the presence of zika-related brain and other abnormalities. These findings support the recommendation for comprehensive medical and developmental follow-up of infants exposed to ZIKV prenatally. Early neuroimaging might identify brain abnormalities related to congenital zika infection even among infants with a normal head circumference [111]. The WHO stated in March 2016 that there was scientific consensus that ZIKV was a cause of the neurological disorder GBS and of microcephaly and other congenital brain abnormalities based on rapid evidence assessments. Decisions about causality require systematic assessment to guide public health actions. WHO convened a multidisciplinary panel of experts who reached consensus statements to update the WHO position on causality. The expert panel concluded that:

- 1) the most likely explanation of available evidence from outbreaks of ZIKV infection and clusters of microcephaly is that ZIKV infection during pregnancy is a cause of congenital brain abnormalities, including microcephaly; and
- 2) the most likely explanation of available evidence from outbreaks of ZIKV infection and GBS is that ZIKV infection is a trigger of GBS.

The expert panel recognized that ZIKV alone may not be sufficient to cause either congenital brain abnormalities or GBS but agreed that the evidence was sufficient to recommend increased public health measures [112]. The establishment of a comprehensive surveillance system to monitor pregnant women with ZIKV infection will provide data to further elucidate the full range of potential outcomes for fetuses and infants of mothers with asymptomatic and symptomatic ZIKV infection during pregnancy. In February 2016, ZIKV disease and congenital ZIKV infections became nationally notifiable conditions in the US. Cases in pregnant women with laboratory evidence of ZIKV infection who have either:

- 1) symptomatic infection; or
- 2) asymptomatic infection with diagnosed complications of pregnancy;

can be reported as cases of ZIKV disease to ArboNET, CDC's national arboviral diseases surveillance system. Under existing interim guidelines from the Council for State and Territorial Epidemiologists (CSTE), asymptomatic ZIKV infections in pregnant women who do not have known pregnancy complications are not reportable. ArboNET does not currently include pregnancy surveillance information (e.g., gestational age or pregnancy exposures) or pregnancy outcomes. To understand the full impact of infection on the fetus and neonate, other systems are needed for reporting and active monitoring of pregnant women with laboratory evidence of possible ZIKV infection during pregnancy. Thus, in collaboration with state, local, tribal, and territorial health departments, CDC established two surveillance systems to monitor pregnancies and congenital outcomes among women with laboratory evidence of ZIKV infection in the US and territories:

- 1) the US Zika Pregnancy and Infant Registry (USZPIR), which monitors pregnant women residing in US and territories except Puerto Rico; and
- 2) the Zika Active Pregnancy Surveillance System (ZAPSS), which monitors pregnant women residing in Puerto Rico.

As of May 12th, 2016, the surveillance systems were monitoring 157 and 122 pregnant women with laboratory evidence of possible ZIKV infection from participating US and territories, respectively. Tracking and monitoring clinical presentation of ZIKV infection, all prenatal testing, and adverse consequences of ZIKV infection during pregnancy are critical to better characterize the risk for congenital infection, the performance of prenatal diagnostic testing, and the spectrum of adverse congenital outcomes. These data will improve clinical guidance, inform counseling messages for pregnant women, and facilitate planning for clinical and public health services for affected families [113]. To better understand the impact of birth defects resulting from ZIKV infection, the CDC surveillance case definition established in 2016 for birth defects potentially related to ZIKV infection was retrospectively applied to population-based birth defects surveillance data collected during 2013-2014 in three areas before the introduction of ZIKV (the pre-zika years) into the WHO's Region of the Americas. These data, from Massachusetts (2013), North Carolina (2013), and Atlanta, Georgia (2013-2014), included 747 infants and fetuses with one or more of the birth defects meeting the case definition (pre-zika prevalence = 2.86 per 1,000 live births). Brain abnormalities or microcephaly were the most frequently recorded (1.50 per 1,000), followed by neural tube defects (NTD) and other early brain malformations (0.88), eye abnormalities without mention of a brain abnormality (0.31), and other consequences of CNS dysfunction without mention of brain or eye abnormalities (0.17). During January 15th-September 22nd, 2016, the USZPIR reported 26 infants and fetuses with these same defects among 442 completed pregnancies (58.8 per 1,000) born to mothers with laboratory evidence of possible ZIKV infection during pregnancy. Although the ascertainment methods differed, this finding was approximately 20 times higher than the proportion of one or more of the same birth defects among pregnancies during the pre-zika years. These data demonstrate the importance of population-based surveillance for interpreting data about birth defects potentially related to ZIKV infection [114]. As of November 2016, the Florida Department of Health (FDH) and the CDC have confirmed more than 4,000 travel-related ZIKV infections in the US with >700 of those in Florida. There have been 139 cases of locally acquired infection, all occurring in Miami, Florida. Within the US territories (e.g., Puerto Rico, USVI), >30,000 cases of ZIKV infection have been reported.

The projected number of individuals at risk for ZIKV infection in Latin America and the Caribbean (LAC) approximates five million. South Florida is an epicenter for ZIKV infection in the US and the year-round warm climate along with an abundance of mosquito vectors that can harbor the Flavivirus raise health care concerns. Federal health officials observed 899 confirmed zika-positive pregnancies and the FDH monitored 110 pregnant women with evidence of zika infection. The University of Miami/Jackson Memorial Hospital is uniquely positioned just North of downtown Miami and within the vicinity of Liberty City, Little Haiti, and Miami Beach, which are currently “hot spots” for ZIKV exposure and transmission. As the FDH works fervently to prevent a zika epidemic in the region, health care providers at the University of Miami and Jackson Memorial Hospital prepare for the clinical spectrum of ZIKV effects as well as the safe peri-operative care of the parturients and their affected newborns [115]. In Colombia, approximately 105,000 suspected cases of ZIKV disease (diagnosed based on clinical symptoms, regardless of laboratory confirmation) were reported during August 9th, 2015-November 12th, 2016, including nearly 20,000 in pregnant women. Colombia’s Instituto Nacional de Salud (INS) maintains national surveillance for birth defects, including microcephaly and other CNS defects. This report provided preliminary information on cases of congenital microcephaly identified in Colombia during epidemic weeks five to 45 (January 31st-November 12th) in 2016. During this period, 476 cases of microcephaly were reported, compared with 110 cases reported during the same period in 2015. The temporal association between reported ZIKV infections and the occurrence of microcephaly, with the peak number of reported microcephaly cases occurring approximately 24 weeks after the peak of the ZIKV disease outbreak, provides evidence suggesting that the period of highest risk is during the first trimester of pregnancy and early in the second trimester of pregnancy. Microcephaly prevalence increased more than fourfold overall during the study period, from 2.1 per 10,000 live births in 2015 to 9.6 in 2016. Ongoing population-based birth defects surveillance is essential for monitoring the impact of ZIKV infection during pregnancy on birth defects prevalence and measuring the success in preventing ZIKV infection and its consequences, including microcephaly [116]. On November 28th, 2016, the Texas Department of State Health Services (DSHS) reported its first confirmed case of local mosquito-borne ZIKV transmission in the city of Brownsville, located in South Texas along the US-Mexico border. On January 1st, 2017, Texas DSHS established enhanced surveillance to identify women with laboratory evidence of possible ZIKV infection during pregnancy and suspected cases of ZIKV-associated birth defects among completed pregnancies [117]. Another report included an analysis of completed pregnancies (which include live births and pregnancy losses, regardless of gestational age) in the 50 US and the DC with laboratory evidence of possible recent ZIKV infection reported to the USZPIR from January 15th to December 27th, 2016. Birth defects potentially associated with ZIKV infection during pregnancy include brain abnormalities and/or microcephaly, eye abnormalities, other consequences of CNS dysfunction, and NTD and other early brain malformations. During the analysis period, 1,297 pregnant women in 44 states were reported to the USZPIR. ZIKV-associated birth defects were reported for 51 (5%) of the 972 fetuses/infants from completed pregnancies with laboratory evidence of possible recent ZIKV infection (95% CI = 4-7%); the proportion was higher when restricted to pregnancies with laboratory-confirmed ZIKV infection (24/250 completed pregnancies [10%, 95% CI = 7-14%]). Birth defects were reported in 15% (95% CI = 8-26%) of fetuses/infants of completed pregnancies with confirmed ZIKV infection in the first trimester. Among 895 liveborn infants from pregnancies with possible recent ZIKV infection, postnatal neuroimaging was reported

for 221 (25%), and ZIKV testing of at least one infant specimen was reported for 585 (65%). These findings highlight why pregnant women should avoid ZIKV exposure. Because the full clinical spectrum of congenital ZIKV infection is not yet known, all infants born to women with laboratory evidence of possible recent ZIKV infection during pregnancy should receive postnatal neuroimaging and ZIKV testing in addition to a comprehensive newborn physical exam and hearing screen. Identification and follow-up care of infants born to women with laboratory evidence of possible recent ZIKV infection during pregnancy and infants with possible congenital ZIKV infection can ensure that appropriate clinical services are available [118]. CDC analyzed data from 15 US jurisdictions conducting population-based surveillance for birth defects potentially related to ZIKV infection. Jurisdictions were stratified into the following three groups:

- 1) those with documented local transmission of ZIKV during 2016;
- 2) one or more cases of confirmed, symptomatic, travel-associated ZIKV disease reported to CDC per 100,000 residents; and
- 3) less than one case of confirmed, symptomatic, travel-associated ZIKV disease reported to CDC per 100,000 residents.

A total of 2,962 infants and fetuses (3.0 per 1,000 live births; 95% CI = 2.9-3.2) met the case definition. In areas with local transmission there was a non-statistically significant increase in total birth defects potentially related to ZIKV infection from 2.8 cases per 1,000 live births in the first half of 2016 to 3.0 cases in the second half ($p = 0.10$). However, when NTD and other early brain malformations were excluded, the prevalence of birth defects strongly linked to congenital ZIKV infection increased significantly, from 2.0 cases per 1,000 live births in the first half of 2016 to 2.4 cases in the second half, an increase of 29 more cases than expected ($p = 0.009$). These findings underscore the importance of surveillance for birth defects potentially related to ZIKV infection and the need for continued monitoring in areas at risk for Zika [119]. Early identification of and intervention for neurodevelopmental problems can improve cognitive, social, and behavioral functioning. Pregnancies with laboratory evidence of confirmed or possible ZIKV infection and infants resulting from these pregnancies are included in the USZPIR and followed through active surveillance methods. An USZPIR report included data on children aged at least one year born in US territories and freely associated states. Receipt of reported follow-up care was assessed, and data were reviewed to identify Zika-associated birth defects and neurodevelopmental abnormalities possibly associated with congenital ZIKV infection. Among 1,450 children of mothers with laboratory evidence of confirmed or possible ZIKV infection during pregnancy and with reported follow-up care, 76% had developmental screening or evaluation, 60% had postnatal neuroimaging, 48% had automated auditory brainstem response (ABR)-based hearing screen or evaluation, and 36% had an ophthalmological evaluation. Among evaluated children, 6% had at least one Zika-associated birth defect identified, 9% had at least one neurodevelopmental abnormality possibly associated with congenital ZIKV infection identified, and 1% had both. One in seven evaluated children had a Zika-associated birth defect, a neurodevelopmental abnormality possibly associated with congenital ZIKV infection, or both reported to the USZPIR. Given that most children did not have evidence of all recommended evaluations, additional anomalies might not have been identified. Careful monitoring and evaluation of children born to mothers with evidence of ZIKV infection during pregnancy is essential for ensuring early detection of

possible disabilities and early referral to intervention services [120]. In February 2016, CDC recommended:

- 1) routine testing for ZIKV infection of asymptomatic pregnant women living in areas with ongoing local ZIKV transmission at the first prenatal care visit;
- 2) re-testing during the second trimester for women who initially test negative; and
- 3) testing of pregnant women with signs or symptoms consistent with ZIKV disease (e.g., fever, rash, arthralgia, or conjunctivitis) at any time during pregnancy.

During January 1st, 2016-April 25th, 2017, US territories with local transmission of ZIKV reported 2,549 completed pregnancies (live births and pregnancy losses at any gestational age) with laboratory evidence of recent possible ZIKV infection; 5% of fetuses or infants resulting from these pregnancies had birth defects potentially associated with ZIKV infection. Among completed pregnancies with positive nucleic acid tests confirming zika infection identified in the first, second, and third trimesters, the percentage of fetuses or infants with possible zika-associated birth defects was 8%, 5%, and 4%, respectively. Among live-born infants, 59% had zika laboratory testing results reported to the pregnancy and infant registries. Identification and follow-up of infants born to women with laboratory evidence of recent possible ZIKV infection during pregnancy permits timely and appropriate clinical intervention services [121].

CDC has developed interim guidelines for health care providers in the US caring for pregnant women during a ZIKV outbreak. These guidelines include recommendations for pregnant women considering travel to an area with ZIKV transmission and recommendations for screening, testing, and management of pregnant returning travelers. Health care providers should ask all pregnant women about recent travel. Pregnant women with a history of travel to an area with ZIKV transmission and who report two or more symptoms consistent with ZIKV disease (acute onset of fever, maculopapular rash, arthralgia, or conjunctivitis) during or within two weeks of travel, or who have ultrasound findings of fetal microcephaly or intracranial calcifications, should be tested for ZIKV infection in consultation with their state or local health department. Testing is not indicated for women without a travel history to an area with ZIKV transmission. In pregnant women with laboratory evidence of ZIKV infection, serial ultrasound examination should be considered to monitor fetal growth and anatomy and referral to a maternal-fetal medicine or infectious disease specialist with expertise in pregnancy management is recommended [122]. Updated guidelines include a new recommendation to offer serological testing to asymptomatic pregnant women (women who do not report clinical illness consistent with ZIKV disease) who have traveled to areas with ongoing ZIKV transmission. Testing can be offered two to 12 weeks after pregnant women return from travel. This update also expands guidance to women who reside in areas with ongoing ZIKV transmission, and includes recommendations for screening, testing, and management of pregnant women and recommendations for counseling women of reproductive age (15-44 years). Pregnant women who reside in areas with ongoing ZIKV transmission have an ongoing risk for infection throughout their pregnancy. For pregnant women with clinical illness consistent with ZIKV disease, testing is recommended during the first week of illness. For asymptomatic pregnant women residing in areas with ongoing ZIKV transmission, testing is recommended at the initiation of prenatal care with mid-second trimester follow-up testing. Local health officials should determine when to implement testing of asymptomatic pregnant women based on information about levels of ZIKV transmission and laboratory capacity.

Health care providers should discuss reproductive life plans, including pregnancy intention and timing, with women of reproductive age in the context of the potential risks associated with ZIKV infection [123]. CDC has updated its interim guidance for US health care providers caring for women of reproductive age with possible ZIKV exposure to include recommendations on counseling women and men with possible ZIKV exposure who are interested in conceiving. This guidance is based on limited available data on persistence of ZIKV RNA in blood and semen. Women who have ZIKV disease should wait at least eight weeks after symptom onset to attempt conception, and men with ZIKV disease should wait at least six months after symptom onset to attempt conception. Women and men with possible exposure to ZIKV but without clinical illness consistent with ZIKV disease should wait at least eight weeks after exposure to attempt conception. Possible exposure to ZIKV is defined as travel to or residence in an area of active ZIKV transmission, or sex (vaginal intercourse, anal intercourse, or fellatio) without a condom with a man who traveled to or resided in an area of active transmission. Women and men who reside in areas of active ZIKV transmission should talk with their health care provider about attempting conception [124]. CDC has updated its interim guidance for US health care providers caring for pregnant women with possible ZIKV exposure, to include the emerging data indicating that ZIKV RNA can be detected for prolonged periods in some pregnant women. To increase the proportion of pregnant women with ZIKV infection who receive a definitive diagnosis, CDC recommends expanding real-time RT-PCR testing. Possible exposures to ZIKV include travel to or residence in an area with active ZIKV transmission, or sex with a partner who has traveled to or resides in an area with active ZIKV transmission without using condoms or other barrier methods to prevent infection. Testing recommendations for pregnant women with possible ZIKV exposure who report clinical illness consistent with ZIKV disease (symptomatic pregnant women) are the same, regardless of their level of exposure (i.e., women with ongoing risk for possible exposure, including residence in or frequent travel to an area with active ZIKV transmission, as well as women living in areas without ZIKV transmission who travel to an area with active ZIKV transmission, or have unprotected sex with a partner who traveled to or resides in an area with active ZIKV transmission). Symptomatic pregnant women who are evaluated earlier than two weeks after symptom onset should receive serum and urine ZIKV RT-PCR testing. Symptomatic pregnant women who are evaluated two to 12 weeks after symptom onset should first receive a ZIKV IgM antibody test; if the IgM antibody test result is positive or equivocal, serum and urine RT-PCR testing should be performed. Testing recommendations for pregnant women with possible ZIKV exposure who do not report clinical illness consistent with ZIKV disease (asymptomatic pregnant women) differ based on the circumstances of possible exposure. For asymptomatic pregnant women who live in areas without active ZIKV transmission and who are evaluated earlier than two weeks after last possible exposure, RT-PCR testing should be performed. If the RT-PCR result is negative, a ZIKV IgM antibody test should be performed two to 12 weeks after the exposure. Asymptomatic pregnant women who do not live in an area with active ZIKV transmission, who are first evaluated two to 12 weeks after their last possible exposure should first receive a ZIKV IgM antibody test; if the IgM antibody test result is positive or equivocal, serum and urine RT-PCR should be performed. Asymptomatic pregnant women with ongoing risk for exposure to ZIKV should receive ZIKV IgM antibody testing as part of routine obstetric care during the first and second trimesters; immediate RT-PCR testing should be performed when IgM antibody test results are positive or

equivocal [125]. CDC has updated the interim guidance for US health care providers caring for pregnant women with possible ZIKV exposure in response to:

- 1) declining prevalence of ZIKV disease in the WHO's Region of the Americas; and
- 2) emerging evidence indicating prolonged detection of ZIKV IgM antibodies.

ZIKV cases were first reported in the Americas during 2015-2016; however, the incidence of ZIKV disease has since declined. As the prevalence of ZIKV disease declines, the likelihood of false-positive test results increases. In addition, emerging epidemiological and laboratory data indicate that, as is the case with other Flaviviruses, ZIKV IgM antibodies can persist beyond 12 weeks after infection. Therefore, IgM test results cannot always reliably distinguish between an infection that occurred during the current pregnancy and one that occurred before the current pregnancy, particularly for women with possible ZIKV exposure before the current pregnancy. These limitations should be considered when counseling pregnant women about the risks and benefits of testing for ZIKV infection during pregnancy. This updated guidance emphasizes a shared decision-making model for testing and screening pregnant women, one in which patients and providers work together to make decisions about testing and care plans based on patient preferences and values, clinical judgment, and a balanced assessment of risks and expected outcomes [126].

Pregnant women in ZIKV-affected areas should protect themselves from mosquito bites by using air conditioning, screens, or nets when indoors, wearing long sleeves and pants, using permethrin-treated clothing and gear, and using insect repellents when outdoors. Pregnant and lactating women can use all US EPA-registered insect repellents according to the product label [127]. After reports of microcephaly and other adverse pregnancy outcomes in infants of mothers infected with ZIKV during pregnancy, CDC issued a travel alert on January 15th, 2016, advising pregnant women to consider postponing travel to areas with active transmission of ZIKV. On January 19th, CDC released interim guidelines for US health care providers caring for pregnant women with travel to an affected area, and an update was released on February 5th. As of February 17th, CDC had received reports of nine pregnant travelers with laboratory-confirmed ZIKV disease; 10 additional reports of ZIKV disease among pregnant women were under investigation. No ZIKV-related hospitalizations or deaths among pregnant women were reported. Pregnancy outcomes among the nine confirmed cases included two early pregnancy losses, two elective terminations, and three live births (two apparently healthy infants and one infant with severe microcephaly); two pregnancies (approximately 18 weeks' and 34 weeks' gestation) were continuing without known complications. Confirmed cases of ZIKV infection were reported among women who had traveled to one or more of the following nine areas with ongoing local transmission of ZIKV: American Samoa, Brazil, El Salvador, Guatemala, Haiti, Honduras, Mexico, Puerto Rico, and Samoa. US health care providers caring for pregnant women with possible ZIKV exposure during pregnancy should follow CDC guidelines for patient evaluation and management. ZIKV disease is a nationally notifiable condition [128]. Guidelines for US health care providers caring for infants born to mothers who traveled to or resided in areas with ZIKV transmission during pregnancy and guidelines to include infants and children with possible acute ZIKV disease contain a recommendation for routine care for infants born to mothers who traveled to or resided in areas with ZIKV transmission during pregnancy but did not receive ZIKV testing, when the infant has a normal head circumference,

normal prenatal and postnatal ultrasounds (if performed), and normal physical examination. Acute ZIKV disease should be suspected in an infant or child aged <18 years who:

- 1) traveled to or resided in an affected area within the past two weeks; and
- 2) has at least two of the following manifestations: fever, rash, conjunctivitis, or arthralgia.

Since maternal-infant transmission of ZIKV during delivery is possible, acute ZIKV disease should also be suspected in an infant during the first two weeks of life:

- 1) whose mother traveled to or resided in an affected area within two weeks of delivery; and
- 2) who has at least two of the following manifestations: fever, rash, conjunctivitis, or arthralgia.

Evidence suggests that ZIKV illness in children is usually mild. As an arboviral disease, ZIKV disease is nationally notifiable. Health care providers should report suspected cases of ZIKV disease to their local, state, or territorial health departments to arrange testing and so that action can be taken to reduce the risk for local ZIKV transmission [129]. Laboratory testing is recommended for:

- 1) infants born to mothers with laboratory evidence of ZIKV infection during pregnancy; and
- 2) infants who have abnormal clinical or neuroimaging findings suggestive of CZS and a maternal epidemiological link suggesting possible transmission, regardless of maternal ZIKV test results.

Recommended infant laboratory evaluation includes both molecular (real-time RT-PCR) and serological (IgM) testing. Initial samples should be collected directly from the infant in the first two days of life, if possible; testing of cord blood is not recommended. A positive infant serum or urine RT-PCR test result confirms congenital ZIKV infection. Positive ZIKV IgM testing, with a negative RT-PCR result, indicates probable congenital ZIKV infection. In addition to infant ZIKV testing, initial evaluation of all infants born to mothers with laboratory evidence of ZIKV infection during pregnancy should include a comprehensive physical examination, including a neurological examination, postnatal head ultrasound, and standard newborn hearing screen. Infants with laboratory evidence of congenital ZIKV infection should have a comprehensive ophthalmological exam and hearing assessment by ABR testing before one month of age. Recommendations for follow-up of infants with laboratory evidence of congenital ZIKV infection depend on whether abnormalities consistent with CZS are present. Infants with abnormalities consistent with CZS should have a coordinated evaluation by multiple specialists within the first month of life; additional evaluations will be needed within the first year of life, including assessments of vision, hearing, feeding, growth, and neurodevelopmental and endocrine function. Families and caregivers will also need ongoing psycho-social support and assistance with coordination of care. Infants with laboratory evidence of congenital ZIKV infection without apparent abnormalities should have ongoing developmental monitoring and screening by the primary care provider; repeat hearing testing

is recommended [130]. However, there are few published reports describing postnatally acquired ZIKV disease among children. During January 2015-July 2016, a total of 158 cases of confirmed or probable postnatally acquired ZIKV disease among children aged <18 years were reported to CDC from US. The median age was 14 years (range = one month to 17 years), and 88 (56%) were female. Two (1%) patients were hospitalized; none developed GBS, and none died. All reported cases were travel-associated. Overall, 129 (82%) children had rash, 87 (55%) had fever, 45 (29%) had conjunctivitis, and 44 (28%) had arthralgia. Health care providers should consider a diagnosis of ZIKV disease in children who have an epidemiological risk factor and clinically compatible illness, and should report cases to their state or local health department [131]. It is critical that pediatricians understand its epidemiology, clinical presentation, clinical sequelae, and management. The field of clinical research in ZIKV has rapidly evolved over recent months. It is critical that pediatricians continue to stay up-to-date with the continuously evolving understanding of the clinical aspects of ZIKV to ensure optimal identification and management of affected infants and children. Given the recent changes in CDC guidelines to limit screening of asymptomatic pregnant women in the US with possible ZIKV exposure, comprehensive ZIKV clinical knowledge becomes even more crucial [132-134].

In the past six years, ZIKV has emerged from obscurity onto the world stage-traversing and transcending clinical specialties, basic science disciplines, and public health efforts. Due to the fact that ZIKV can be sexually transmitted, there is a potential risk for disease transmission at several stages of assisted reproduction. Such a possibility poses a serious challenge to couples pursuing fertility with assisted reproductive technologies (ART). ZIKV can be transmitted from infected men and women through vaginal, oral, or anal intercourse. ZIKV RNA has been detected in blood, semen, cervical mucus, and vaginal fluid. Currently, the CDC recommends that infected men wait six months, and infected women eight weeks, prior to attempting pregnancy. Reproductive tissue donors should wait six months before giving a specimen. Further study of ZIKV transmission in different reproductive tissues and establishment of validated testing methods for viral disease transmissibility are urgently needed. Reproductive technologists need to establish screening, testing, and laboratory protocols aimed to reduce the risk of ZIKV transmission during assisted reproduction [135]. Indeed, the spread of ZIKV has serious implications for the specialty of reproductive endocrinology and infertility. Patients, practices, and labs – worldwide and specifically in the US – have been impacted by this teratogenic, sexually transmitted, largely asymptomatic virus. While the WHO's PHEIC designation has lapsed as major epidemics have subsided and understanding of risks is in part clarified, the acute and long-term threat to pregnant patients is not over. The risk of wider spread in the US is not insignificant, the subtler and long-ranging consequences beyond microcephaly are not fully known, large geographic areas of risk still contain naïve populations, and whether zika will continue to be an intermittent risk in endemic areas is uncertain. Staying up to date with the burgeoning research on ZIKV is an important objective for all health care providers [136-138].

Given the extensive and variable fetal and newborn presentations/complications associated with prenatal ZIKV infection, and the dearth of information provided, knowledge gaps are evident. Studies on this matter involve both rare outcomes and rare exposures and methodological choices are not straightforward. Cohort studies will surely offer more robust evidences, but their efficiency must be enhanced. A study can be statistically more efficient than another if its estimates are more accurate (precise and valid), even if the studies involve

the same number of subjects. Sample size and specific design strategies can enhance or impair the statistical efficiency of a study, depending on how the subjects are distributed in subgroups pertinent to the analysis. In most ZIKV during pregnancy cohort studies to date there is an *a priori* identification of the source population (pregnant women, regardless of their exposure status) which is then sampled or included in its entirety (census). Subsequently, the group of pregnant women is classified according to exposure (presence or absence of ZIKV during pregnancy), respecting the exposed/unexposed ratio in the source population. The sample selection can be done from the *a priori* identification of groups of pregnant women exposed and unexposed to ZIKV during pregnancy. This method will allow for an oversampling (even 100%) of pregnant women with ZKV during pregnancy and an optimized sampling from the general population of pregnant women unexposed to ZIKV during pregnancy, saving resources in the unexposed group and improving the expected number of incident cases (outcomes) overall [139]. Mathematical modeling analysis using reported outcome data suggests that surveillance for these outcomes should begin as soon as an outbreak is detected and should continue for 40 weeks after the outbreak ends [140]. A non-human primate (NHP) model of congenital ZIKV infection can help fill the gaps in knowledge where tissue studies are required to define viral pathogenesis and identify targets for therapeutic intervention. This model system has already identified critical features of ZIKV pathogenesis in congenital infection. Before translating these NHP studies to human clinical trials, we must understand the similarities and differences between human and NHP fetal immune system development, neural development, and infant assessment tools. Because of the overall similarity between fetal and infant development in humans and NHP, this NHP model can complement human clinical trials by defining immune correlates of protection and evaluating therapeutic interventions [141]. Further research and comprehensive reporting may provide a better understanding of ZIKV infection in pregnancy and attendant maternal/fetal complications. This knowledge could inform the creation of effective and evidence-based strategies, guidelines, and recommendations aimed at the management of maternal ZIKV infection. Adherence to current best practice guidelines for prenatal care among health providers is encouraged in the context of maternal ZIKV infection [142-148].

DIAGNOSIS

Diagnosis of ZIKV infection should be suspected in individuals with the appropriate symptomatology and exposure history. Diagnostic tests are available and vary based on symptom duration [149]. Diagnostic testing for ZIKV infection can be accomplished using both molecular and serological methods. For persons with suspected ZIKV disease, a positive real-time RT-PCR result confirms ZIKV infection, but a negative RT-PCR result does not exclude infection. In these cases, IgM and neutralizing antibody testing can identify additional recent ZIKV infections. However, ZIKV antibody test results can be difficult to interpret because of cross-reactivity with other Flaviviruses, which can preclude identification of the specific infecting virus, especially when the person previously was infected with or vaccinated against a related Flavivirus. This is important because the results of ZIKV and DENV testing will guide clinical management. Pregnant women with laboratory evidence of ZIKV infection should be evaluated and managed for possible adverse pregnancy outcomes and be reported to the

USZPIR or the Puerto Rico ZAPSS for clinical follow-up. All patients with clinically suspected dengue should have proper management to reduce the risk for hemorrhage and shock. If serological testing indicates recent Flavivirus infection that could be caused by either ZIKV or DENV, patients should be clinically managed for both infections because they might have been infected with either virus [150]. A confirmed case of ZIKV infection is defined as a positive result for ZIKV testing by RT-PCR for ZIKV in a blood or urine specimen. A presumptive case is defined as a positive result by ZIKV IgM ELISA and a negative result by DENV IgM ELISA, or a positive test result by ZIKV IgM ELISA in a pregnant woman. An unspecified Flavivirus case is defined as positive or equivocal results for both ZIKV and DENV by IgM ELISA [151-155].

The RNA of the ZIKV has been identified in samples of brain tissue, placenta, and amniotic fluid of children with microcephaly and in the still-born infants of women infected by zika during pregnancy. The RT-PCR test is recommended to detect viral RNA, and serological tests (IgM ELISA and neutralizing antibodies) should be conducted to confirm infection by zika. The differential diagnosis includes infection by DENV and CHIKV [156]. Urine and saliva have been previously established as useful tools in ZIKV diagnosis. The detection of infectious ZIKV particles in urine and saliva of patients during the acute phase may represent a critical factor in the spread of the virus. The epidemiological relevance of this finding, regarding the contribution of alternative non-vectorial ZIKV transmission routes, needs further investigation [157]. Rhesus macaques are susceptible to infection by an Asian-lineage ZIKV closely related to strains currently circulating in the Americas. Following subcutaneous inoculation, ZIKV RNA is detected in plasma one day post infection (d.p.i.) in all animals, and is also present in saliva, urine, and CSF. Non-pregnant and pregnant animals remain viremic for 21 days and for up to at least 57 days, respectively. Neutralizing antibodies are detected by 21 d.p.i. Re-challenge 10 weeks after the initial challenge results in no detectable virus replication, indicating protective immunity against homologous strains. Therefore, Asian-lineage ZIKV infection of Rhesus macaques provides a relevant animal model for studying pathogenesis and evaluating potential interventions against human infection, including during pregnancy [158-160].

CDC has developed interim guidelines for health care providers in the US who are caring for infants born to mothers who traveled to or resided in an area with ZIKV transmission during pregnancy. These guidelines include recommendations for the testing and management of these infants. Pediatric health care providers should work closely with obstetric providers to identify infants whose mothers were potentially infected with ZIKV during pregnancy (based on travel to or residence in an area with ZIKV transmission), and review fetal ultrasounds and maternal testing for ZIKV infection. ZIKV testing is recommended for:

- 1) infants with microcephaly or intracranial calcifications born to women who traveled to or resided in an area with ZIKV transmission while pregnant; or
- 2) infants born to mothers with positive or inconclusive test results for ZIKV infection.

For infants with laboratory evidence of a possible congenital ZIKV infection, additional clinical evaluation and follow-up is recommended. Health care providers should contact their state or territorial health department to facilitate testing [161]. CDC has updated its interim guidance for US health care providers caring for infants with possible congenital ZIKV infection in response to recently published updated guidance for health care providers caring

for pregnant women with possible ZIKV exposure, unknown sensitivity and specificity of currently available diagnostic tests for congenital ZIKV infection, and recognition of additional clinical findings associated with congenital ZIKV infection. All infants born to mothers with possible ZIKV exposure during pregnancy should receive a standard evaluation at birth and at each subsequent well-child visit including a comprehensive physical examination, age-appropriate vision screening and developmental monitoring and screening using validated tools, and newborn hearing screen at birth, preferably using ABR methodology. Specific guidance for laboratory testing and clinical evaluation are provided for three clinical scenarios in the setting of possible maternal ZIKV exposure:

- 1) infants with clinical findings consistent with CZS regardless of maternal testing results;
- 2) infants without clinical findings consistent with CZS who were born to mothers with laboratory evidence of possible ZIKV infection; and
- 3) infants without clinical findings consistent with CZS who were born to mothers without laboratory evidence of possible ZIKV infection.

Infants in the first two scenarios should receive further testing and evaluation for ZIKV, whereas for the third group, further testing and clinical evaluation for ZIKV are not recommended. Health care providers should remain alert for abnormal findings (e.g., postnatal-onset microcephaly and eye abnormalities without microcephaly) in infants with possible congenital ZIKV exposure without apparent abnormalities at birth [162].

The rapid spread of ZIKV across the WHO's Region of the Americas has had a direct effect on the US health care delivery system. CDC recommends ZIKV testing for potentially exposed persons with signs or symptoms consistent with ZIKV disease, and recommends that health care providers offer testing to asymptomatic pregnant women within 12 weeks of exposure. During January 3rd-March 5th, 2016, ZIKV testing was performed for 4,534 persons who traveled to or moved from areas with active ZIKV transmission; 3,335 (73.6%) were pregnant women. Among persons who received testing, 1,541 (34.0%) reported at least one ZIKV-associated sign or symptom (e.g., fever, rash, arthralgia, or conjunctivitis), 436 (9.6%) reported at least one other clinical sign or symptom only, and 2,557 (56.4%) reported no signs or symptoms. Among 1,541 persons with one or more ZIKV-associated symptoms who received testing, 182 (11.8%) had confirmed ZIKV infection. Among the 2,557 asymptomatic persons who received testing, 2,425 (94.8%) were pregnant women, seven (0.3%) of whom had confirmed ZIKV infection. Although risk for ZIKV infection might vary based on exposure-related factors (e.g., location and duration of travel), in the current setting in US, where there is no local transmission, most asymptomatic pregnant women who receive testing do not have ZIKV infection [163]. Hospitals in NYC have been implementing prevention and response efforts consistent with CDC guidance. As of September 21st, 2016, a total of 715 cases of laboratory-confirmed ZIKV disease had been diagnosed in New York state among travelers who returned from affected areas, their sexual contacts, or infants infected in utero. This represents the highest number of reported cases in any state to date, and underscores the importance of health care systems preparing to care for patients with possible ZIKV disease. Building upon a framework that was established in 2014 to screen patients for possible exposure to EBOV disease, NYC Health Hospitals, the largest municipal health care delivery system in the US, implemented a Zika Preparedness and Response Action Plan (Zika Action

Plan) to address the threat from zika and ensure appropriate patient care. The plan developed by NYC Health Hospitals includes universal travel screening, signage depicting areas with active ZIKV transmission, clinical and epidemiological evaluation for possible ZIKV exposure, diagnostic testing for ZIKV infection and linking of infected patients to appropriate specialists, and education on ZIKV disease and preventive measures (e.g., avoiding travel to areas with active ZIKV transmission) [164]. The first patients with laboratory-confirmed cases of ZIKV disease in American Samoa had symptom onset in January 2016. In response, the ASDoH implemented mosquito control measures, strategies to protect pregnant women, syndromic surveillance based on electronic health record (EHR) reports, ZIKV testing of persons with one or more signs or symptoms of ZIKV disease (fever, rash, arthralgia, or conjunctivitis), and routine testing of all asymptomatic pregnant women in accordance with CDC guidance. All collected blood and urine specimens were shipped to the Hawaii Department of Health Laboratory for ZIKV testing and to CDC for confirmatory testing. Early in the response, collection and testing of specimens from pregnant women was prioritized over the collection from symptomatic non-pregnant patients because of limited testing and shipping capacity. The weekly numbers of suspected ZIKV disease cases declined from an average of six per week in January-February 2016 to one per week in May 2016. By August, the EHR-based syndromic surveillance indicated a return to pre-outbreak levels. The last ZIKV disease case detected by real-time RT-PCR occurred in a patient who had symptom onset on June 19th, 2016. In August 2016, ASDoH requested CDC support in assessing whether local transmission had been reduced or interrupted and in proposing a timeline for discontinuation of routine testing of asymptomatic pregnant women. An end date (October 15th, 2016) was determined for active mosquito-borne transmission of ZIKV and a timeline was developed for discontinuation of routine screening of asymptomatic pregnant women in American Samoa (conception after December 10th, 2016, with permissive testing for asymptomatic women who conceived through April 15th, 2017) [165].

The detection of suspected cases by the surveillance system depends on the case definition adopted. As the laboratory diagnosis of zika infection still relies on the use of expensive and complex molecular techniques with low sensitivity due to a narrow window of detection, most suspected cases are not confirmed by laboratory tests, mainly reserved for pregnant women and newborns. In this context, an accurate definition of a suspected zika case is crucial in order for the surveillance system to gauge the magnitude of an epidemic. In areas where multiple arboviruses circulate, the presence of rash with pruritus or conjunctival hyperemia, without any other general clinical manifestations such as fever, petechia, or anorexia is the best zika case definition [166]. The identify-isolate-inform (3I) tool, originally conceived for initial detection and management of EBOV disease patients in the emergency department, and later adjusted for measles and Middle East Respiratory Syndrome (MERS), can be adapted for real-time use for any emerging infectious disease. Following an assessment of epidemiological risk, including travel to countries with mosquitoes that transmit ZIKV, patients are further investigated if clinically indicated. If after a rapid evaluation, zika or other arthropod-borne diseases are the only concern, isolation (contact, droplet, and airborne) is unnecessary. The modified 3I tool can facilitate rapid analysis and triggering of appropriate actions for patients presenting to the emergency department at risk for zika [167]. Determining the viral cause of a rash presents significant diagnostic challenges. ZIKV has re-emerged in the Pacific with local transmission from imported cases. Climate change, global warming, and spillover of zoonotic viruses are contributing to the emergence and spread of viral diseases. Important clues to the diagnosis of

viral exanthems include their distribution and morphology, geographic location, and potential exposure to vector-borne or blood-borne viruses. Diagnosis is commonly made via serology, nucleic acid tests or, rarely, viral culture. Skin biopsy is not usually required [168]. Defining the tissue pathology associated with the various disease manifestations provides insight into pathogenesis and diagnosis, and potentially future prevention and treatment, of ZIKV infections. Pathological findings associated with ZIKV infection are characteristic but not specific. In CZS, tissue pathology is due to direct viral infection of neural structures, whereas in GBS, pathology is likely due to a post-viral, aberrant host-directed immune response. Both fetal and placental pathology specimens are useful for ZIKV diagnosis by molecular and immunohistochemical (IHC) assays; however, the implications of ZIKV detection in placentas from second- and third-trimester normal live births are unclear, as the potential postnatal effects of late gestational exposure remain to be seen [169]. Detection of ZIKV RNA in clinical and tissue specimens can provide definitive laboratory evidence of recent ZIKV infection. Whereas duration of viremia is typically short, prolonged detection of ZIKV RNA in placental, fetal, and neonatal brain tissue has been reported and can provide key diagnostic information by confirming recent ZIKV infection. In accordance with recent guidance, CDC provides ZIKV testing of placental and fetal tissues in clinical situations where this information could add diagnostic value. A report described the evaluation of formalin-fixed paraffin-embedded (FFPE) tissue specimens tested for ZIKV infection in 2016 and the contribution of this testing to the public health response. Among 546 live births with possible maternal ZIKV exposure, for which placental tissues were submitted by the 50 states and DC, 60 (11%) were positive by ZIKV RT-PCR. Among 81 pregnancy losses for which placental and/or fetal tissues were submitted, 18 (22%) were positive by ZIKV RT-PCR. ZIKV RT-PCR was positive on placental tissues from 38/363 (10%) live births with maternal serological evidence of recent unspecified Flavivirus infection and from 9/86 (10%) with negative maternal ZIKV IgM where possible maternal exposure occurred >12 weeks before serum collection. These results demonstrate that ZIKV RT-PCR testing of tissue specimens can provide a confirmed diagnosis of recent maternal ZIKV infection [170].

CZS is a recognizable pattern of structural anomalies and functional disabilities secondary to CNS and, perhaps, PNS damage. Although many of the components of this syndrome, such as cognitive, sensory, and motor disabilities, are shared by other congenital infections, there are five features that are rarely seen with other congenital infections or are unique to congenital ZIKV infection:

- 1) severe microcephaly with partially collapsed skull;
- 2) thin cerebral cortices with subcortical calcifications;
- 3) macular scarring and focal pigmentary retinal mottling;
- 4) congenital contractures; and
- 5) marked early hypertonia and symptoms of extrapyramidal involvement.

Although the full spectrum of adverse reproductive outcomes caused by ZIKV infection is not yet determined, a distinctive phenotype – the CZS – has emerged. Recognition of this phenotype by clinicians for infants and children can help ensure appropriate etiological evaluation and comprehensive clinical investigation to define the range of anomalies in an affected infant as well as determine essential follow-up and ongoing care [171]. Neuropathological findings in CZS helped to understand its pathogenetic mechanisms. The

most relevant post-mortem findings in the CNS of fetuses and neonates infected with ZIKV early in gestation are microcephaly with *ex vacuo* ventriculomegaly and large head circumference associated with obstructive hydrocephalus due to severe mid-brain and aqueduct distortion. Babies with severe brain lesions are born with arthrogryposis. Histologically, there is extensive destruction of the hemispheric parenchyma, calcifications, various disturbances of neuronal migration, reactive gliosis, microglial hyperplasia, and occasional perivascular cuffs of lymphocytes, also in the meninges. Hypoplastic lesions secondary to the lack of descending nerve fibers include small basis pontis, pyramids, and spinal corticospinal tracts. Cerebellar hypoplasia is also common. Severe nerve motor nerve cell loss is observed in the anterior horn of the spinal cord. A spectrum of neuropathological changes, from severe microcephaly to obstructive hydrocephalus, is observed. The severity of the lesions is directly related to the gestational age, the most severe occurring when the mother is infected in the first trimester. Infection of progenitor cells at the germinal matrix is demonstrated. The lack of spinal motor neurons is responsible for fetal akinesia and consequent arthrogryposis [172, 173].

In 1984, Chervenak et al. proposed criteria for the prenatal ultrasound diagnosis of microcephaly as at least three standard deviations (SD) from the mean. Despite improvements in medicine, these criteria have not been reevaluated in 30 years. The 1984 ultrasound criteria for microcephaly remain valid. Physicians today have two alternatives: either use the three-SD cutoff as recommended by Chervenak et al. and endorsed by the SMFM or develop a new dataset for one's population with statistical validation [174-176]. A wide range of clinical-radiological findings of congenital ZIKV infections were reported in the literature, such as microcephaly, overlapping sutures, cortical migrational and *corpus callosum* abnormalities, intracranial calcifications, ventriculomegaly, brain stem and cerebellar malformations, spinal cord involvement, and joint contractures. ZIKV is also related to other severe neurological manifestations in grown-up individuals such as GBS and encephalomyelitis [177]. The most frequently observed radiological findings are microcephaly and decreased brain parenchymal volume associated with lissencephaly, ventriculomegaly secondary to the lack of brain tissue (not hypertensive), and coarse and anarchic calcifications mainly involving the subcortical cortical transition, and the basal ganglia. Although it cannot be concluded that there is a definitive pathognomonic radiographic pattern of microcephaly caused by ZIKV, gross calcifications and anarchic distribution involving the subcortical cortical transition and the basal ganglia, in association with lissencephaly and in the absence of hypertensive ventriculomegaly, are characteristic of this type of infection [178]. Neuroimaging investigation contributes to the prenatal detection of microcephaly and other brain abnormalities in cases of intrauterine ZIKV infection. Neuroimaging is based antenatally on two-dimensional and three-dimensional ultrasound and fetal magnetic resonance imaging (MRI), whereas computed tomography (CT) scan is performed postnatally. Although neuropathology associated with intrauterine ZIKV infection is characterized by non-specific findings of brain disorder, reduced cortical gyration and white-matter hypomyelination or dysmyelination and cerebellar hypoplasia have been consistently observed in the majority of fetuses and newborns. Prenatal or postnatal genetic workup should be carried out to exclude cases of primary microcephaly. Follow-up should rely upon MRI and CT scan as well as neuropediatrician to better define developmental outcome in survivors [179-181].

ETHICAL AND SOCIAL ISSUES

While until recently the small and isolated zika outbreaks in Eastern Asia and Pacific Islands had been overlooked, the large-scale outbreak that started in Brazil in 2015 and the increase of microcephaly cases in the same place and time made media headlines. Considered as harmless until recently, zika has given rise to an important global crisis that poses not only health challenges but also environmental, economic, social, and ethical challenges for states and people around the world [182]. In the first phases of an infectious outbreak, health authorities have to face the challenge of communicating uncertainty. Just when the attention of the public is at the top, information about what is going on is usually still missing. This is the case of zika crisis in Latin America, where an association with microcephaly in newborns and neurological complications have been described. Despite this, and even if the risk would be mainly limited to pregnant women, zika threat is perceived by the general public as greater than others, such as those from dengue or flu. Some peculiarities of this outbreak explain such a gap between real and perceived risk. Nevertheless, in such a situation, WHO and other health authorities have the duty to act out of an abundance of caution, aware of the lose-lose game they are going through: if they raise alarm, and this subsided, they could be accused to fuel people's fears because of economic interests, as it happened in 2009 A (H1N1) pandemic; if they underestimate the threat, they could find themselves in front of a global tragedy [183].

The zika pandemic provides biomedical scientists, clinicians, public health advocates, and governments a unique opportunity to advance reproductive justice. The circumstances in which pregnancies occur are morally relevant to women's reproductive life decisions, to the provision of reproductive health care, and to the development of reproductive health policy. Whether the zika pandemic might foster context-driven reproductive pandemic planning and response is yet to be determined. Maintaining the status quo will surely increase a range of global health disparities and further stratify reproduction, producing predictable and preventable outcomes in which some people receive the necessary care and resources to achieve family building while others are neglected. Women and men should be able to count on biomedical researchers to answer the questions that need answering without undue influence from political agendas. Women should be able to continue pregnancies and count on public health assistance and help for children with zika-related disabilities, or prevent or end a zika-affected pregnancy. Pandemic responses that don't further these ends are morally unacceptable [184]. Public health officials, policymakers, and international organizations are considering interventions to address health consequences of the zika epidemic. To date, public health responses have focused on mosquito vector eradication, sexual and reproductive health services, knowledge and technology including diagnostic test and vaccine development, and health system preparedness [185]. As ZIKV continues to spread, decisions regarding resource allocations to control the outbreak underscore the need for a tool to weigh policies according to their cost and the health burden they could avert. For example, to combat zika outbreak the US President requested the allocation of \$ 1.8 billion from Congress in February 2016. The commitments and the proposal are likely to be cost-effective, whereas the cost-effectiveness of genetically modified mosquitoes depends on the country of implementation. Current estimates suggest that the health burden from microcephaly and GBS warrants substantial expenditures focused on ZIKV control. Results justify the funding committed in Costa Rica and Brazil and many aspects of the budget outlined in the US President's proposal. As data continue to be collected, new

parameter estimates can be customized in real-time to provide updated estimates on cost-effectiveness of interventions and inform policy decisions in country-specific settings [186].

Much of the fear and uncertainty around zika epidemics stem from potential association between ZIKV complications on infected pregnant women and risk of their babies being born with microcephaly and other neurological abnormalities. However, much remains unknown about its mode of transmission, diagnosis, and long-term pathogenesis. Worries of these unknowns necessitate the need for effective and efficient psycho-social programs and medical-legal strategies to alleviate and mitigate ZIKV related burdens. In this light, local and global efforts in maintaining fundamental health principles of moral, medical and legal decision-making policies, and interventions to preserve and promote individual and collective Human Rights, autonomy, protection of the most vulnerable, equity, dignity, integrity, and beneficence that should not be confused and relegated by compassionate humanitarian assistance and support. Further long-term cross-borders operational research is required in elucidating zika-related population-based epidemiology, ethical-medical, and societal implications in guiding evidence-based local and global ZIKV maternal-child health complications related approaches and interventions. Meeting basic health needs (i.e., preventive measures), together with research and development conducive to a vaccine for global zika immunization program in most vulnerable and affected countries and worldwide or treatment for the ZIKV should be priorities [187]. Since a vaccine or anti-viral therapy is not yet available, current means of disease prevention involve protection from mosquito bites, excluding pregnant females from traveling to zika-endemic territories, and practicing safe sex in those countries. Importantly, in countries where zika is reported as endemic, caution is advised in planning to conceive a baby until such time as the apparent association between infection with the virus and microcephaly is either confirmed or refuted. The question arises as to what advice is appropriate to give in more economically developed countries distant to the current epidemic and in which zika has not yet been reported. Despite understandable concern among the general public that has been fueled by the media, in regions where zika is not present, such as North America, Europe, and Australia, at this time any outbreak (initiated by an infected traveler returning from an endemic area) would very probably be contained locally. Since *Aedes* spp. has very limited spatial dispersal, overlapping high population densities of mosquitoes and humans would be needed to sustain a focus of infection. However, as *A. aegypti* is distinctly anthropophilic, future control strategies for zika should be considered in tandem with the continuing threat to human well-being that is presented by dengue, yellow fever, and Japanese encephalitis, all of which are transmitted by the same vector species [188].

Zika's impact on infants may shift the terms of debate about abortion access [189]. The focus on abortion legalization in the aftermath of the zika outbreak is distracting for policy and lawmakers from what needs to be done to address the outbreak effectively [190]. The high number of cases in Brazil has been attributed to a failure of the state to contain the epidemic and protect the affected people, especially women. Therefore, the public health crisis created by zika exposed a stark conflict between Brazil's constitutional right to health and the long-standing violation of reproductive rights in the country. Although health is considered to be a right of all in Brazil, women struggle with barriers to reproductive services and lack of access to safe and legal abortions. In response to the epidemic, women's rights advocates have filed a lawsuit with Brazil's supreme court that requires the decriminalization of abortion upon the diagnosis of ZIKV. However, the selective decriminalization of abortion may lead to negative social consequences and further stigmatization of people with disabilities. A solution to the

reproductive health crisis in Brazil must reconcile women's right to choose and the rights of people with disabilities [191].

The ZIKV epidemic and the possible devastating teratogenic effects of the virus represent a challenge. Health authorities have the responsibility to create programs that provide adequate preventive, medical, and psychological services to the affected population. The estimated risk of microcephaly in infants of mothers infected with ZIKV is 1% to 13% when the infection occurs in the first trimester of pregnancy. There is insufficient data to estimate the risk of microcephaly when infection occurs in the second or third trimester. Pregnant women and those in reproductive age are advised to avoid traveling to places where there is local transmission of the ZIKV. Human rights advocates have requested comprehensive sexual and reproductive health services that include expanded access to contraceptive methods including emergency contraception and safe abortion services. These strategies created a debate between the abortion rights and the right of the disabled. The discussion rests on the assumption that there are lives that are not worth living. Most people focus on the most severely affected patients, but few consider that the spectrum of disabilities associated with congenital zika infection is broad. The rights of children with disabilities and their dignity as individuals should be respected [192]. The uncertainty surrounding the possibility of congenital anomalies due to ZIKV infection during pregnancy bring a renewed debate about the rights of women to control their reproductive decisions. Current strategies, resources, and services aimed at prevention priorities fall short of responding to a clear framework regarding sexual reproductive health, rights, and justice. A comprehensive approach to reproduction, in times of zika, needs to empower women of reproductive age and their families to make decisions and to act on those decisions [193-195].

PREVENTION

As ZIKV continues to spread and further research is conducted regarding its teratogenic effects, the need for concise and effective education is critical to raise awareness, decrease the potential for maternal exposure, and reduce fetal risks. Therefore, the need for appropriate recognition, treatment, management, and prevention of the ZIKV disease prompts the necessity for further education and high-quality level research to be conducted and utilized [196]. Currently, no vaccine or medication exists to prevent or treat ZIKV infection. Persons residing in or traveling to areas of active ZIKV transmission should take steps to prevent ZIKV infection through prevention of mosquito bites [197]. Widespread ZIKV transmission in the Region of the Americas since 2015 has heightened the urgency of preparing for the possibility of expansion of mosquito-borne transmission of ZIKV during the mosquito seasons. CDC and other US government agencies have been working with state and local government partners on prevention and early detection of ZIKV infection and will increase these activities as part of their preparation for the anticipated emergence of mosquito-borne transmission of ZIKV in the continental US [198]. Health care providers must be equipped to provide appropriate advice to reproductive-aged patients for protection against the potentially devastating consequences of prenatal ZIKV exposure. All travelers should use personal protective measures against mosquito bites including insect repellents and protection of living areas against mosquito entry. Pregnant women should avoid travel to areas designated as being of concern because of

ongoing ZIKV transmission. Women planning a pregnancy should consult with their health care provider and consider postponing travel to these areas. All other travelers may wish to consider deferring travel to designated areas based on risk tolerance, values, and preferences. In the absence of clear data, we make the assumption that viral shedding is unlikely to persist beyond six months for men and two months for non-pregnant women. Health care providers should take a travel history from their pregnant patients including relevant information related to the travel history of their partners. Screening and management recommendations are provided for all travelers including potentially contagious male partners, pregnant women (symptomatic and asymptomatic), and the fetus or infant of potentially infected women. Robust quantitative assessments for the full spectrum of ZIKV-associated risks are not possible. This reflects, among other things, uncertainties related to the likelihood of infection among travelers to ZIKV-affected areas, vertical transmission from mother to fetus, sexual transmission (from symptomatic or asymptomatic partners), and serious ZIKV-associated sequelae among travelers. Given this uncertainty, as well as the potentially severe effects of ZIKV infection on the fetus, recommendations are conservative [199].

Control measures include using insect repellents, aerial spraying of insecticides, eliminating mosquito breeding sites, covering water tanks, and using mosquito nets or door and window screens [200]. Reviews assessing the safety and toxicity of N,N-diethyl-metoluamide (DEET) conducted by the US EPA and the Canadian Pest Management Regulatory Agency (PMRA) conclude that DEET has low acute toxicity and does not appear to pose a significant health concern to humans when used as directed. Some experimental animal and limited epidemiological data suggest that prenatal pyrethroid (permethrin) exposure may adversely affect learning and behavior, but this level of evidence pales in comparison to the known risks of ZIKV to the fetal brain. The available evidence has led to the strong recommendation by the CDC for use of these products by pregnant women as personal protection against mosquito bites in the fight against ZIKV infection. This message has been affirmed by obstetrics and gynecology professional organizations. Because ZIKV is unlikely to be the last disease requiring vector control, those with environmental health expertise must continue to join with infectious disease specialists to communicate the potential vulnerability of the youngest (fetuses, infants, and young children) to vector-borne disease, both to the disease itself and to the strategies employed to mitigate the spread of such disease [201].

Outbreaks of mosquito-borne viral illnesses commonly occur after storms. As storms are predicted to worsen in intensity and frequency, mosquito-borne viruses, including the ZIKV are expected to spread, and with devastating consequences. An overlooked vector of the ZIKV, however, is men whose semen can transmit the virus at the time of sexual intercourse. Thus, although spread is primarily by *Aedes* spp. mosquitoes, instances of sexual transmission of ZIKV have been reported, and replicative virus has been isolated from semen of one man with hematospermia. On February 5th, 2016, CDC published recommendations for preventing sexual transmission of ZIKV. Updated prevention guidelines were published on February 23rd, with a further update on April 1st, 2016. During February 6th-22nd, 2016, CDC received reports of 14 instances of suspected sexual transmission of ZIKV. Among these, two laboratory-confirmed cases and four probable cases of ZIKV disease have been identified among women whose only known risk factor was sexual contact with a symptomatic male partner with recent travel to an area with ongoing ZIKV transmission. Two instances have been excluded based on additional information, and six others are still under investigation. State, territorial, and local public health departments, clinicians, and the public should be aware of current recommendations for

preventing sexual transmission of ZIKV, particularly to pregnant women. Men who reside in or have traveled to an area of ongoing ZIKV transmission and have a pregnant partner should abstain from sexual activity or consistently and correctly use condoms during sex with their pregnant partner for the duration of the pregnancy [202]. The above recommendations apply to all men and women who have traveled to or reside in areas with active ZIKV transmission and their female or male sex partners. These recommendations are updated to include time intervals after travel to areas with active ZIKV transmission or after ZIKV infection for taking precautions to reduce the risk for sexual transmission. This guidance defines potential sexual exposure to ZIKV as any person who has had sex (i.e., vaginal intercourse, anal intercourse, or fellatio) without a condom with a man who has traveled to or resides in an area with active ZIKV transmission [203]. The recommendations are now updated to reduce the risk for sexual transmission of ZIKV from both men and women to their sex partners. This guidance defines potential sexual exposure to ZIKV as having had sex with a person who has traveled to or lives in an area with active ZIKV transmission when the sexual contact did not include a barrier to protect against infection. Such barriers include male or female condoms for vaginal or anal sex and other barriers for oral sex. Sexual exposure includes vaginal sex, anal sex, oral sex, or other activities that might expose a sex partner to genital secretions [204].

CDC has updated its interim guidance for persons with possible ZIKV exposure who are planning to conceive and interim guidance to prevent transmission of ZIKV through sexual contact, now combined into a single document. Possible ZIKV exposure is defined as travel to or residence in an area of active ZIKV transmission, or sex without a condom with a partner who traveled to or lived in an area of active transmission. Based on new though limited data, CDC now recommends that all men with possible ZIKV exposure who are considering attempting conception with their partner, regardless of symptom status, wait to conceive until at least six months after symptom onset (if symptomatic) or last possible ZIKV exposure (if asymptomatic). Recommendations for women planning to conceive remain unchanged: women with possible ZIKV exposure are recommended to wait to conceive until at least eight weeks after symptom onset (if symptomatic) or last possible ZIKV exposure (if asymptomatic). Couples with possible ZIKV exposure, who are not pregnant and do not plan to become pregnant, who want to minimize their risk for sexual transmission of ZIKV should use a condom or abstain from sex for the same periods for men and women described above. Women of reproductive age who have had or anticipate future ZIKV exposure who do not want to become pregnant should use the most effective contraceptive method that can be used correctly and consistently [205]. CDC has also updated the interim guidance for men with possible ZIKV exposure who:

- 1) are planning to conceive with their partner; or
- 2) want to prevent sexual transmission of ZIKV at any time.

CDC now recommends that men with possible ZIKV exposure who are planning to conceive with their partner wait for at least three months after symptom onset (if symptomatic) or their last possible ZIKV exposure (if asymptomatic) before engaging in unprotected sex. CDC now also recommends that for couples who are not trying to conceive, men can consider using condoms or abstaining from sex for at least three months after symptom onset (if symptomatic) or their last possible ZIKV exposure (if asymptomatic) to minimize their risk for sexual transmission of ZIKV. All other guidance for ZIKV remains unchanged. The definition

of possible ZIKV exposure remains unchanged and includes travel to or residence in an area with risk for ZIKV transmission or sex without a condom with a partner who traveled to or lives in an area with risk for ZIKV transmission [206].

Current recommendations for preventing the sexual transmission of zika are inadequate and need to emphasize male reproductive responsibility, via expanding services for men's family planning and developing novel male contraceptives. To prevent the sexual transmission of zika, the WHO recommends that couples use condoms or abstain from sexual activity for at least six months when traveling in zika-infected areas. Strict adherence to these recommendations is neither practical nor adequate to address zika's sexual transmission. As up to 13% of couples who use condoms experience unintended pregnancy, semen and consequent viral exposure is imminent. The use of contraception beyond just barrier methods is essential. However, the burden of contraception largely falls upon women and efforts to prevent vertical transmission are often aimed at educating women, when the outcome is equally undesirable among their male partners. These short-comings highlight the lack of attention to men's options for family planning. Educating men about the full range of contraceptive options, correcting misconceptions about the efficacy of withdrawal and barrier contraceptive methods, increasing access to vasectomy, and developing novel male contraceptive options would allow shared responsibility for the prevention of unintended pregnancy and zika-related morbidity. Gaps in recommendations to prevent the sexual transmission of zika provide an opportunity to develop men's family planning initiatives and the range of accessible contraceptives to men [207]. Broad recommendations by government agencies and public health officials regarding avoidance of pregnancy for women at risk of acquiring ZIKV have theoretical and documented implications for abortion and contraception demand and access. Additional research is needed to better understand the impact of ZIKV health recommendations and advisories on women's decision making around pregnancy, as well as their impact on demand, access, and safety of family planning services [208, 209].

Because of the potential risks associated with ZIKV infection during pregnancy, CDC has recommended that health care providers discuss prevention of unintended pregnancy with women and couples who reside in areas of active ZIKV transmission and do not want to become pregnant. However, limitations in access to contraception in some of these areas might affect the ability to prevent an unintended pregnancy. In fact, in all these countries a large proportion of babies are still born out of unplanned pregnancies. Public health messaging on zika prevention has predominantly targeted women who know they are pregnant or intend to become pregnant, but not teenage females for whom unintended pregnancy is more likely. Vulnerabilities among this population to reproductive risks associated with zika are further amplified by restrictive abortion laws in several zika-impacted states. Key to prevention is enhanced, targeted public health messaging centered on teens nationally and particularly in certain high-risk regions [210]. Teenage girls are particularly at high risk, as they often lack access to preventive contraception methods, or the knowledge to use them appropriately. Nearly 20% of children born in Brazil today (~560,000 live births) are by teenage mothers. Birth incidence is far higher in the tropical and poorer northern states. However, in absolute terms most births occur in the populous southeastern states, matching to a large extent the geographic distribution of dengue (an indicator of suitable climatic and sociodemographic conditions for the circulation of *Aedes* mosquitoes). These findings indicate that recommendation to delay pregnancy will leave over half a million pregnant adolescents in Brazil vulnerable to infection every year if not accompanied by effective education and real

access to prevention [211]. Individual health care providers may encounter health system barriers to providing evidence-based care. In Mexico, states with the highest unmet need for contraception are low-lying coastal states. The government recently announced an investment to combat Zika but made no mention of family planning initiatives to assist women in preventing pregnancy. In Texas, the DSHS has issued recommendations to help women and men avoid mosquito bites; the issue of whether women should plan or avoid pregnancy is not addressed. Puerto Rico has the largest number of confirmed cases of ZIKV in the US and territories. Recently, the CDC Foundation launched the Zika Contraception Access Network (Z-CAN), which provides contraceptives at no cost to participating clinics in Puerto Rico. ZIKV highlights weaknesses in health systems that make it difficult for women to use contraception if they want to delay births. Women across the globe, with or without ZIKV, need access to contraception to prevent unintended pregnancy, and health care providers require functioning health systems that offer support to ensure access is a reality [212].

In response to the ZIKV threat, the WHO is strategically targeting prevention of infection in pregnant women and funding contraception in epidemic regions. The damaging effects of ZIKV can be reduced using a seasonal window of opportunity for conception that may minimize maternal exposure. Like other acute viral infections – including the related Flavivirus, DENV – the transmission of ZIKV is anticipated to be seasonal. By seasonally planning pregnancy, this aspect of pathogen ecology can be leveraged to align sensitive periods of gestation with the low-transmission season [213]. However, the effectiveness of this policy in reducing prenatal ZIKV infection has yet to be quantified. With 50% adherence to recommendations to delay pregnancy by nine to 24 months, the cumulative incidence of prenatal ZIKV infections is likely to decrease by 17% to 44%, whereas recommendations to delay pregnancy by six or fewer months are likely to increase prenatal infections by 2% to 7%. This paradoxical exacerbation of prenatal ZIKV exposure is due to an elevated risk for pregnancies to shift toward the peak of the outbreak. Pregnancy delays can have a substantial effect on reducing cases of microcephaly but risks exacerbating the ZIKV outbreak if the duration is not sufficient. Duration of the delay, population adherence, and the timing of initiation of the intervention must be carefully considered [214].

ZIKV infection during pregnancy remains a serious health threat in Puerto Rico. As of March 16th, 2016, the highest number of ZIKV disease cases in the US and territories were reported from Puerto Rico. The number of cases will likely rise with increasing mosquito activity in affected areas, resulting in increased risk for transmission to pregnant women. High rates of unintended and adolescent pregnancies in Puerto Rico suggest that, in the context of this outbreak, access to contraception might need to be improved. CDC estimates that 138,000 women of reproductive age (aged 15-44 years) in Puerto Rico do not desire pregnancy and are not using one of the most effective or moderately effective contraceptive methods, and therefore might experience an unintended pregnancy. CDC and other federal and local partners are seeking to expand access to contraception for these persons. Such efforts have the potential to increase contraceptive access and use, reduce unintended pregnancies, and lead to fewer adverse pregnancy and birth outcomes associated with ZIKV infection during pregnancy. The assessment of challenges and resources related to contraceptive access in Puerto Rico might be a useful model for other areas with active transmission of ZIKV [215]. From January 1st, 2016 through March 29th, 2017, Puerto Rico reported approximately 3,300 pregnant women with laboratory evidence of possible ZIKV infection. There is currently no vaccine or intervention to prevent the adverse effects of ZIKV infection during pregnancy; therefore, prevention has

been the focus of public health activities, especially for pregnant women. CDC and the PRDH analyzed data from the Pregnancy Risk Assessment Monitoring System – Zika Postpartum Emergency Response (PRAMS-ZPER) survey conducted from August through December 2016 among Puerto Rico residents with a live birth. Most women (98.1%) reported using at least one measure to avoid mosquitoes in their home environment. However, only 45.8% of women reported wearing mosquito repellent daily, and 11.5% reported wearing pants and shirts with long sleeves daily. Approximately one third (38.5%) reported abstaining from sex or using condoms consistently throughout pregnancy. Overall, 76.9% of women reported having been tested for ZIKV by their health care provider during the first or second trimester of pregnancy. These results can be used to assess and refine ZIKV infection prevention messaging and interventions for pregnant women and to reinforce measures to promote prenatal testing for zika [216]. As of May 2nd, 2017, the USVI, comprising St. Thomas, St. John, and St. Croix, had reported 1,021 probable or confirmed cases of ZIKV disease in its population of approximately 100,000; 222 symptomatic and asymptomatic pregnant women in the USVI had tested positive for ZIKV. In January 2016, USVI Department of Health (DOH) initiated zika response measures, including surveillance, vector control, and a communications program. Interventions included education and outreach, distribution of zika prevention kits to pregnant women in the USVI, and provision of free ZIKV laboratory testing and vector control services. In November 2016, USVI DOH staff members conducted interviews with convenience samples of community members and pregnant women to gather feedback about current and proposed interventions. Pregnant women reported taking a median of two actions to protect themselves from zika, with repellent use being the most commonly reported action. Community members reported taking a median of one action and were supportive of several proposed vector control approaches. Whereas multiple pregnant women and community members reported hearing messages about the cause and consequences of ZIKV infections, few recalled messages about specific actions they could take to protect themselves. Integrating evaluation into response measures permits ongoing assessment of intervention effectiveness and supports improvement to serve the population's needs [217].

CDC prevention efforts include mosquito surveillance and control, targeted education about ZIKV and condom use to prevent sexual transmission, and guidance for providers on contraceptive counseling to reduce unintended pregnancy. To estimate the prevalence of contraceptive use among non-pregnant and postpartum women at risk for unintended pregnancy and sexually active female high school students living in the 41 states where mosquito-borne transmission might be possible, CDC used 2011-2013 and 2015 survey data from four state-based surveillance systems: the Behavioral Risk Factor Surveillance System (BRFSS, 2011-2013), which surveys adult women; the PRAMS (2013) and the Maternal and Infant Health Assessment (MIHA, 2013), which surveys women with a recent live birth; and the Youth Risk Behavior Survey (YRBS, 2015), which surveys students in grades nine to 12. CDC defines an unintended pregnancy as one that is either unwanted (i.e., the pregnancy occurred when no children, or no more children, were desired) or mistimed (i.e., the pregnancy occurred earlier than desired). The proportion of women at risk for unintended pregnancy who used a highly effective reversible method, known as long-acting reversible contraception (LARC), ranged from 5.5% to 18.9% for BRFSS-surveyed women and 6.9% to 30.5% for PRAMS/MIHA-surveyed women. The proportion of women not using any contraception ranged from 12.3% to 34.3% (BRFSS) and from 3.5% to 15.3% (PRAMS/MIHA). YRBS data indicated that among sexually active female high school students, use of LARC at last

intercourse ranged from 1.7% to 8.4%, and use of no contraception ranged from 7.3% to 22.8%. In the context of zika preparedness, the full range of contraceptive methods approved by the Food and Drug Administration (FDA), including LARC, should be readily available and accessible for women who want to avoid or delay pregnancy. Given low rates of LARC use, states can implement strategies to remove barriers to the access and availability of LARC including high device costs, limited provider reimbursement, lack of training for providers serving women and adolescents on insertion and removal of LARC, provider lack of knowledge and misperceptions about LARC, limited availability of youth-friendly services that address adolescent confidentiality concerns, inadequate client-centered counseling, and low consumer awareness of the range of contraceptive methods available [218, 219]. To support state and territory response to the threat of zika, CDC's Interim Zika Response Plan outlined activities for vector control, clinical management of exposed pregnant women and infants, targeted communication about ZIKV transmission among women and men of reproductive age, and primary prevention of zika-related adverse pregnancy and birth outcomes by prevention of unintended pregnancies through increased access to contraception. The most highly effective, reversible contraception includes intrauterine devices and implants, known as LARC. On September 28th, 2016, the Association of Maternal and Child Health Programs (AMCHP) and CDC facilitated a meeting in Atlanta, Georgia, of representatives from 15 states to identify state-led efforts to implement seven CDC-published strategies aimed at increasing access to contraception in the context of ZIKV. Qualitative data were collected from participating jurisdictions. The number of states reporting implementation of each strategy ranged from four to 11. Participants identified numerous challenges, particularly for strategies implemented less frequently. Examples of barriers were discussed and presented with corresponding approaches to address each barrier. Addressing these barriers could facilitate increased access to contraception, which might decrease the number of unintended pregnancies affected by ZIKV [220]. Ensuring access to and promoting use of effective contraception have been identified as important strategies for preventing unintended pregnancy. The importance of ensuring resources to prevent unintended pregnancy in the context of public health emergencies was highlighted during the 2016 ZIKV outbreak when ZIKV infection during pregnancy was identified as a cause of serious birth defects. Accordingly, CDC outlined strategies for state, local, and territorial jurisdictions to consider implementing to ensure access to contraception. To update previously published contraceptive use estimates among women at risk for unintended pregnancy and to estimate the number of women with ongoing or potential need for contraceptive services, data on contraceptive use were collected during September-December 2016 through the BRFSS. Results from 21 jurisdictions indicated that most women aged 18-49 years were at risk for unintended pregnancy (range across jurisdictions = 57.4-76.8%). Estimates of the number of women with ongoing or potential need for contraceptive services ranged from 368 to 617 per 1,000 women aged 18-49 years. The percentage of women at risk for unintended pregnancy using a most or moderately effective contraceptive method ranged from 26.1% to 65.7%. Jurisdictions can use this information to estimate the number of women who might seek contraceptive services and to plan and evaluate efforts to increase contraceptive use. This information is particularly important in the context of public health emergencies, such as the recent ZIKV outbreak, which have been associated with increased risk for adverse maternal-infant outcomes and have highlighted the importance of providing women and their partners with resources to prevent unintended pregnancy [221].

ZIKV RNA has been detected in a number of body fluids, including blood, urine, saliva, and amniotic fluid, and whereas transmission associated with occupational exposure to these body fluids is theoretically possible, it has not been documented. Although there are no reports of transmission of ZIKV from infected patients to health care personnel or other patients, minimizing exposures to body fluids is important to reduce the possibility of such transmission. CDC recommends standard precautions in all health care settings to protect both health care personnel and patients from infection with ZIKV as well as from blood-borne pathogens (e.g., HIV and HCV). Because of the potential for exposure to large volumes of body fluids during the labor and delivery process and the sometimes unpredictable and fast-paced nature of obstetric care, the use of standard precautions in these settings is essential to prevent possible transmission of ZIKV from patients to health care personnel [222]. Multiple arbovirus epidemics continue to occur and challenge blood safety policy makers in non-endemic developed countries. There is new documentation of transfusion transmission of DENV and Ross River Virus (RRV), and new or increased concern about CHIKV and ZIKV. Considering that the virus causes a viremia in which 80% of infected individuals have no symptoms, the potential for transfusion transmission from an asymptomatic blood donor is high if utilizing donor screening alone without testing. Platelet units have been shown to infect two patients via transfusion in Brazil. Although there was an investigational nucleic acid test available for testing donors, not all blood centers were initially required to participate. Subsequently, the US FDA issued a guidance in August 2016 that recommended universal nucleic acid testing for the ZIKV on blood donors. Ultimately, this current global threat, as described by the WHO, will inevitably be followed by future outbreaks of other bloodborne pathogens; the principles and practices of perioperative patient blood management will reduce the risks from not only known, but also unknown risks of blood transfusion for our patients [223]. Pathogen inactivation has been shown to inactivate almost all bacterial species and several epidemic arboviruses that pose a transfusion transmission risk. The two available platelet pathogen inactivation technologies show different levels of pathogen inactivation as measured by *in vitro* infectivity assays; the clinical significance of this finding is not known. Pathogen inactivation can mitigate infectious risk and should do so more completely than other interventions such as donor questioning, donor/component recall, or donor testing. However, pathogen inactivation increases the cost of the pathogen-reduced blood component, which is a significant obstacle in the current healthcare environment. This may inhibit the ability to move forward with an effective new paradigm for blood safety that fulfills the implicit public trust in the blood system [224, 225].

There are no licensed vaccines for ZIKV; however, many vaccine platforms/approaches that have been utilized for other Flavivirus vaccines are being applied to ZIKV [226]. On the basis of previous studies, it has long been stated that 17D yellow fever vaccine generally did not induce complement-fixing (CF) antibodies, and that the presence of CF antibodies could be used in epidemiological studies to distinguish individuals infected with wild YFV from vaccinated persons. The practical significance of these studies is that they demonstrated that in a high percentage of persons with prior Flavivirus exposure, anamnestic serological responses to yellow fever vaccine result in CF antibodies similar to those induced by natural YFV infection. In Africa and tropical America, where the background of flaviviral immunity is high, it is imperative that sero-epidemiological investigations during or after yellow fever outbreaks be conducted prior to vaccination [227]. Various companies are working to develop a vaccine and antiviral medicine against ZIKV. Despite promising results, the road remains long until a vaccine or antiviral medicines are available [228]. To further complicate the matters, ZIKV is

genetically similar (about 40%) with the four serotypes of DENV, so much so that it can almost be called a fifth dengue serotype. This homology poses the risk of causing cross-reactive immune responses and subsequent antibody-dependent enhancement (ADE) of infection in case of secondary infections or for immunized individuals. All of these factors complicate the development of a single preventive vaccine candidate or a pharmacological intervention that will completely eliminate or cure ZIKV infection. A combinatorial approach including immunization and treatment might prove to be the winning strategy [229]. Until we reach a firm conclusion, past experience with another virus that causes damage to newborns offers some hope. The development and almost universal use of rubella vaccine has all but eliminated the congenital rubella syndrome in the world. Rapid development of ZIKV vaccine might well do the same for this epidemic [230-235].

Recent efforts towards the development of a vaccine have been remarkable from both the private and public sectors and include new candidate vaccines ranging from the classical live-attenuated or inactivated vaccines to more sophisticated approaches such as messenger RNA (mRNA) or genetically engineered viral platforms. Previous successes with licensed Flavivirus vaccines indicate that a protective ZIKV vaccine should be an achievable goal. Nevertheless, numerous pre- and post-licensure challenges need to be taken into account, such as the interaction of vaccine-induced immune responses with other Flaviviruses, in particular with dengue, where antibody-dependent enhancement could become an issue, and the importance of a rapid induction of protective responses during pregnancy [236]. As a result, there are currently 45 ZIKV vaccine candidates in development. Both traditional (purified inactivated, live attenuated, viral-vectored, and recombinant sub-unit) and novel (deoxy-ribonucleic acid [DNA], self-replicating RNA, and mRNA) vaccine platforms are being utilized. For emergency use, vaccines that are appropriate for women of child-bearing age (including pregnant women) are being developed. Live vaccines that may be contraindicated in pregnancy are also in development for potential inclusion in national immunization programs in childhood or pre-teenage age groups. WHO developed a target product profile for zika vaccines for use in an emergency. Although ZIKV vaccine development had a quick head start, further development may be hampered because of the inability to conduct large efficacy trials with the decline in cases globally and unpredictability of new outbreaks. Furthermore, there are complex ethical issues involved in conducting efficacy trials in pregnant women [237-239]. As evidenced from recent epidemics, both EBOV and ZIKV infections are potentially catastrophic when occurring in pregnant women. Production of efficacious vaccines has been a public health priority for both infections. Unfortunately, during the clinical trials and subsequent deployment of a vaccine for the EBOV, pregnant and lactating women were, and continue to be, excluded from receiving the life-saving vaccine. The most serious consequence of ZIKV infection, CZS, results from fetal infection during pregnancy. Thus, pregnant women have a major stake in the ongoing development of a vaccine for ZIKV. The exclusion of pregnant women from the development, clinical trials, and administration of a potential zika vaccine unfairly deprives them and their infants of the protection they need against this potentially catastrophic intrauterine infection. When creating policy about these issues, it is important to critically evaluate vaccine safety in pregnancy in the context of the substantial risk of infection for the pregnant woman and her fetus in the absence of immunization [240].

The National Institute of Allergy and Infectious Diseases (NAID), part of the National Institutes of Health (NIH), has launched a vaccine trial at the beginning of August 2016 to control ZIKV transmission: patients who received the vaccine were expected to return within

44 weeks to determine if the vaccine is safe. Results show that high levels of sexual transmission create larger cases of infection associated with the peak of infected humans arising in a shorter period of time, even when a vaccine is available in the population. However, a high level of transmission of zika from vectors to humans compared with sexual transmission represents that ZIKV will take longer to invade the population providing a window of opportunities to control its spread, for instance, through vaccination [241]. Development of new vaccines and drugs is challenging and several points particular to ZIKV infections augment this difficulty:

- 1) cross-reactions between ZIKV and other Flaviviruses: the impact of ZIKV vaccination on subsequent Flavivirus infections, and vice-versa, is unknown;
- 2) drugs against ZIKV should be safe in pregnant women; and
- 3) evaluation of the efficacy of vaccine and drugs against ZIKV in clinical trials phase II-IV will be complicated due to the decline of ZIKV circulation [242, 243].

A rapid response by academia, government, public health infrastructure, and industry has enabled the expedited development and testing of a suite of vaccine platforms aiming to control and eliminate ZIKV-induced disease. Analysis of key immunization and pathogenesis studies in multiple animal models, including during pregnancy, has begun to define immune correlates of protection. Nonetheless, the deployment of ZIKV vaccines, along with the confirmation of their safety and efficacy, still has major challenges, one of which is related to the waning of the epidemic [244].

TREATMENT

After the WHO declared ZIKV as a PHEIC, intense research for the development of vaccines and drugs has been undertaken, leading to the development of several candidate compounds targeting ZIKV proteins, i.e., the envelope and capsid structural proteins, the non-structural protein 3 (NS3) helicase/protease, and the NS5 methyltransferase/RNA-dependent RNA polymerase. Research for effective drugs against ZIKV is still in a very early discovery phase. Notwithstanding the intense efforts for the development of new drugs and the identification of several promising candidates by using different approaches, including computational methods, so far only a few candidates have been experimentally tested. An important caveat of anti-Flavivirus drug development is represented by the difficult of reproducing the *in vivo* microenvironment of the replication complex, which may lead to discrepancies between *in vitro* results and *in vivo* experimental evaluation. Moreover, anti-ZIKV drugs have the additional requirement of an excellent safety profile in pregnancy and ability to diffuse to different tissues, including the CNS, the testis, and the placenta [245]. Drug trials in pregnancy raise several scientific, ethical, and logistic challenges, which are compounded further in ZIKV because of limited knowledge of the disease pathophysiology and a product development pipeline in its infancy. The major challenges in choosing therapeutics to prevent congenital ZIKV disease and conducting clinical trials of these treatments must be characterized and planned for now so that clinical trials can progress expediently and effectively in the future [246]. Genomic and molecular investigations are

underway to investigate ZIKV pathology and its recent enhanced pathogenicity, as well as to design safe and potent vaccines, drugs, and therapeutics. Anti-ZIKV therapeutics include drugs targeting virus entry into cells and the helicase protein, nucleosides, inhibitors of NS3 protein, small molecules, methyltransferase inhibitors, IFN, repurposed drugs, drugs designed with the aid of computers, neutralizing antibodies, convalescent serum, antibodies that limit antibody-dependent enhancement, and herbal medicines. Additionally, covalent inhibitors of viral protein expression and anti-Toll-like receptor (TLR) molecules. To counter ZIKV-associated disease, we need to make rapid progress in developing novel therapies that work effectually to inhibit ZIKV [247-249].

CONCLUSION

The worldwide invasion of arboviruses in recent decades is responsible for emerging public health threats. Some factors like climate change, urbanization, and uncontrolled population growth are fueling their widespread. Arboviruses incorporate a vast collection of genetically diverse viral pathogens including that of dengue, zika, and chikungunya. These viruses are peculiar as they are zoonotic and are a serious harm to the society, with no particular therapy to neutralize their effect. So, it is the need of the hour to develop an effective treatment against infections caused by them [250]. The CDC have developed specific molecular and serologic testing protocols and algorithms for follow-up care of suspected cases. Currently, the mainstay of management is conservative care while researchers attempt to develop a vaccine. Strategies to contain ZIKV include vector control, travel restriction for women who are pregnant or trying to become pregnant, and avoidance of mosquito bites in endemic regions of the world. The global epidemic of ZIKV infection has emerged as an important public health problem affecting pregnant women and their infants. Many questions remain regarding the risk factors, pathophysiology, epidemiology, and timing of maternal-fetal transmission and disease. These include mechanisms of fetal brain damage and microcephaly, the role of covariables, such as viral burden, duration of viremia, and host genetics, on vertical transmission, and the clinical and pathological spectrum of CZS. Additional questions include defining the potential long-term physical and neurobehavioral outcomes for infected infants, whether maternal or fetal host genetics influence the clinical outcome, and whether ZIKV infection can cause maternal morbidity. Recent advances in epidemiology, clinical medicine, pathology, and experimental studies have provided a great amount of new information regarding vertical ZIKV transmission and the mechanisms of congenital microcephaly, brain damage, and CZS in a relatively short time. However, much work still needs to be performed to more completely understand the maternal and fetal aspects of this new and emerging viral disease [251]. The future outlook regarding the current ZIKV outbreak in the Americas remains uncertain. What is certain is our need to promptly and efficiently address research gaps in our understanding of clinical outcomes from infection and environmental factors that influence emergence meanwhile improving diagnostic, therapeutic, and preventive measures against the disease [252]. There is a need to perform updated systematic reviews on ZIKV infection periodically because this epidemic is bringing up new evidence with extraordinary speed. Many challenges are still far from being solved regarding the epidemiology, case definition, clinical and laboratory diagnosis, and preventive measures. An approach using “omics” and new biomarkers for

diagnosis, and a ZIKV-vaccine for treatment, might finally give us the tools to solve these challenges [253].

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Chapter 3

DISTRIBUTION OF ZIKA VIRUS INFECTION SPECIFIC IGG IN WESTERN PROVINCE OF ZAMBIA: A POPULATION-BASED STUDY

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ABSTRACT

Zika virus (ZIKV) is a flavivirus that causes disease with similar but milder symptoms to dengue fever. There is no information on Zika virus infection in Zambia. Hence, the objective of the study was to determine the prevalence and correlates for Zika virus infection in Western province of Zambia. A cross-sectional study using a standardised questionnaire was conducted. Logistic regression analyses were conducted to determine factors associated with Zika virus infection. Out of 1824 respondents, 44.8% were males and 36.0% were aged below 15 years. Altogether, 10.2% of the participants had Zika virus infection. Factors associated with the infection were age and education. Participants aged less than 15 years were 53% (AOR = 0.47, 95% CI [0.34, 0.65]) less likely to be infected compared to those aged 45 years or older. Compared to participants who had attained secondary or higher levels of education, those who had attained primary level of education were 1.28 (95% CI [1.04, 1.58]) times more likely to have the infection. Zika virus infection is prevalent among the residents of Western Province in Zambia. There is need to

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strengthen strategies to address the emerging challenge of Zika virus infection such as laboratory diagnostic capacities.

Keywords: Zika virus infection, correlates, Western province, Zambia

INTRODUCTION

Zika virus (ZIKV) is a flavivirus that causes disease with similar but milder symptoms to dengue fever. Clinical pictures range from asymptomatic cases to an influenza-like syndrome associated with fever, headache, malaise and cutaneous rash (1). Other less frequent symptoms and signs of ZIKV include myalgia, headache, retro-orbital pain, oedema and vomiting (2). Its natural transmission cycle in Africa involves primarily mosquitoes (*Aedes* species) and monkeys. While serological and entomological Zika virus infection have been reported in different areas in Africa including Burkina Faso, Ivory Coast, Egypt, Central African Republic, Mozambique, Nigeria, Uganda and Senegal, entomological and virological surveillance for arboviruses have been conducted since 1972 in Senegal and revealed presence of Zika virus (3). The virus was first identified in 1947 in rhesus monkey serum from Zika forest in Uganda (4). However ZIKV antibodies have been detected in humans across Africa, and Asia (5, 6). In Cameroon, the infection with Zika virus spread to neighbouring Nigeria resulting in an epidemic with symptoms including jaundice (7), an indicator for potential global spread (8).

Despite the widespread distribution of Zika virus, there have been very few reported human cases until the Yap outbreak in 2007 (9). In particular, there is no documented information on Zika virus infection in Western province of Zambia. Following a risk assessment for Yellow fever in Western province of Zambia resulting from a re-classification of the province by World Health Organization as a low-risk area for Yellow fever, a study was undertaken using data collected from the Yellow fever survey to determine the prevalence and correlates for Zika virus infection in Western province of Zambia.

METHODS

Western Province has a common border with Angola. The geography of the province is dominated by the Barotse Floodplain of the Zambezi river. This floodplain is inundated from December to June; this is important to agriculture as it provides natural irrigation for the grasslands. Much of the province is covered by sand believed to come from the Kalahari Desert, grasslands and woodlands.

Western Province had seven districts with a total of 1,902 Standard Enumeration Areas (SEAs). Fishing and cattle rearing were the main occupation in the province. The population of the province was 881,524 according to national census of 2010 (10).

Western province borders with Angola and had seven districts divided into 1,902 SEAs. The population stood at 881,524 with a population density of 7.0 (10). Crop and livestock production as well as fishing were the main economic activities.

Sample Sizes

The sample size calculation was based on the assumption that the seroprevalence for Yellow fever was 7% based on the study conducted by Robinson (11). A Statcal program in Epi Info v6.04 was used to estimate the sample size. The sample size was equally allocated to North-Western and Western provinces and powered to avoid chance findings, that is, 1,800 participants from each province.

Sampling

A multi-stage sampling technique was used for participants in both districts. Firstly, wards were randomly selected from each constituency. In the second stage of sampling, standard enumeration areas (SEAs) proportional to the ward size were systematically sampled. All survey participants aged nine months or older in a selected household were eligible to be enrolled in the study.

Ethical Approval

The study protocol was reviewed and approved by the Tropical Diseases Research Centre Research Ethics Committee, and permission to conduct the study was obtained from the Ministry of Health, Zambia. Informed consent was obtained from survey participants after the interviewer had explained the benefits and risks of participating in the study. Entry forms were viewed only by those approved to be part of the survey.

Data Collection

A detailed semi structured questionnaire was used to collect information. The questionnaires were pre-tested to validate the appropriateness of the questions to capture the required information. During the data collection process, questionnaires were checked for inconsistencies and completeness on a daily basis.

Data Management and Analysis

All data were entered into a computer using Epi-Info data entry screen that had consistency and range checks embedded in it. The Epi data file was exported to SPSS for data analysis.

Further editing was conducted by running frequencies during the analysis stage. Odds ratios were used to estimate magnitudes of associations using unadjusted odds ratios (OR) and adjusted odds ratios (AOR) together with their 95% confidence intervals (CI).

RESULTS

A total of 1,824 participants out of which 44.8% were males participated in the survey. The age distribution was similar between sexes with 36.0% of the participants belonging to the under 15 years age group. A significant association between education and sex was observed ($p = 0.002$) with 34.3% of males and 27.1% of females having attained secondary or higher levels of education.

Altogether, 10.2% of the participants had Zika virus infection with no sex difference ($p = 0.957$). Table 1 shows the description of the sample. While age, education and visiting Angola were significantly associated with Zika virus infection in bivariate analyses, only age and education remained significantly associated with the infection in multivariate analysis. Participants aged less 15 years were observed to be 53% (AOR = 0.47, 95% CI [0.34, 0.65]) less likely to have the infection compared to participants of age 45 years or older. Meanwhile, participants who had attained primary level of education were 28% (AOR = 1.28, 95% CI [1.04, 1.58]) more likely to have the infection compared to participants who had attained secondary or higher levels of education (Table 2).

Table 1. Sample description for Western province for Zika virus infection

Factor	Total n (%)	Male n (%)	Female n (%)	p value
Age (years)				
<15	655 (36.0)	312 (38.3)	342 (34.0)	0.122
15 – 24	390 (21.4)	180 (22.1)	210 (20.9)	
25 – 34	245 (13.5)	94 (11.5)	151 (15.0)	
35 – 44	213 (11.5)	93 (11.4)	120 (11.9)	
45+	318 (17.5)	136 (16.7)	182 (18.1)	
Sex				
Male	815 (44.8)			
Female	1005 (55.2)			
Education				
None	388 (21.5)	152 (18.9)	235 (23.6)	0.002
Primary	868 (48.2)	376 (46.8)	492 (49.3)	
Secondary or higher	546 (30.3)	276 (34.3)	270 (27.1)	
Zika virus infection				
Yes	186 (10.2)	82 (10.1)	103 (10.2)	0.957
No	1638 (89.8)	733 (89.9)	902 (89.9)	

DISCUSSION

Zika virus which had not been previously reported in Western province of Zambia was prevalent affecting 10.2% of the survey participants. Compared to other findings, the prevalence in the current study is much lower than what has been reported elsewhere in Africa. The findings in Nigeria, reporting rates of 31 and 56% (12, 13) and in Yap island of Federated States of Micronesia reporting a rate of 73% (14) were based on small sample sizes, and this may partly explain the differences in the rates reported.

While age was significantly associated with Zika infection in the current study, no significant association was observed between age and the infection on the Yap Island (14). However, in a study conducted in Kenya, age was significantly associated with the infection. The risk of infection linearly increased with age (15). Older persons may have been more exposed to infection than the younger ones partly due to activities such as social gathering and economic activities such as fishing, crop and livestock production that may predispose them to mosquito bites. Repellents may not be affordable in poor communities. Wearing of long sleeved shorts and trousers for men and wearing of wrappers, traditionally known as *chitenge*, by women may be possible alternatives to minimize man-mosquito contact.

The association between education and Zika virus infection was not consistent. While persons who had never been to school were equally likely to be infected as those with secondary or higher levels of education, persons who had attained primary level of education were more likely to be infected compared to persons with higher levels of education (Table 2). Further analysis is needed to consider possible confounding factors such as the wealth index in the relationship between education and infection. Alternatively, persons with primary level of education may be more engaged in activities, for which we are unable to suggest, that may predispose them to mosquito carrying the infection.

Despite the fact that a high proportion of samples collected during the risk assessment tested positive for Zika virus infection (10.2%), no human case of Zika disease had been reported from Western province. This may be due to the lack of knowledge by the medical personnel and partly due to the absence of a case definition for Zika virus infection in the Integrated Diseases Surveillance and Response (IDSR) guidelines which are widely used for syndromic disease surveillance in Zambia. Furthermore, the symptoms of Zika infection apart from being sub-clinical in presentation could mimic malaria which is prevalent in the province. Therefore, suspected malaria cases may mask outbreaks of Zika virus infection. Grard et al. (16) in their study observed that the number of human cases of Zika virus infection were few compared to Dengue virus infection probably due to the occurrence of sub-clinical form of Zika disease that did not require medical attention.

The other challenge contributing to lack of information on arboviral disease is the limitations on availability of diagnostic methods, thereby making it difficult to identify these emerging diseases in the country. Brady et al. in assessing the distribution of dengue in the entire continent alluded to this challenge in many healthcare settings coupled with scanty information or data on surveillance and research generated reports in many resource poor countries (17).

The discovery of ZIKV in the Western Province of Zambia strongly indicates the need to develop laboratory capacity for diagnosis of flaviviruses and strengthen Integrated Disease Surveillance and Response (IDSR). Strengthening the capacities of the laboratory is key to confirming clinically suspicious cases. There is need to strengthen control measures for malaria as the vector is similar and as such the country should scale-up the use of Insecticide-treated nets (ITNs) and in-door residual spraying to reduce human mosquito contact. Other measures include health education to promote personal protection using long-sleeved clothes and repellents. Furthermore, although research efforts have focused on many of these viruses, other medically important members of the mosquito-borne flaviviruses, such as Zika virus, have received far less attention.

Table 2. Factors associated with Zika virus infection in Western Province in bivariate and multivariate logistic regression analyses

Factor	OR (95% CI)	AOR (95% CI)
Age (years)		
<15	0.51 (0.38, 0.69)	0.47 (0.34, 0.65)
15 – 24	0.74 (0.53, 1.02)	0.81 (0.57, 1.13)
25 – 34	1.19 (0.85, 1.67)	1.24 (0.88, 1.74)
35 – 44	1.03 (0.71, 1.50)	1.03 (0.71, 1.48)
45+	1	1
Sex		
Male	0.99 (0.85, 1.15)	
Female	1	
Education		
None	0.92 (0.71, 1.19)	1.02 (0.77, 1.34)
Primary	1.23 (1.00, 1.51)	1.28 (1.04, 1.58)
Secondary or higher	1	1
Use of mosquito nets		
Yes	0.98 (0.84, 1.15)	–
No	1	
In-door residual spraying		
Yes	0.90 (0.72, 1.14)	–
No	1	
Visited Angola		
Yes	1.67 (1.12, 2.50)	–
No	1	

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Chapter 4

CORRELATES OF ZIKA VIRUS INFECTION SPECIFIC IGG IN NORTH-WESTERN PROVINCE OF ZAMBIA: RESULTS FROM A POPULATION-BASED CROSS-SECTIONAL STUDY

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ABSTRACT

Arthropod-borne viruses (arboviruses) have become significant public health problems, with the emergency and re-emergence of arboviral diseases nearly worldwide. The objective of this study was to determine the prevalence and the risk factors for Zika virus infection in North-Western Province of Zambia. A cross-sectional study using a standardised questionnaire was used. Bivariate logistic regression analyses were conducted to obtain odds ratios and their 95% confidence intervals. In total, 1,755 survey participants were recruited. Overall, 48.8% of the survey participants were males. Males tended to have higher education levels than females, with 37.4% of males and 26.0% of females having attained secondary or higher levels of education ($p < 0.001$). Altogether, 1.8% of participants had Zika virus infection, with no sex difference (2.1% of males and 1.4% of females, $p = 0.957$). Visiting Angola was the only factor that was significantly associated with Zika virus infection. Participants who visited Angola were 2.82 (95% CI [1.82, 4.38])

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times more likely to have Zika virus infection compared to participants who had not visited Angola. Zika virus infection is prevalent among residents of North-Western Province in Zambia. Strengthening of disease surveillance, clinical management of cases and laboratory diagnostic capacities are necessary to curb the infection.

Keyword: Zika virus infection, prevalence, correlates, North-Western province, Zambia

INTRODUCTION

Zambia is undergoing rapid developmental changes particularly in infrastructure. The opening up of new mines in recent years in North-Western province means that mining has become a major occupation for the province. This has resulted in population movement especially from the 'traditional' Copperbelt province, creating a sudden increase in the total population of the province with resultant challenges for the health sector. Sudden population increase has resulted in over-crowding making a suitable environment for disease spread including the arboviruses.

Among the emerging infectious diseases, the arboviral diseases group has particularly warranted attention in the global health landscape with its potential for epidemics and its unprecedented spread (1). Despite the significance and increasing public health impact on individuals worldwide, arboviruses remain poorly understood and controlled. While increasingly well characterised in industrialised countries, the epidemiology of these viruses is a major challenge to developing countries and surveillance often is usually in the form of reports during outbreaks due to the poor population-based surveillance systems (2). The majority of the surveillance systems were designed to detect known pathogens (61.5%), while 19.9% were for both known and unknown pathogens and only a small proportion were designed to detect unknown pathogens (3).

The diagnosis of Zika virus infection is based on detection of specific antibodies (4) or virus isolation from animals or mosquitoes which is time consuming; however, rapid diagnostic methods have been developed for the African and Asian strains (5). Several serological surveys have been carried out in Africa and notable among these is the Portuguese Guinea survey which demonstrated frequent antibodies to group B viruses particularly Yellow Fever, Zika and Wesselbrons; a finding similar to previous surveys in the same region (6). Based on serological analyses, flaviviruses were classified into eight antigenic complexes; however, many viruses including the prototype of the group Yellow Fever and many new viruses could not be affiliated to any complex partly due to extensive geographical distribution, diversity of arthropod vector or vertebrate hosts and also confusion in virus nomenclature (7).

No information on Zika virus infection has been reported in Zambia. The objective of the study was, thus, to determine the prevalence and correlates for Zika virus infection in North-Western province of Zambia in order to contribute to the body of knowledge on the epidemiology of Zika virus infection in Zambia.

METHODS

North-Western Province borders with Angola in the West and Democratic Republic of Congo (DRC) in the North. North-Western Province was one of Zambia's nine provinces at the time of the survey before the creation of the tenth province. It covers an area of 125,826 km² and had a population of 695,599 (8).

A total of 1,178 Standard Enumeration Areas (SEAs) were demarcated in North-Western province in the eight districts of the province. North-Western province is located in Agro-ecological zone III which is suitable for cultivating rice, cassava, pineapples and bananas (9).

Sample Size

The data was obtained from a survey on Yellow fever. The sample size calculation was based on the assumption that the sero-prevalence for Yellow fever was 7% based on the study conducted by Robinson (10). A Statcal program in Epi Info v6.04 was used to estimate the sample size. After adjusting for 80% response rate, a sample size of 3600 was obtained. The sample size was equally divided into the two provinces and powered to avoid chance findings, that is, 1,800 participants from each province.

Sampling

A multi-stage sampling technique was used for participants in all districts. Firstly, wards were randomly selected from each constituency. In the second stage of sampling, standard enumeration areas (SEAs) proportional to the ward size were systematically sampled. All survey participants aged nine months or older in a selected household were eligible to be enrolled in the study.

Ethical Approval

The study protocol was reviewed and approved by the Tropical Diseases Research Centre Research Ethics Committee, and permission to conduct the study was obtained from the Ministry of Health, Zambia. Informed consent was obtained from survey participants after the interviewer had explained the benefits and risks of participating in the study. Entry forms were viewed only by those approved to be part of the survey.

Data Collection

A detailed semi structured questionnaire was used to collect information. During the data collection process, questionnaires were checked for inconsistencies and completeness on a daily basis.

Table 1. Sample description for North-Western Province for Zika virus infection

Factor	Total n (%)	Male n (%)	Female n (%)	p value
Age (years)				
<15	533 (30.4)	267 (31.3)	265 (29.5)	0.102
15 – 24	348 (19.8)	164 (19.2)	182 (20.3)	
25 – 34	348 (19.8)	151 (17.7)	197 (22.0)	
35 – 44	247 (14.1)	121 (14.2)	125 (13.9)	
45+	278 (15.8)	150 (17.6)	128 (14.3)	
Sex				
Male	854 (48.8)			
Female	897 (51.2)			
Education				
None	370 (21.4)	160 (19.1)	209 (23.6)	<0.001
Primary	812 (47.0)	365 (43.5)	445 (50.3)	
Secondary or higher	545 (31.6)	314 (37.4)	230 (26.0)	
Zika virus infection				
Yes	31 (1.8)	18 (2.1)	13 (1.4)	0.957
No	1724 (98.2)	836 (97.9)	884 (98.6)	

Data Management and Analysis

All data were entered in Epi-Info data entry screen that had consistency and range checks embedded in it. Epi data files were exported to SPSS for data analysis. Further editing was conducted by running frequencies during the analysis stage. Odds ratios and their 95% confidence intervals were used to estimate magnitudes of associations.

RESULTS

In total, 1755 survey participants were recruited. Overall, 48.8% of the survey participants were males. Males tended to have higher education levels than females, with 37.4% of males and 26.0% of females having attained secondary or higher levels of education ($p < 0.001$). Altogether, 1.8% of participants had Zika virus infection, with no sex difference (2.1% of males and 1.4% of females, $p = 0.957$). Table 1 below describes the sample population.

Visiting Angola was the only factor that was significantly associated with Zika virus infection. Participants who visited Angola were 2.82 (95% CI [1.82, 4.38]) times more likely to have Zika virus infection compared to participants who had not visited Angola. Age, sex, education, use of mosquito net and in-door residual spraying were not significantly associated with Zika virus infection.

DISCUSSION

Information concerning the distribution of Zika virus in North-Western Province was non-available until after the risk assessment was conducted in April/May 2013 by the Ministry of

Health with the support of World Health Organization. The risk assessment carried out in North-Western Province was conducted to respond to the re-classification of the province as a low-risk area for arboviruses particularly Yellow Fever. There was no assessment carried out in the province since 1950 when the last sero-survey was conducted during the colonial era. The sero-survey of 1950 concentrated in Barotseland (Western), Balovale (North-Western) and Ndola in present day Copperbelt Province (10).

Zika virus which had not been previously reported in North-Western Province appeared to be an important flavivirus affecting 1.8% of the survey participants. Our finding indicates that Zika virus was circulating among people of North-Western Province. Our finding is much lower than those reported in Kainji lake basin of Nigeria of 56% (11), Oyo State of Nigeria of 31% (12), Yap island of Federated States of Micronesia of 73% (13). Differences in rates of infection may partly be due to different laboratory tests, power of the studies and epidemiological stages of the infection in study areas.

The risk assessment revealed that international travel especially outside of the province was highly associated with acquisition of infection for Zika virus. The survey participants with a history of travel to neighbouring countries especially to the Republic of Angola had twice as much risk of acquiring Zika infection than their counterparts without history of travel (Table 2). However, travel to the Democratic Republic of Congo was not found to be significant probably due to host ecology and host behaviour (14). Stoddard et al, using mathematical models, illustrated the importance of human movement in the transmission of pathogens especially for populations most at risk to vector-borne diseases such as Zika virus (15).

Table 2. Factors associated with Zika virus infection in North-Western Province in bivariate logistic regression analysis

Factor	OR (95% CI)
Age (years)	
<15	0.37 (0.14, 0.99)
15 – 24	0.38 (0.12, 1.21)
25 – 34	1.15 (0.54, 2.47)
35 – 44	1.19 (0.93, 3.96)
45+	1
Sex	
Male	1.21 (0.84, 1.73)
Female	1
Education	
None	0.82 (0.43, 1.58)
Primary	1.36 (0.84, 2.22)
Secondary or higher	1
Use of mosquito net	
Yes	1.27 (0.87, 1.86)
No	1
In-door residual spraying	
Yes	0.96 (0.64, 1.43)
No	1
Visited Angola	
Yes	2.82 (1.82, 4.38)
No	1

Despite the fact that 1.8% of samples collected during the risk assessment tested positive for Zika virus infection, no human case of Zika disease has been reported from North-Western Province. This may be due to the lack of knowledge by the medical personnel and also due to the absence of case definition in the Integrated Diseases Surveillance and Response (IDSR) guidelines which are widely used for syndromic diseases surveillance in health facilities. Furthermore, the symptoms of Zika infection apart from being sub-clinical in presentation could be mimicking malaria which has been known to be prevalent in the province. Therefore, suspected malaria cases might have continued to mask outbreaks of Zika virus infection.

The other challenge contributing to lack of information on arboviral disease presence or absence is the limitation on availability of diagnostic methods, thereby making it difficult to identify these emerging diseases in the country. Until 2013 in preparation for the risk assessment on Yellow Fever, the laboratory capacity to diagnose arboviruses had not been developed.

In conclusion, Zika virus infection is prevalent among residents of North-Western Province in Zambia. Strengthening of disease surveillance, clinical management of cases and laboratory diagnostic capacities are necessary to curb the infection.

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Chapter 5

ZIKA VIRUS: BASICS ABOUT THE DISEASE*

Sarah A. Lister

INTRODUCTION

In late 2015, health officials in Brazil recognized a marked increase in the number of infants born with microcephaly (from Greek, meaning “small head”), a birth defect that may accompany significant, permanent brain damage.¹ Although not conclusive, the increase in microcephaly is suspected to be related to the emergence of Zika virus infections in Brazil early in 2015.²

Zika virus is related to the viruses that cause yellow fever, dengue, West Nile, and Japanese encephalitis viruses.³ Historically, Zika virus was found in Africa.⁴ Since 2007, Zika transmission has also occurred in Southeast Asia and the Western Pacific. In the Americas, transmission was first identified in Brazil in May 2015. As of January 29, 2016, the Pan American Health Organization (PAHO) had received reports of local transmission in 25 countries and territories in the Americas, including Puerto Rico and the U.S. Virgin Islands.⁵

Symptoms of Zika virus infection are usually mild, and the illness often resolves quickly. However, the possible link to microcephaly has raised the level of public health concern in the Americas and elsewhere, and has spurred wide-ranging efforts to protect pregnant women from infection. *Although some aspects of Zika virus infection (such as the behavior of the mosquitoes that carry it) are well understood, many other aspects of this emerging infection in the Americas are unknown.* While study of the Zika virus ramps up, health officials will have to base their decisions on the best available evidence, which could have substantial gaps for the time being.

On February 1, 2016, the World Health Organization (WHO) determined that the rapid spread of Zika infections in the Americas and the suspected link to a serious birth defect constituted a “Public Health Emergency of International Concern” under the International Health Regulations.⁶ Under this designation, nations are expected to cooperate, expand monitoring, and share information in order to stem the spread of the disease.

* This is an edited, reformatted and augmented version of a Congressional Research Service publication R44368, prepared for Members and Committees of Congress, dated February 4, 2016.

This report discusses scientific and technical aspects of Zika virus infection, including modes of transmission, symptoms, diagnosis and treatment, and prevention. Policy concerns and official actions are evolving and will be discussed in forthcoming CRS products. Unless otherwise cited, information in this report is drawn from the Zika virus information pages of the U.S. Centers for Disease Control and Prevention (CDC),⁷ WHO,⁸ and PAHO,⁹ where readers can also find the most current public health recommendations for the general public, travelers, and pregnant women.

Transmission

The primary means of Zika virus transmission is through the bite of an infected mosquito. Zika infections are not commonly transmitted directly between people, although the following direct transmission routes have been reported: from an infected pregnant woman to the fetus, through sexual contact with an infected person, and through blood transfusion from an infected blood donor. Zika virus has been detected in breast milk of infected mothers, but transmission via breast milk has not been reported.¹⁰

Zika virus is carried by and transmitted through the bite of a specific genus of mosquito, *Aedes*, primarily *A. aegypti* (see *Figure 1*), and possibly *A. albopictus* (also known as the Asian tiger mosquito). Both species have noticeable black and white stripes. These are the same species that transmit the viruses that cause yellow fever, dengue, and chikungunya. As a result, the behavior, geographic ranges, and control methods for these mosquitoes are generally well understood. Neither species is native to the Americas. Both were unintentionally introduced. Their geographic ranges vary from year to year, but in the United States they are limited to warmer regions. *A. aegypti* is found in the South and parts of the Southwest. The range of *A. albopictus* is broader, including parts of the central United States, Ohio Valley, and Eastern Seaboard as far north as New York City. Both species are aggressive daytime biters, well adapted to urban environments.¹¹



Source: James Gathany, CDC Public Health Image Library, image #9261, <http://phil.cdc.gov>.

Figure 1. The *Aedes aegypti* mosquito, the most common vector of Zika virus.

Health officials are most concerned about areas where Zika virus *transmission* has been reported, rather than where human infections have been reported. Because *Aedes* mosquitoes are present in the United States, CDC says that “... [w]ith the recent outbreaks [in Central and South America], the number of Zika virus disease cases among travelers visiting or returning to the United States will likely increase. These imported cases may result in local spread of the virus in some areas of the United States. CDC has been monitoring these epidemics and is prepared to address cases imported into the United States and cases transmitted locally.”¹²

The WHO says that an *animal reservoir* for the Zika virus is “unknown.”¹³ An animal reservoir is a non-human animal that also harbors a pathogen and contributes to its spread. (Examples are birds and West Nile virus, deer and the Lyme disease bacteria, and bats and Ebola virus.) Research on possible animal reservoirs of Zika virus is underway. If an animal reservoir is present, it could amplify the number of infected mosquitoes and increase the risk of transmission to humans.

Symptoms

According to CDC, “About 1 in 5 people infected with Zika will get sick. For people who get sick, the illness is usually mild. For this reason, many people might not realize they have been infected. The most common symptoms of Zika virus disease are fever, rash, joint pain, or conjunctivitis (red eyes). Symptoms typically begin 2 to 7 days after being bitten by an infected mosquito.”¹⁴ Zika virus usually remains in the blood of an infected person for about a week, but it can persist for longer periods in some people.

WHO is concerned that the spike in cases of microcephaly in Brazil is consistent with the recent introduction of the Zika virus into populations that lack immunity to it. Health officials are investigating possible links between Zika infection and other neurological and congenital disorders, including Guillain-Barré syndrome (GBS), an immune disorder often associated with bacterial or viral infections, characterized by muscle weakness and sometimes paralysis.¹⁵

Diagnosis and Treatment

CDC reports that no commercially available diagnostic tests are available for Zika virus disease. Zika virus testing is performed at CDC and a few state health departments. Clinicians are advised to contact their state health department to facilitate testing.¹⁶

No specific therapy or vaccine against Zika infection is approved by the U.S. Food and Drug Administration (FDA) for use in the United States, nor is one available elsewhere in the world. For the time being, treatment of infected persons is supportive, including treating symptoms and maintaining hydration. CDC, the National Institutes of Health (NIH), and pharmaceutical companies are working to develop tests, potential treatments, and vaccines.¹⁷ Officials caution that it is highly unlikely that a vaccine would be available in less than one or two years.

Preventing Transmission

Absent a vaccine, avoiding bites from infected mosquitoes is the primary means to prevent transmission.¹⁸ According to WHO, “At present, the most important protective measures are the control of mosquito populations and the prevention of mosquito bites in at-risk individuals, especially pregnant women.”¹⁹ Official recommendations for areas in which infected mosquitoes may be present are (1) for everyone, routine use of protective clothing and repellants, and staying indoors with windows closed or screened; and (2) for pregnant women, consideration of postponing travel to affected areas, and diligent protection against mosquito bites if travel is unavoidable.²⁰

End Notes

- ¹ Pronounced my-kroh-SEF-uh-lee. See <http://www.merriam-webster.com/dictionary/microcephaly>. For more information see U.S. Centers for Disease Control and Prevention (CDC), “Facts about Microcephaly,” <http://www.cdc.gov/ncbddd/birthdefects/microcephaly.html>.
- ² See CRS Insight IN10433, *Zika Virus: Global Health Considerations*, by Tiaji Salaam-Blyther.
- ³ This paragraph is drawn from CDC, “Zika Virus Spreads to New Areas—Region of the Americas, May 2015–January 2016,” *MMWR*, vol. 65, no. 3, January 29, 2016, <http://www.cdc.gov/mmwr/volumes/65/wr/pdfs/mm6503.pdf>.
- ⁴ Zika virus was first isolated in 1947 from a monkey in the Zika forest of Uganda.
- ⁵ PAHO is the Regional Office for the Americas of the World Health Organization (WHO). See <http://www.paho.org>.
- ⁶ WHO, “WHO Announces a Public Health Emergency of International Concern,” press release, February 1, 2016, <http://www.who.int/en/>. For more information on the International Health Regulations, see “International Health Regulations (IHR)” in CRS Report R43829, *Ebola: Selected Legal Issues*, by Jared P. Cole et al.
- ⁷ CDC, “Zika Virus,” <http://www.cdc.gov/zika/index.html>.
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- ⁹ Pan American Health Organization (PAHO), “Zika virus infection,” http://www.paho.org/hq/index.php?option=com_content&view=article&id=11585&Itemid=41688.
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- ¹¹ CDC, “Surveillance and Control of Aedes aegypti and Aedes albopictus in the United States,” <http://www.cdc.gov/chikungunya/resources/vector-control.html>.
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- ¹⁵ WHO, “WHO statement on the first meeting of the International Health Regulations (2005) (IHR 2005) Emergency Committee on Zika virus and observed increase in neurological disorders and neonatal malformations,” February 1, 2016, <http://www.who.int/mediacentre/news/statements/2016/1st-emergency-committee-zika/en/>.
- ¹⁶ CDC, “Zika Virus for Healthcare Providers,” <http://www.cdc.gov/zika/hc-providers/index.html>.
- ¹⁷ Comments of Dr. Tony Fauci, Director of the NIH National Institutes of Allergy and Infectious Diseases, Transcript for CDC Telebriefing: Zika Virus Travel Alert, January 28, 2016, <http://www.cdc.gov/media/archives.htm>.
- ¹⁸ Mosquito control approaches are beyond the scope of this report. For more information on U.S. domestic mosquito control activities, see CDC, “Mosquito Control,” <http://www.cdc.gov/westnile/faq/mosquitoControl.html>.
- ¹⁹ WHO, “WHO Announces a Public Health Emergency of International Concern,” press release, February 1, 2016, <http://www.who.int/en/>.
- ²⁰ CDC, “Zika Virus Disease Q&A: What can people do to prevent becoming infected with Zika?” <http://www.cdc.gov/zika/disease-qa.html>; and CDC, “Interim Guidelines for Pregnant Women during a Zika Virus Outbreak—United States, 2016,” *MMWR*, vol. 65, no. 2, January 22, 2016, <http://www.cdc.gov/mmwr/volumes/65/wr/mm6502e1.htm>.

Chapter 6

ZIKA VIRUS IN LATIN AMERICA AND THE CARIBBEAN: U.S. POLICY CONSIDERATIONS*

Clare Ribando Seelke, Tiaji Salaam-Blyther and June S. Beittel

ABSTRACT

Congress is debating how to respond to an ongoing outbreak of Zika virus, a mosquito-borne illness that has no treatment or vaccine and can cause microcephaly—a severe birth defect—and other neurological complications. As of June 16, 2016, 60 countries and territories had reported mosquito-borne transmission of the virus, 39 of which are in Latin America and the Caribbean and are reporting cases of Zika for the first time. Brazil, which has registered the most confirmed cases of Zika in Latin America, will host the summer Olympics in August 2016. Scientists expect that travel destinations in the Caribbean will see more cases as the summer’s warm, rainy season continues. More than 750 U.S. citizens, including pregnant women, have become infected through either travel or sexual transmission.

Frequent business and tourist travel, combined with the close proximity and similar climates of Latin America and the southern United States, means that mosquito-borne Zika infections are likely in the United States. Zika is primarily spread by *Aedes* mosquitoes—primarily *Aedes aegypti* but also *Aedes albopictus*, the latter of which is present in a majority of U.S. states. Local (or mosquito-borne) transmission has not yet occurred in the continental United States but is occurring in Puerto Rico and the U.S. Virgin Islands.

On February 8, 2016, the Obama Administration submitted an emergency request for almost \$1.9 billion in supplemental funding to respond to the Zika outbreak, including \$526 million for international efforts. On April 6, 2016, the Administration announced that it would reprogram \$589 million in unobligated funds, including \$510 million in Ebola supplemental funds, for efforts to address the Zika outbreak. The U.S. Agency for International Development (USAID) is reprogramming \$215 million of that funding—including a \$78 million transfer to the U.S. Centers for Disease Control and Prevention (CDC)—for international efforts. In mid-May 2016, both the House and the Senate passed supplemental appropriations measures for Zika response. The House bill, H.R. 5243, would

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provide \$622.1 million in Zika funding and rescind an equal amount of budget authority. The Senate measure (S.Amdt. 3900 to H.R. 2577, the combined FY2017 Military Construction-Veterans Affairs and Transportation-Housing and Urban Development appropriations bills) would provide \$1.1 billion in Zika response funding without rescissions. On June 23, 2016, the House agreed to a conference agreement (see H.Rept. 114-640) that would provide \$1.1 billion for Zika response, including \$175.1 million for State Department and USAID activities. On June 28, 2016, the Senate voted not to invoke cloture on the conference agreement.

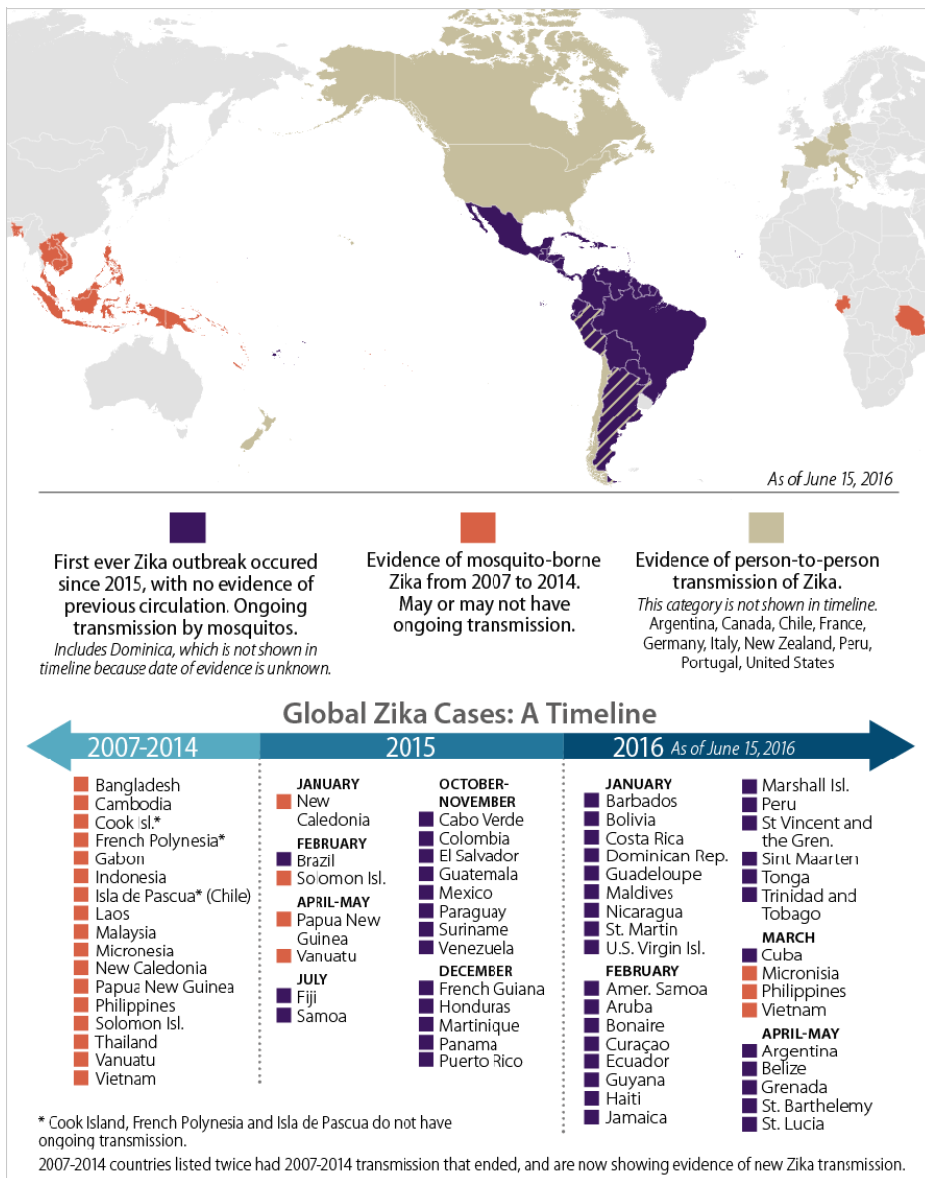
The number of people in the Western Hemisphere affected by Zika is unknown, but as many as 4 million people may be at risk of infection in 2016, and nearly all countries in Latin America and the Caribbean have recorded cases of the virus. Zika responses in the region have been led by Brazil and Colombia, multilateral organizations such as the World Health Organization (WHO)/Pan American Health Organization (PAHO), and the U.S. government. Health experts have expressed concerns about the capacity of health systems—particularly in Central America and the Caribbean—to prevent, diagnose, and care for Zika cases and associated complications, particularly among pregnant women. Related issues of interest to Congress include how to balance support for U.S. initiatives and multilateral approaches, the proper scope and components of U.S. health assistance to the region, and funding for pandemic preparedness and research on neglected tropical illnesses in Latin America.

INTRODUCTION

As of June 16, 2016, the World Health Organization (WHO) reported that 60 countries worldwide had experienced mosquito-borne transmission of Zika, 46 of which had never had Zika cases before (see Figure 1).¹ The U.S. Centers for Disease Control and Prevention (CDC) has concluded that Zika causes microcephaly (a serious birth defect involving brain damage) and is associated with Guillain-Barré syndrome (GBS, a neurological condition) and other neurological and autoimmune conditions. Latin America and the Caribbean have been most affected by this outbreak (Figure 1). Since February 2016, new cases in the southern parts of the region have decreased, but an increase in new cases is expected in the Caribbean during the summer months as mosquitoes hatch and bite and as the United States will likely experience an increase in travel-associated cases and possibly local transmission (Figure 2).² Policymakers are concerned about the spread of Zika into the continental United States, as well as the potential that visitors traveling to the Olympic Games in Brazil in August could contract the virus and bring it back to their home countries.

In February 2016, WHO Director-General Margaret Chan announced that the International Health Regulations (2005) Emergency Committee on Zika virus had determined that the Zika outbreak was a Public Health Emergency of International Concern (PHEIC).³ Shortly thereafter, President Barack Obama requested that Congress provide almost \$1.9 billion in emergency appropriations to fund domestic and international responses to the outbreak. A conference agreement on Zika funding was approved by the House but remains pending in the Senate. As Congress considers funding the Zika request and then exercises oversight over U.S. Zika responses in Latin America and the Caribbean, Members may consider issues such as the following:

- **Balance between U.S. bilateral and multilateral Zika responses.** Although U.S. health assistance (bilateral and regional) to Latin America in general has declined, U.S. support for the Pan American Health Organization (PAHO)⁴ has increased. While considering the President's Zika request and the FY2017 budget request, Congress may discuss how much support to provide for multilateral responses led by PAHO and for bilateral efforts.
- **U.S. health programs in Latin America as a part of U.S. policy toward the region.** On average, roughly 10%-20% of the funds provided by the State Department and the U.S. Agency for International Development (USAID) for Latin America between FY2009 and FY2016 have been aimed at health programs. The WHO has recommended that women and men in countries with local transmission⁵ of Zika be correctly informed and oriented to consider delaying pregnancy.⁶ Congress may consider what levels of health funding would be appropriate given the WHO recommendation and the limited access to sexual education and contraception in the region.
- **Regional apportionment and components of global health budget.** Less than 5% of all U.S. global health funds are provided to Latin America, and the vast majority of these funds are for HIV/AIDS programs. On average, health indicators in the region—particularly those related to maternal and child health, family planning, and reproductive health—are better than in other low- and middle-income countries, although inequities exist.⁷ Congress might reexamine the apportionment of global health funding and consider whether investments in the region are sufficient to meet emerging health concerns.
- **Funding for pandemic preparedness.** The United States committed to support 30 countries and the Caribbean Community (CARICOM)⁸ in bolstering their pandemic preparedness through the Global Health Security Agenda (GHSA).⁹ Two of the 30 countries are in Latin America and the Caribbean (Haiti and Peru). Congress included almost \$600 million in emergency Ebola appropriations to the CDC in support of GHSA and \$50 million in FY2016 appropriations to USAID for pandemic preparedness activities. Congress might consider funding levels for those programs and where those funds are allotted.
- **Investments in research and development of neglected diseases.** In recent years, *Aedes* mosquitoes have caused three disease outbreaks (dengue, chikungunya, and Zika) in Latin America and the Caribbean, all of which have been imported into the United States, with the latter being only travel-associated at the time of this report. These and other diseases lack vaccines to prevent transmission, treatment regimens, and effective vector control tools. Congress might evaluate options to address threats from new and reemerging diseases, including those that are mosquito-borne.



Source: Adapted by the Congressional Research Service (CRS) from World Health Organization (WHO), *Zika Virus, Microcephaly, Guillain-Barré Syndrome*, June 16, 2016.

Notes: Zika virus is not necessarily present throughout the countries/territories shaded in this map.

Figure 1. Global Transmission of Zika: 2007-2016.

This report provides background information on the Zika virus, discusses challenges faced by governments and implementing partners in the Latin America and Caribbean region that are attempting to control the ongoing outbreak, and analyzes the above issues in the context of the U.S. Zika response.

BACKGROUND

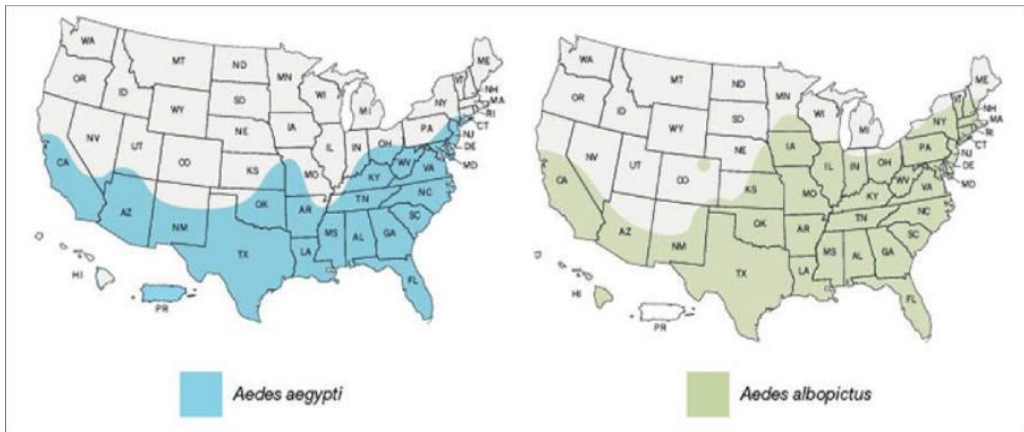
Zika was discovered in the Zika forest of Uganda in 1947. From that time until 2007, when the first large Zika outbreak was recorded, Zika virus infection primarily caused mild symptoms (fever, skin rash, conjunctivitis, muscle and joint pain) that resolved within one week.¹⁰ In 2007, the first large Zika outbreak was recorded on the Micronesian island of Yap. Household surveys detected 185 cases.¹¹

Scientists are studying the virulence of the Zika virus and the extent to which human activity affects global spread.¹² Retrospective studies of a 2013-2014 outbreak in French Polynesia linked Zika infection with GBS for the first time.¹³ The current outbreak, which began in Brazil, has been accompanied with a spike in microcephaly and GBS cases, as well as other neurological and autoimmune disorders.¹⁴ As of June 16, 2016, WHO has reported more than 1,600 cases of Zika-related microcephaly worldwide—almost all in Brazil. In addition, 13 Zika-affected countries have recorded an increased incidence of GBS.¹⁵

***Aedes*-Related Outbreaks in Latin America and the Caribbean**

In the last century, Latin American and Caribbean countries have transformed from largely rural to mostly urban societies, with some 80% of all people in Latin America and the Caribbean now living in urban areas. Throughout the region, millions of people live in densely populated urban slums and poor rural communities where homes and other facilities lack air conditioning or window screens. Lack of proper plumbing and poor sanitation facilitate mosquito breeding, as mosquitoes can lay their eggs in standing water.¹⁶ *Aedes* mosquitoes thrive in such conditions, biting during the day and breeding indoors and out.¹⁷

Health threats to Latin American and Caribbean populations may also be exacerbated by the 2015-2016 El Niño weather pattern, which is reflected in unusually warm water in the eastern equatorial Pacific Ocean. The present El Niño phenomenon, which has been particularly strong, has produced multiyear droughts in some areas (Colombia, Venezuela, and northern Central America) and extreme flooding in others (Argentina, Uruguay, and Paraguay). The warm, wet weather has facilitated the proliferation of mosquitoes, and human responses to drought conditions have provided favorable conditions for mosquito breeding because more people have been storing water.¹⁸ Studies have also linked climate change with greater health threats, such as increasing prevalence of malaria, chikungunya, and dengue fever.¹⁹ In recent years, *Aedes* mosquitoes have spread three disease outbreaks across the Americas: chikungunya,²⁰ dengue,²¹ and Zika. All three of these diseases have been imported into the United States and can become locally transmissible because the *Aedes* mosquito resides in large segments of the United States (see **Figure 2**).



Source: CDC, “Estimated Range of *Aedes aegypti* and *Aedes albopictus* in the United States, 2016 Maps,” <http://www.cdc.gov/zika/vector/range.html>, accessed on May 23, 2016.

Notes: Maps represent an estimate of the potential range of the *Aedes* mosquito. They are not intended to represent risk for spread of Zika.

Figure 2. Estimated Range of *Aedes* Mosquito in the United States.

Zika in Latin America and the Caribbean

Scientists are unsure how many people have been infected by Zika in the Western Hemisphere, but as many as 4 million people may be at risk of infection, and nearly all countries have recorded cases.²² As of June 16, 2016, Brazil had 159,914 suspected Zika cases, almost 40,000 of which have been confirmed through diagnostic testing (see **Figure 3**).²³ Since the outbreak began in Colombia, the country had recorded 82,935 suspected cases, more than 8,000 of which have been confirmed.²⁴ Two key factors complicate efforts to count Zika cases:

1. About 75% of infected people do not develop symptoms.
2. The virus is detectable for less than seven days in infected people’s blood.²⁵

Zika: From Latin America to the United States and U.S. Territories

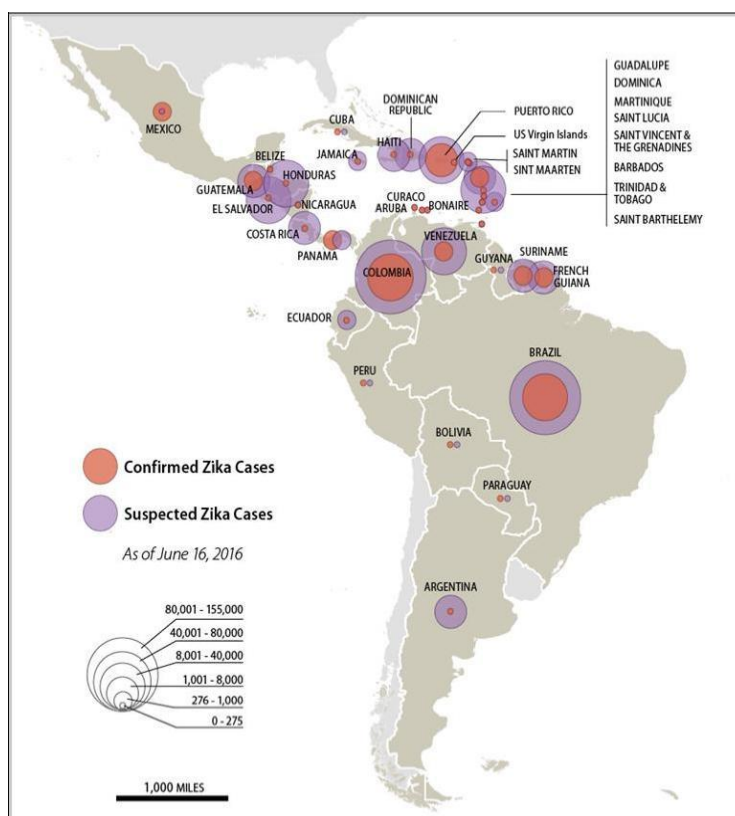
As of June 16, 2016, all Zika cases detected in the continental United States²⁶ (755) have been either acquired abroad or sexually transmitted, although the U.S. Virgin Islands, American Samoa, and Puerto Rico have experienced local transmission by mosquito. CDC and other health experts are preparing for the likelihood that the continental United States may experience locally acquired Zika cases this summer.²⁷ Given the broad range of the *Aedes* mosquitoes (see Figure 2) and the fact that mosquito-borne diseases have been imported into the United States previously, a successful response to the Zika outbreak may require U.S.-Latin American cooperation in surveillance, research, and response over several years.

The Zika Virus Outbreak in the U.S. Territories²⁸

Although Puerto Rico and the U.S. Virgin Islands are U.S. territories, when it comes to the Zika virus, their locations, climate, and ecology put them in company with much of Latin America and the Caribbean. PAHO is tracking the spread of the Zika virus in Puerto Rico, the U.S. Virgin Islands, and countries across Latin America and the Caribbean. On June 2, 2016, PAHO commented, “A downward trend of cases of Zika virus disease in Central and South America continues to occur while in most Caribbean countries and territories the trend continues to rise.”

As of June 15, 2016, none of the 50 U.S. states had identified local transmission of Zika virus. However, local transmission was first identified in Puerto Rico in December 2015 and in the U.S. Virgin Islands in January 2016. Puerto Rico has now identified more than 1,300 laboratory-confirmed cases, including more than 140 pregnant women. Puerto Rico is working with the CDC to actively monitor these women and assure that they and their babies receive the best possible pre- and post-natal care. Full-term babies from the earliest of these pregnancies will be due in mid- to late summer. More than 20 locally acquired cases of the Zika virus have been identified in the U.S. Virgin Islands. Puerto Rico’s Zika outbreak comes amid a fiscal emergency in the territory. The Obama Administration has requested assistance for Puerto Rico and the other territories as part of emergency supplemental appropriations for the national and global response to the Zika outbreak. Congress is considering an appropriations package at this time.

Sources and additional information: CRS Report R44460, Zika Response Funding: Request and Congressional Action; CRS Report R44275, Puerto Rico and Health Care Finance: Frequently Asked Questions; CRS Report R44095, Puerto Rico’s Current Fiscal Challenges; CDC, “Zika Virus,” <http://www.cdc.gov/zika/>; PAHO, “Zika Virus Infection,” <http://www.paho.org/zika>.



Source: Adapted by WHO/PAHO, Cumulative Zika Suspected and Confirmed Cases Reported by Countries and Territories in the Americas, June 17, 2016.

Figure 3. Confirmed and Suspected Zika Cases by Country; (as of June 16, 2016).

INTERNATIONAL ZIKA RESPONSES IN LATIN AMERICA AND THE CARIBBEAN

Country Efforts

The number of Zika cases, the capacity of health systems to address them and related complications, and the plans to do so vary widely across Latin America and the Caribbean. Haiti, for example, lacks a functioning hospital system, and Venezuela has little capacity to provide basic maternal and child health care at this time. In terms of preparedness and response to epidemiological emergencies, including Zika, recent assessments of core capacities under the International Health Regulations carried out by PAHO have highlighted weaknesses in health system capacity in all Caribbean countries (including Suriname and Guyana), selected countries of Central America (El Salvador, Guatemala, Honduras, and Nicaragua), Bolivia, and Paraguay. The Inter-American Development Bank (IDB) shares this view.²⁹

Experts are concerned that these countries and several others are much less equipped than Brazil and Colombia—two of the six countries in the Americas that PAHO deemed capable of handling a pandemic illness in 2012—to address Zika and related health consequences.³⁰ Inadequate laboratory and diagnostic capacity, poor access to sexual education and contraception, and resistance to national mosquito control efforts due to mistrust of government authorities have hindered efforts in some countries. Gang violence and insecurity have also reportedly prevented health workers from providing services in some parts of El Salvador and Honduras.

Should Zika-associated cases of microcephaly become more common outside of Brazil, the health systems in the region may come under strain. Most countries in Latin America and the Caribbean lack the capacity to treat children born with severe birth defects and do not generally permit abortion.³¹ Lifetime care for a child with microcephaly can be expensive. In the United States, such care can cost up to \$10 million.³² Brazil has struggled to care for infants with microcephaly, many of whose families live hours from one of the few hospitals that can provide care.

*Brazil*³³

Brazil has been at the epicenter of the ongoing Zika outbreak, with 159,914 suspected Zika cases since the beginning of the current outbreak, of which 39,993 have been confirmed as of June 16, 2016.³⁴ On November 11, 2015, Brazil's Ministry of Health declared a Public Health Emergency of National Importance in response to a sharp increase in the number of infants born with microcephaly. Whereas fewer than 200 cases of microcephaly were reported annually in Brazil prior to 2015, Brazil's Ministry of Health detected 1,581 microcephaly cases between the start of 2015 and June 16, 2016.³⁵

In Brazil, as in most countries in Latin America, a diagnosis of microcephaly in utero does not meet the government's standards under which abortion is permissible.³⁶ As a result, Brazil's Ministry of Health has issued guidelines for providing physical and occupational therapy to children born with microcephaly and is certifying hospitals capable of providing care to those infants. Brazil's congress passed a law to provide a small monthly stipend to families caring for microcephalic children, many of whom are led by single mothers who have lost the ability to maintain employment due to the type of care microcephalic babies require. Many observers

are concerned that there may be a rise in illegal abortions (and possibly maternal mortality due to unsafe abortions) in Brazil as a result of increasing diagnoses of microcephaly.³⁷

The Brazilian government has launched a National Plan to Combat the *Aedes* Mosquito and Microcephaly, which includes research, prevention, and mosquito control efforts, as well as health assistance for pregnant women and children. Brazil has several world-class research institutions with vast experience in tropical diseases, and the country's national public health institutions are working with local and international partners to develop more efficient diagnostic kits, antiviral drugs, and a Zika vaccine.³⁸ The government has dispatched 220,000 troops and 300,000 health agents to communities around the country to educate the population and eliminate mosquito breeding grounds. Officials have placed particular focus on mosquito-control efforts in Rio de Janeiro, which is scheduled to host the 2016 Summer Olympic Games in August (see text box).

**2016 Summer Olympics in Rio de Janeiro, Brazil,
and the Zika Outbreak**

Ongoing debate concerns whether the arrival of hundreds of thousands of tourists to Rio de Janeiro, Brazil, in August for the summer Olympics will hasten the global spread of the Zika virus. For some time, Brazilian officials have sought to assuage the fears of athletes and fans by pointing out that the Olympics will be occurring during the dry, winter season in Brazil, when fewer mosquitoes are present, and that all the venues and hotel areas will be regularly fumigated in preparation for the games. Regardless of those assurances, some health officials have called for the Olympics to be postponed or called off entirely. They have expressed concern that the strain of Zika present in Brazil, which has been linked to microcephaly and neurological problems, has been exported to Cape Verde, Africa. In May 2016, 150 health experts and bioethicists wrote an open letter to the WHO director-general urging her to recommend postponing the Olympics. In addition, some widely known athletes have indicated that they will skip the Rio Olympics due to concerns about the Zika virus.

Others oppose the proposal, noting that nonpregnant travelers going to Brazil can take sensible precautions to avoid mosquito bites and, upon returning home, use mosquito repellent (to avoid infecting mosquitoes) and use condoms to prevent sexual transmission of Zika. WHO made earlier statements indicating that "cancelling or changing the location of the 2016 Olympics will not significantly alter the international spread of Zika virus." On June 14, 2016, the WHO Emergency Committee on Zika met and reaffirmed its decision that "there is a very low risk of further international spread of Zika virus as a result of the Olympic and Paralympic Games as Brazil will be hosting the Games during the Brazilian winter when the intensity of [local] transmission of arboviruses, such as dengue and Zika viruses, will be minimal." U.S. epidemiologists calculated that the Olympic visitors would account for only 0.25% (i.e., less than 1%) of the total risk for spreading Zika through air travel.

Sources: Lena H. Sun, "150 Experts Say Olympics Must Be Moved or Postponed Because of Zika," *Washington Post*, May 27, 2016; WHO, "WHO Statement on the Third Meeting of the International Health Regulations (2005) (IHR(2005)) Emergency Committee on Zika Virus and Observed Increase in Neurological Disorders and Neonatal Malformations," June 14, 2016; Reuters, "New Research Finds Low Risk of Zika Virus at Olympics," June 7, 2016.

Some observers have expressed concerns about the adequacy of Brazil's efforts, particularly in low-income areas. Although Brazil has one of the most advanced public health systems in Latin America, significant gaps in prenatal care and birth outcomes exist in the poor north and northeast regions, where many Afro-descendants live (and many Zika cases have been concentrated) compared to the wealthier south.³⁹ Reports also indicate that many states in Brazil's northeast region ran out of mosquito larvicide last year and that the country's fiscal

challenges and political instability have inhibited efforts to address the Zika outbreak this year.⁴⁰

Colombia

Colombia has the second-most cases of reported Zika virus infection in the Western Hemisphere. As of June 16, 2016, Colombia had reported 82,935 suspected cases, roughly 8,000 of which have been confirmed.⁴¹ Two-thirds of Colombia's municipalities have reported suspected or confirmed cases, and Colombia's National Institute of Health estimates that between 200,000 and 300,000 people may contract the disease in the country by the end of 2016.⁴²

Similar to Brazil, Colombia has a relatively sophisticated public health system. Over the past several years, Colombia has spent a little over \$15 million annually on combating contagious pathogens. It plans to maintain that funding level in 2016.⁴³ The Colombian Ministry of Health issued a Zika virus risk-based preparedness and response plan in January 2016 that included four key elements:

1. Strengthening the national system of epidemiological surveillance;
2. Training health personnel on early detection, diagnosis, and management of Zika cases;
3. Coordinating Zika awareness, prevention, and response activities; and
4. Bolstering health care services to improve capacity to address Zika cases and related illnesses and to implement guidelines for comprehensive care of patients.

In January 2016, the Colombian Minister of Health visited the main cities around the country to raise awareness about Zika and build support for countering the disease among local health officials. The Ministry of Health also released policy recommendations that advised couples in affected areas to use contraceptive methods to prevent possible sexual transmission of the virus and postpone pregnancy. The government allotted an additional \$1.4 million to purchase necessary supplies and improve institutional support to prevent and combat Zika.

As of June 16, 2016, Colombia had seven confirmed cases of microcephaly.⁴⁴ Some experts predict that Colombia may see an increase in microcephaly cases in the coming months because its Zika outbreak began roughly six months after Brazil's. Others maintain that numbers may not grow significantly because abortion is legal under certain circumstances in the country, and women may opt to abort after a prenatal microcephaly diagnosis.⁴⁵ Colombian officials predict that about 300 Zika-linked microcephaly cases may be diagnosed between May and September 2016.⁴⁶ The government is working with the U.S. CDC to monitor and treat women infected with the Zika virus.

Multilateral Organizations

WHO and PAHO

In 2015, Brazil experienced an unusual spike in microcephaly cases. Evidence later emerged linking Zika with microcephaly. The phenomenon prompted WHO Director-General Margaret Chan to convene an emergency committee on Zika virus to discuss four key issues:

1. The association of Zika infection with birth malformations and neurological syndromes;
2. The potential for further international spread of the virus given the wide geographical distribution of the mosquito vector;
3. The lack of population immunity in newly affected areas; and
4. The absence of vaccines, specific treatments, and rapid diagnostic tests.⁴⁷

Upon recommendations of the emergency committee, Chan declared that the Zika outbreak was a PHEIC in February 2016. A PHEIC declaration signals that the health event may require immediate international action and often prompts a coordinated, multinational response. Also in February, the WHO released a Strategic Response Framework and Joint Operations Plan to guide the international response to the outbreak, neonatal malformations, and neurological conditions associated with the virus from January to June 2016. The plan focused on the following:

- Strengthening disease surveillance,
- Building laboratory capacity to detect the virus,
- Bolstering mosquito control,
- Providing care for infected persons, and
- Defining and supporting priority research areas.⁴⁸

The plan asked for donors to provide \$25 million to WHO (to coordinate and support global responses to the outbreak and scientific studies on the virus) and PAHO (to coordinate and support implementation of responses in the Americas). The funds were intended to support national activities related to disease surveillance; responses to the Zika outbreak, microcephaly, and GBS; and research from January through June 2016. The remaining \$31 million in funds was requested for partner organizations, including the U.N. Children's Fund (UNICEF) and U.N. Population Fund.⁴⁹

Due to sluggish contributions, in May 2016, U.N. Secretary General Ban Ki-moon announced the establishment of a U.N. Zika Response Multi-Partner Trust Fund to attract support for unfunded priorities outlined in the aforementioned WHO strategic framework. Contributions to the fund can be made by U.N. member states, regional organizations, nongovernmental organizations, businesses, and individuals. As of June 22, 2016, donors had provided more than \$4 million (16%) to the WHO/PAHO portion of the request.⁵⁰ In addition, WHO released \$3.8 million in emergency support for the Zika response. WHO and PAHO do not count the emergency funds toward fundraising goals, as these funds are to be reimbursed.

On June 16, 2016, WHO issued a revised \$122 million Zika Strategic Response Plan to guide international efforts from July 2016 through December 2017.⁵¹ The plan describes how the WHO/PAHO and 60 partner organizations aim to bolster detection, prevention, care and support, and research on Zika and related complications. It prioritizes support for women of childbearing age and their partners in communities affected by Zika. In addition, the plan urges countries and donors to bolster investments in counseling, reproductive health services, abortions (where legal), and postnatal follow-up and care for women who have been infected with Zika and for children born with microcephaly. It also calls for expanding services and research on GBS.

Since 2015, PAHO has been working with the U.S. CDC, USAID, National Institutes of Health (NIH), U.S. Department of Defense (DOD), and other leading research entities in the region to fill in significant knowledge gaps about Zika, its transmission, and its complications and to develop new diagnostic tests and hasten progress toward creating a vaccine. PAHO has partnered with governments, U.N. entities, multilateral development banks, and private organizations on disease surveillance, mosquito abatement, community engagement and education campaigns on personal protection, and services for those affected. PAHO, the World Bank, and the IDB have developed an assessment tool that measures country capacity to handle anticipated Zika cases and accompanying complications.⁵²

World Bank

In recent years, the World Bank has scaled back many of its health programs in Latin America to focus on regions with greater health needs, particularly sub-Saharan Africa. Nevertheless, the World Bank has maintained health strengthening projects in Argentina, Brazil, Nicaragua, and El Salvador and is launching a new project in Panama.⁵³ It is also analyzing countries to forecast the impact of major events, such as Zika, on economies in affected countries. In February 2016, the World Bank initially estimated that the costs of the Zika outbreak to Latin America would be moderate at \$3.5 billion, or roughly 0.06% of regional gross domestic product.⁵⁴ Officials have since stated, however, that the impact could be greater, particularly for Caribbean countries dependent on tourism from non-Zika affected countries.⁵⁵

Since the Zika outbreak began in Latin America, the World Bank has made \$150 million available for assistance. Most of the funding would be made available by restructuring existing projects to include components focused on Zika or by including Zika-related activities in new projects. Nine countries in Latin America qualify for International Development Association⁵⁶ aid based on their low per-capita incomes. These countries can therefore access a “crisis response” window that would provide new money (including grants or credits). As of mid-June 2016, Guyana had asked for \$5 million from the crisis response window.⁵⁷ El Salvador had allocated \$4 million from an existing health project, and Nicaragua had allocated \$1 million from an existing project for Zika responses. Two states in Brazil had requested \$20 million. Another large effort with the federal government of Brazil had been put on hold due to the political challenges unfolding in the country.

Inter-American Development Bank

The IDB has an active portfolio of loans and grants for health programs in Latin America totaling roughly \$2.7 billion. The largest programs are in Brazil, Mexico, the Dominican Republic, El Salvador, Nicaragua, and Panama. Most IDB programs focus on health system strengthening to enable governments to improve the provision of maternal and child health care and to better address noncommunicable diseases. The IDB is considering a request from the Caribbean Health Agency and a joint proposal from four South American countries to help improve those countries’ compliance with International Health Regulations (2005)⁵⁸ and assist in planning for health emergencies.

In response to the Zika outbreak, the IDB has offered to reorient up to \$180 million of its current portfolio of water, sanitation, and health programs to address Zika.⁵⁹ Of the \$60 million in health financing made available, the IDB had received requests to reorient \$19 million as of mid-May 2016. IDB efforts to address Zika are generally focused on providing family planning

through community health workers to rural areas, training primary care workers to detect nervous-system problems, and distributing supplies to prevent mosquito bites. The IDB is also supporting communications campaigns and efforts to increase surveillance capacity and vector control. In addition, the IDB has partnered with New York University to launch a crowdsourcing project that would enable governments to seek and partner with global health experts for responses to Zika and other infectious disease outbreaks.

U.S. Government

Supplemental Request

In February 2016, President Barack Obama requested almost \$1.9 billion in emergency supplemental funding to address the Zika outbreak (**Table A-1**), the bulk of which was requested for the Department of Health and Human Services (HHS) primarily for domestic response. The Administration requested that \$376 million of those funds be used by USAID and the Department of State for international responses and \$150 million be used for CDC international responses.

Reprogrammed Funds for USAID and CDC Programs

On April 6, 2016, the Administration announced that it would reprogram \$589 million in unspent Ebola funds to address the Zika outbreak. USAID is reprogramming \$215 million of that funding to support short-term efforts in Latin America, including a transfer of \$78 million to CDC for Zika activities in the region and a \$4 million transfer to the Department of State for a contribution to the International Atomic Energy Agency. The largest components of USAID funds are to support vector control programs (\$50 million) and to fund a grand challenge to encourage innovative responses to vector control, diagnostics, surveillance, and personal protection (\$30 million). By far the largest component of USAID's transfer to CDC is to support surveillance, epidemiology, and public health studies (\$44 million) (See **Table A-2**).

USAID and CDC are determining where to program their activities based on the anticipated numbers of cases in each country (based on experience with dengue and chikungunya) and the anticipated needs of countries in the region. Central America and the Caribbean are top priorities. Unlike the supplemental request, which included \$10 million for operating costs, the reprogrammed funds will be implemented with existing staff and resources (although USAID may use program funding to hire some staff).⁶⁰ The funds are expected to last for eight months to a year at most.⁶¹

*Congressional Action on the Budget Request*⁶²

In mid-May 2016, both the House and the Senate passed supplemental appropriations measures for Zika response. The House bill, H.R. 5243, would provide \$622.1 million in Zika funding and rescind an equal amount of budget authority. The Senate measure (S.Amdt. 3900 to H.R. 2577, the combined FY2017 Military Construction-Veterans Affairs and Transportation-Housing and Urban Development appropriations bills) would provide \$1.1 billion in Zika response funding without rescissions. On June 23, 2016, the House agreed to a conference agreement (see H.Rept. 114-640) that would provide \$1.1 billion for Zika response,

including \$175.1 million for State Department and USAID activities. On June 28, 2016, the Senate voted not to invoke cloture on the conference agreement.

ISSUES TO CONSIDER

Congress is considering a range of domestic and international responses to the Zika outbreak. In the global context, as summarized above (see “Congressional Action on the Budget Request”), Members are debating the appropriate response to the outbreak.⁶³ In the international context, Congress may consider how to balance support for U.S. bilateral and multilateral Zika responses. U.S. foreign assistance to Latin America and the Caribbean has been declining since FY2011, and USAID has been phasing out many global health programs in the region. The Zika outbreak may prompt broader discussions about whether to bolster U.S. global health investments in the Western Hemisphere, including in reproductive health. Discussions about controlling the Zika outbreak may also focus on U.S. support for global pandemic preparedness efforts, as well as research and development for diagnostics, treatments, and prevention measures for certain neglected diseases.

Balancing Support for Multilateral and Bilateral Zika Responses

U.S. bilateral and regional health assistance for Latin America and the Caribbean has declined by roughly 34% during the Obama Administration. In contrast, U.S. annual assessed contributions to PAHO have increased from \$59.1 million in FY2009 to \$65.7 million in FY2015. U.S. voluntary and assessed contributions currently represent roughly 37% of the organization’s \$200 million annual budget.⁶⁴ The U.S. government also provides annually assessed and voluntary contributions to WHO, UNICEF, the World Bank, and the IDB, which have launched Zika responses in the region. PAHO has struggled to fund missions that bring experts to the region, which cost between \$15,000 and \$20,000 per expert. PAHO maintains that those missions, partnerships with NIH and other research entities, and training for health and vector control workers are greatly needed in the region.⁶⁵

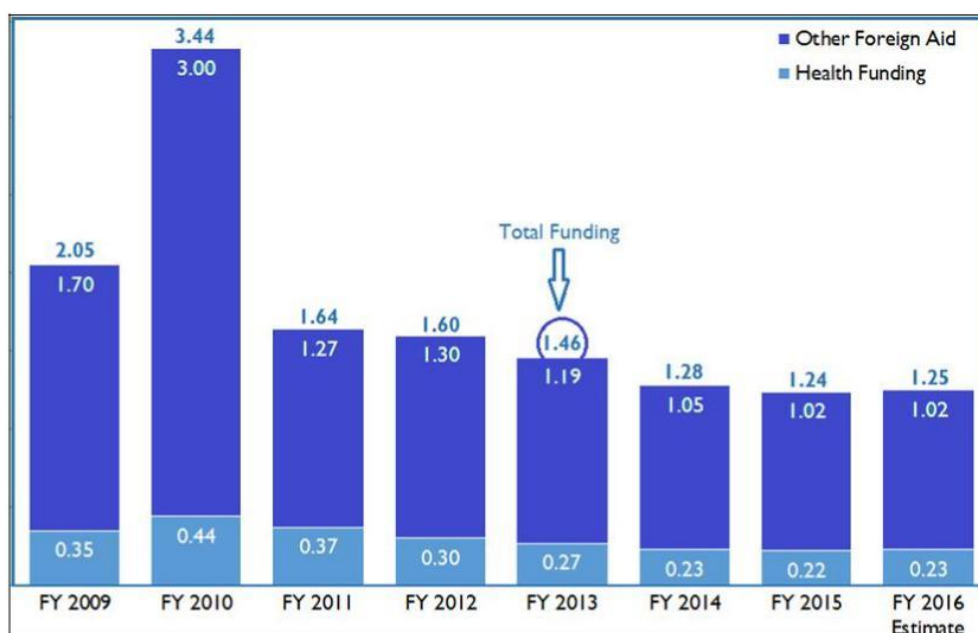
WHO and PAHO have launched a revised plan to support countries in their response to the Zika outbreak (see “WHO and PAHO,” above) through 2017. As of mid-June 2016, WHO and PAHO have received \$4 million to support what they estimate will cost \$122 million overall.⁶⁶ The WHO has not reported voluntary contributions from the U.S. government for the plan, although the Administration’s February 2016 Zika budget request includes \$10 million for the WHO/PAHO response and the Administration reprogrammed \$14 million of Ebola funds for voluntary contributions to WHO, PAHO, and UNICEF for Zika activities in April 2016.⁶⁷

Given the relatively small U.S. health investment in the region (as discussed below), Congress may consider providing funds for the WHO/PAHO Zika plan. Congressional discussions may also identify the appropriate mix of funds, if any, to provide for bilateral and multilateral Zika responses. In a region with relatively deep engagement with multilateral organizations, some would argue for providing increased funds to those entities rather than investing bilaterally. Others believe that it is harder to control how multilateral contributions are spent and ensure that related activities align with U.S. priorities.

Health as a Component of USAID Development Assistance to Latin America and the Caribbean⁶⁸

Current U.S. policy in Latin America is designed to promote economic and social opportunity, ensure the safety of the region's citizens, strengthen effective democratic institutions, and secure a clean energy future. As part of broader efforts to advance these priorities in the region, USAID and the State Department provide foreign assistance. USAID funding in Latin America and the Caribbean has been declining, particularly since FY2011—the period following the release of the 2010 President's Policy Directive on Global Development (PPD-6).⁶⁹ Although the directive sought to elevate development as a “core pillar” of American foreign policy, it also directed U.S. agencies to “be more selective about where and in which sectors [they] work.”

The PPD-6 prompted USAID to conduct a comprehensive review of its assistance programs. Following a review of the health sector, USAID determined that many of the countries in the region “had achieved remarkable progress, were far ahead of other presence countries [i.e., those with a USAID mission], and could effectively sustain progress without further USAID assistance.”⁷⁰ This finding led to a gradual reduction in health funding in the region (Figure 4). Health assistance fell from a high of \$445.6 million in FY2010 to an estimated \$231.3 million in FY2016.



Source: Created by CRS from correspondence with USAID Budget Office, June 8, 2016.

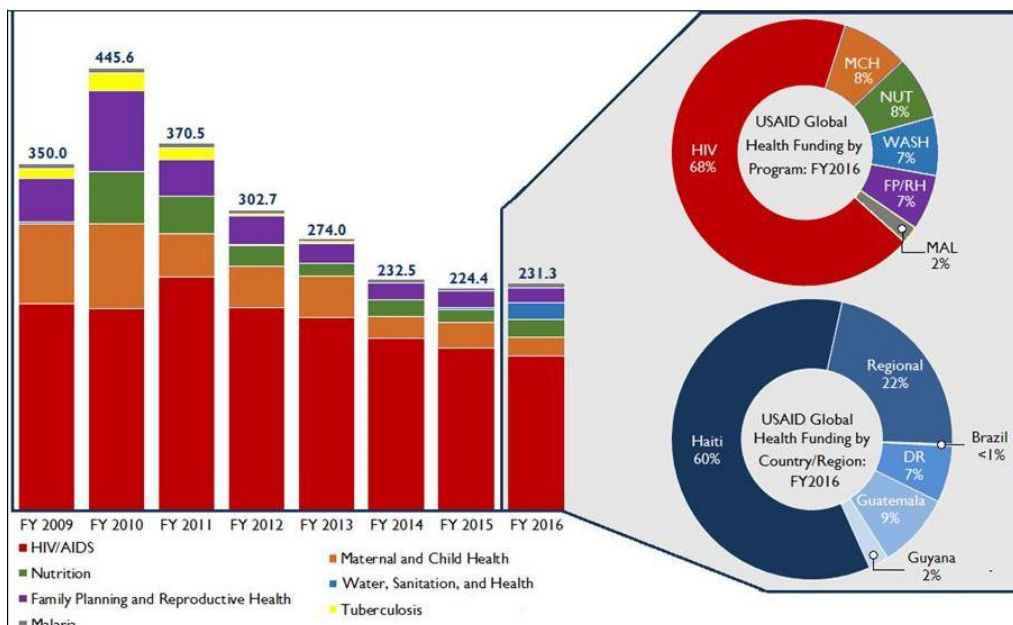
Figure 4. Department of State and USAID Assistance to Latin America and Caribbean: FY2009–FY2016; (current U.S. \$ in millions).

USAID continues to support programs related to maternal and child health, reproductive health, and family planning in Guatemala and Haiti, but it has discontinued such programs in all other countries across the region.⁷¹ Policymakers concerned about development in Latin America may consider whether health should once again become a larger component of U.S.

assistance programs, as inadequate access to health services can exacerbate poverty and inequality. A recent report by the U.N. Development Program urged governments and donors in the region to focus on addressing exclusion experienced by Afro-descendant and indigenous populations, women who suffer from domestic violence, and rural populations.⁷² These issues may be of particular concern in Central America, where the Administration significantly increased development assistance in FY2016 (and in the FY2017 request) but did not include any funds for bilateral health programs in Honduras or El Salvador.

U.S. Global Health Support in Latin America

With support from USAID and other donors, many Latin American countries have made notable progress in improving the delivery of primary health care services—including access to contraception and basic prenatal care—over the past few decades. Since the 1960s, these developments have led to a 41% decline in maternal mortality, a 70% decline in infant mortality, and a drop in fertility rates from six children per family to between two and three children per family.⁷³



Source: Created by CRS from correspondence with USAID Budget Office, June 8, 2016.

Notes: FY2009-FY2015 are enacted levels and FY2016 is an estimate. MCH = maternal and child health; NUT = nutrition; WASH = water, sanitation, and hygiene; FP/RH = family planning/reproductive health; MAL = malaria; DR = Dominican Republic; Regional = regional programs.

Figure 5. USAID Global Health Funding in Latin America and the Caribbean: FY2009-FY2016; (current U.S. \$ in thousands and percentage).

As a result of this progress, USAID global health engagement in the region has been steadily declining. In FY2009, for example, USAID implemented global health programs in 14 countries in the region. By FY2016, USAID global health programs were operating in five

countries (Brazil, Dominican Republic, Guatemala, Guyana, and Haiti). In three of those countries (Brazil, Dominican Republic, and Guyana), USAID global health programs focused only on HIV/AIDS by FY2016.⁷⁴ In FY2009, maternal and child health (23%) and reproductive health and family planning programs (13%) together constituted almost 40% of USAID health programs in Latin America. By 2016, maternal and child health (8%) and family planning and reproductive health programs (7%) amounted to 15% of all USAID health spending in the region.

In 2016, health ministers in several countries urged women to consider postponing pregnancy in response to Zika. Delaying or avoiding pregnancy is a problem in some communities where access to affordable and reliable contraception is limited and domestic violence (including spousal rape) is a major problem.⁷⁵ Experts estimate that some 56% of pregnancies in Latin America are unplanned, particularly among adolescents in poor communities who often do not have access to sexual education or counseling.⁷⁶ Health experts, including WHO Director-General Margaret Chan, have decried this constraint.⁷⁷ The WHO/PAHO Zika Strategic Response Plan, Revised for July 2016-December 2017 urges donors to prioritize providing counseling, reproductive services, and pre- and postnatal care to pregnant women and their partners who may be affected by Zika and related birth defects.

Some groups have advocated for many countries in Latin America and the Caribbean to legalize abortions for severe microcephaly cases and have urged the United States to bolster investments in family planning and reproductive health services across the region.⁷⁸ Others oppose the legalization of abortion and argue that additional resources should be spent on finding effective treatments and vaccines.

Pandemic Preparedness

In recent years, a succession of new and reemerging infectious diseases have caused outbreaks and pandemics that have together affected millions of people worldwide: Severe Acute Respiratory Syndrome (2003), Avian Influenza H5N1 (2005), Pandemic Influenza H1N1 (2009), Middle East Respiratory Syndrome coronavirus (2013), Ebola (2014), and Zika (2015). In 2014, former HHS Secretary Kathleen Sebelius and WHO Director-General Margaret Chan announced the Global Health Security Agenda (GHSA), a global effort to accelerate implementation of the International Health Regulations (2005), particularly in resource-poor countries that lack the capacity to comply with the regulations.⁷⁹ The regulations describe measures to strengthen global capacity to respond to public health events with potential international impact.

Some analysts have asserted that Zika transmission has exposed weaknesses in Latin American countries' pandemic preparedness.⁸⁰ As of 2012, PAHO maintained that only six countries (Brazil, Canada, Colombia, Costa Rica, and the United States) were prepared to handle a pandemic.⁸¹ Through GHSA, the United States has committed to support 30 countries—two of which (Haiti and Peru) are in the Western Hemisphere—and CARICOM.⁸²

Congress provided \$597 million to CDC through emergency Ebola appropriations for the GHSA. The legislation did not specify where the funds were to be used. In light of the growing disease threats posed by *Aedes* mosquitoes in the Western Hemisphere, policymakers might

consider whether to expand U.S. support for GHSA implementation to other countries in Latin America.

Given entreaties from health experts to bolster pandemic preparedness efforts to minimize the effects of future outbreaks, Congress might consider whether ongoing funding for pandemic preparedness is sufficient. Since FY2014, Congress has been appropriating \$72.5 million annually to USAID and an average of \$58 million annually to CDC for global health security efforts. The Administration is seeking \$72.5 million for USAID and \$65.2 million for CDC to implement global health security programs in FY2017.

Research and Development for Neglected Diseases

Several U.S. government agencies are supporting development of vaccines and treatments for the Zika virus.⁸³ DOD is conducting preclinical research on a Zika vaccine candidate and plans to start human testing by the end of 2016. Under its innovations Broad Agency Announcement, the Biomedical Advanced Research Development Authority (BARDA) within HHS has received a number of proposals to develop new Zika vaccines using vaccine platforms that could also be used for other emerging infectious disease threats. BARDA, NIH, and DOD are collaborating to support the development of a vaccine at DOD's Walter Reed Army Institute of Research. Initial small-scale development is proceeding, and preclinical evaluation will begin soon.

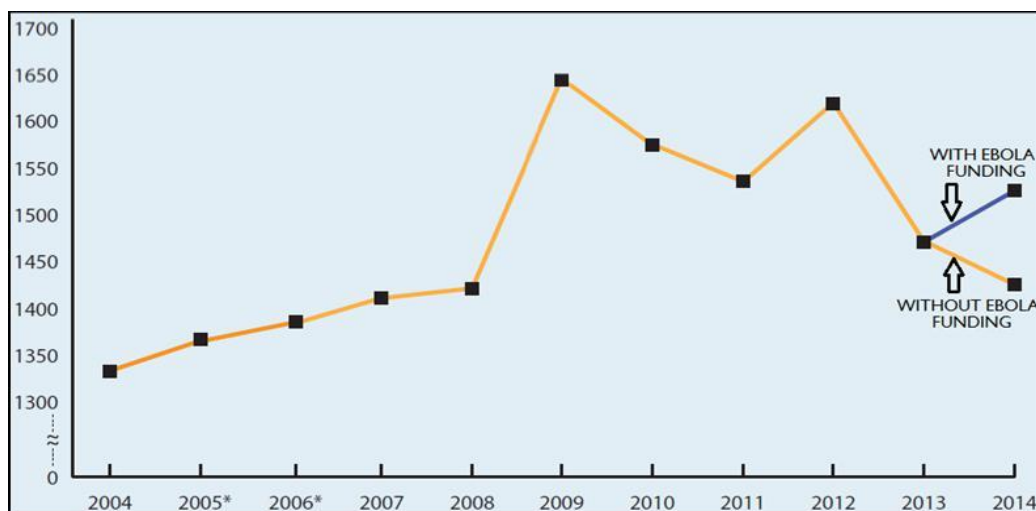
The WHO and other health experts have called for increasing investments in research and development for "neglected tropical diseases" such as dengue and chikungunya, as well as for Zika.⁸⁴ A consortium of health experts estimates that the international community would need to double current investments in health research and development for neglected diseases from \$3 billion in 2014 to \$6 billion by 2020 to meet global health goals.⁸⁵

According to the Global Health Technologies Coalition, the United States accounts for roughly 70% of public investment and 45% of global investment in global health research and development.⁸⁶ The United States provides the highest amounts of funding for research and development for 26 of the 30 most neglected diseases. As of 2012, it supported more than half of the global health products in the development pipeline. Since peaking in 2009, however, U.S. funding for global health research and development has fluctuated (Figure 6).

In April 2016, Congress enacted P.L. 114-146, Adding Zika Virus to the FDA Priority Review Voucher Program Act, to add the Zika virus to the list of tropical diseases eligible for the Food and Drug Administration's (FDA) Priority Review Voucher program, which allows companies to fast-track a product through the FDA regulatory process. Congress has not yet enacted legislation, however, that would provide additional funding for Zika-related research. Some analysts recommend that Congress revise authorizing language to ensure that products granted an FDA priority voucher are affordable.⁸⁷

Health experts also argue that the Zika outbreak highlights the need to increase research into effective vector control measures. At the 69th World Health Assembly, WHO Director-General Chan asserted that "the spread of Zika, the resurgence of dengue, and the emerging threat from chikungunya are the price being paid for a massive policy failure that dropped the ball on mosquito control in the 1970s."⁸⁸ Inappropriate and inconsistent use of insecticides has led to insecticide resistance and the growth of *Aedes* populations across the Western

Hemisphere.⁸⁹ This phenomenon has prompted some to advocate for increased U.S. investments in vector control in the region. Brazil has become the first country in the world to approve the large-scale use of genetically modified mosquitoes in vector control programs, and the FDA is reviewing a request to conduct trials of the technology in the United States.⁹⁰



Source: Adapted by CRS from Global Health Technologies Coalition, *Achieving a Bold Vision for Global Health: Policy Solutions to Advance Global Health R&D*, 2016, p. 6.

Notes: Estimates. Data sources: Global Health Technologies Coalition, “Saving Lives and Creating Impact: Why Investing in Global Health Research Works,” Policy Cures; 2012; and M. Moran et al., “Neglected Disease Research and Development: The Ebola Effect,” G-FINDER, Policy Cures, 2015.

Figure 6. U.S. Investment in Research and Development for Neglected Diseases; (2014 US\$ in millions).

The Zika budget request includes \$100 million for USAID to incentivize the development of Zika vaccines, diagnostics, and vector control measures. It is unclear whether these funds will be sufficient to encourage market development of these products.

POSSIBLE FUTURE ACTIONS

The future of the Zika supplemental funding request is uncertain at this time due to a number of controversial measures that are in the conference report. Some observers are concerned that there may not be enough time for the House and Senate to resume negotiations to reconcile their responses to the Zika outbreak before they adjourn in July.⁹¹ In the meantime, U.S. agencies are using reprogrammed funds to complement current efforts by Latin American governments, PAHO, the private sector, and other donors. The size and scope of U.S.-funded initiatives to address Zika in Latin America and the Caribbean is also uncertain.

APPENDIX A. SUPPORTING DOCUMENTATION

Table A-1. Zika Funding Request

Department/Agency	Key Activities	Funding Level
Department of Health and Human Services (HHS)		\$1.509billion
U.S. Centers for DiseaseControl and Prevention (CDC)	• Support domestic Zika responses	\$743 million, of which \$150million is for international efforts
	• Enhance domestic mosquito control programs	
	• Establish domestic rapid response teams	
	• Improve domestic laboratory and surveillance capacity	
	• Expand the domestic CDC Pregnancy Risk Assessment Monitoring System, improve domestic Guillain-Barré syndrometracking, and ensure the ability of domestic birth defect registries to detect Zika-related risks	
	• Increase research linking Zika virus infections and microcephaly	
	• Enhance international capacity for Zika surveillance, expand the Field Epidemiology Training Program (FETP), laboratory testing, health care provider training, and vector surveillance and control in countries at highest risk of Zika virus outbreaks	
	• Improve diagnostics for Zika virus and support developments for vector control	
Centers for Medicare and Medicaid Services	• Temporary one-year increase in Puerto Rico’s Medicaid federal medical assistance percentage to support health services for pregnant women at risk of infection or diagnosed with Zika and for children with microcephaly and other related health costs	\$250 million
Vaccine Research and Diagnostic Development and Procurement	• Research, rapid advanced development and commercialization of new vaccines and diagnostic tests for Zika virus	\$200 million
	• Establish an Urgent and Emerging Threat Fund to address Zika and other outbreaks	\$210 million
	• Support Puerto Rico’s community health centers in preventing, screening, and treating Zika, expand home visiting services for low-income pregnant women at risk of Zika infection, and provide targeted maternal and child health services	
U.S. Agency for International Development (USAID)	• Implement vector management activities in countries at risk of Zika infection	\$335 million
	• Stimulate private sector research and development of vaccines, diagnostics, and vector control innovations	
	• Support training of health care workers in affected countries	
	• Support for pregnant women’s health	
	• Establish Zika education campaigns	
	• Issue a Global Health Security Grand Challenge calling for innovative diagnostics, vector control, personal protection, community engagement, and surveillance for Zika and other infectious diseases	
	• Provide flexibility in the use of remaining USAID Ebola funds	
U.S. Department of State	• Support for U.S. citizens and State Department employees in affected countries, public diplomacy, communications, and other operations activities • Support World Health Organization and Pan American Health Organization efforts to minimize Zika threats in affected countries	\$41 million

Source: White House, “Preparing for and Responding to the Zika Virus at Home and Abroad,” February 8, 2016.

Notes: The FETP is CDC’s program to train epidemiologists worldwide. For more information, see <http://www.cdc.gov/globalhealth/healthprotection/fetp/index.htm>.

Table A-2. Funding Redirected from USAID Ebola Emergency Operations for International Zika Responses (current US\$ in millions)

Department/Agency	Key Activities	Funding Level
USAID/U.S. Department of State	Social and behavioral change communications	\$17.0
	Vector management	\$50.0
	Grand challenge for development	\$30.0
	Market incentives	\$10.0
	Maternal and child health interventions and service delivery	\$17.0
	Support for international organizations (PAHO, UNICEF)	\$13.0
<i>USAID Total</i>		<i>\$137.0</i>
CDC	Implement Vector Management and Control	\$7.0
	Maternal and Child Health Interventions	\$1.0
	Innovations	\$5.0
	Surveillance, Epidemiology, and Studies	\$44.0
	Laboratory Capacity and Equipment	\$15.0
	Management and Coordination of Response	\$6.0
<i>CDC Total</i>		<i>\$78.0</i>

Source: USAID Congressional Notification, "Zika Response: Initial Time-Critical Activities," April 6, 2016.

APPENDIX B. ONLINE RESOURCES ON ZIKA VIRUS

Organization	Source Title	Document/Link
Centers for Disease Control and Prevention (CDC)	Zika Travel Information	http://wwwnc.cdc.gov/travel/page/zika-travel-information
CDC	Zika Virus Index	http://www.cdc.gov/zika/index.html
Pan American Health Organization (PAHO)	Zika Resources for Health Authorities	http://www.paho.org/hq/index.php?option=com_content&view=article&id=11601&Itemid=41694&lang=en
PAHO	Zika Resources for General Public	http://www.paho.org/hq/index.php?option=com_content&view=article&id=11602&Itemid=41695&lang=en
PAHO	Epidemiological Alerts and Updates	http://www.paho.org/hq/index.php?option=com_content&view=article&id=1239&Itemid=2291&lang=en
World Bank	Zika Virus Fact Sheet	http://www.worldbank.org/en/topic/health/brief/world-bank-group-and-zika-fact-sheet
UNICEF, WHO, PAHO, IFRC	Risk communication and community engagement for Zikavirus prevention and control	http://www.unicef.org/cbsc/files/Zika_Virus_Prevention_and_Control_UNICEF_English.pdf
United Nations Population Fund (UNFPA)	Zika virus: Frequently asked questions	http://www.unfpa.org/resources/zika-virus-frequently-asked-questions
UNFPA	UNFPA on the Zika Virus Outbreak	http://www.unfpa.org/press/unfpa-zika-virus-outbreak
World Health Organization (WHO)	Zika Strategic Response Framework & Joint Operations Plan	http://apps.who.int/iris/bitstream/10665/204420/1/ZikaResponseFramework_JanJun16_eng.pdf?ua=1
WHO	Zika Virus Situation Reports	http://who.int/emergencies/zika-virus/situation-report/en/

END NOTES

¹ WHO, Situation Report: Zika Virus, Microcephaly, Guillain-Barré Syndrome, June 16, 2016.

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³ WHO, “WHO Statement on the First Meeting of the International Health Regulations (2005) Emergency Committee on Zika Virus and Observed Increase in Neurological Disorders and Neonatal Malformations,” February 1, 2016.

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⁹ For background on GHSA, see CRS In Focus IF10022, The Global Health Security Agenda and International Health Regulations, by Tiaji Salaam-Blyther.

¹⁰ WHO, Zika Virus, April 15, 2016.

¹¹ M. K. Kindhauser et al., “Zika: The Origin and Spread of a Mosquito-Borne Virus,” Bulletin of the World Health Organization, February 9, 2016.

¹² Lulan Wang et al., “From Mosquitoes to Humans: Genetic Evolution of Zika Virus,” Cell Host & Microbe, vol. 19 (May 11, 2016).

¹³ Kindhauser, “Zika.” GBS is a condition in which a person’s immune system attacks the peripheral nerves. Many people who develop GBS recover fully, including those with severe GBS. Severe cases of GBS are rare but can result in death. For more information on GBS, see WHO, Guillain-Barré Syndrome, March 14, 2016.

¹⁴ Microcephaly is a condition in which a baby is born with a small head or the head stops growing after birth. Some babies born with mild microcephaly can live normal lives, while most babies born with severe microcephaly can experience epilepsy, cerebral palsy, learning disabilities, hearing loss, and vision problems over their lifetimes. See WHO, Microcephaly, March 2, 2016.

¹⁵ WHO, Situation Report.

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¹⁷ CRS In Focus IF10353, Mosquitoes, Zika Virus, and Transmission Ecology, by M. Lynne Corn, Tadlock Cowan, and Robert Esworthy.

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¹⁹ Sonia Altizer et al., “Climate Change and Infectious Diseases: From Evidence to a Predictive Framework,” Science, vo. 341 (August 15, 2013), p. 514.

²⁰ Chikungunya is a virus that can cause fever and severe joint pain, which can be debilitating. Other symptoms typically include nausea, fatigue, rash, and muscle pain. There is no antiviral treatment or vaccine. From 2013 through 2015, more than 1.7 million people contracted the disease, and 258 people died. During that time period, CDC estimated that 3,113 cases were imported into the United States and 11 cases were contracted locally in Florida. From the beginning of 2016 through June 17, 2016, PAHO/WHO reported 130,138 chikungunya cases in the region, of which 18,220 have been confirmed. On May 31, 2016, the Texas Department of State Health Services reported the first locally acquired chikungunya case, indicating that mosquitoes within the United States now carry the virus. WHO, “Fact Sheet: Chikungunya,” April 2016; U.S. Department of Defense, Chikungunya in the Americas Surveillance Summary #49, September 9, 2015; WHO/PAHO, Number of Reported Cases of Chikungunya Fever in the Americas, by Country or Territory 2016, June 17, 2016; Texas Department of State Health Service, “DSHS Announces First Texas Acquired Chikungunya Case,” press release, May 31, 2016.

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Chapter 7

ZIKA RESPONSE FUNDING: REQUEST AND CONGRESSIONAL ACTION*

Susan B. Epstein and Sarah A. Lister

ABSTRACT

The second session of the 114th Congress is considering whether and how to provide funds to control the spread of the Zika virus throughout the Americas. Zika infection, primarily spread by *Aedes* mosquitoes, has been linked to severe birth defects and other health concerns. Local transmission of the Zika virus has occurred in American Samoa, Puerto Rico, and the U.S. Virgin Islands, and is expected on the U.S. mainland this summer, in areas where *Aedes* mosquitoes are present.

On February 22, 2016, the Obama Administration submitted a request for more than \$1.89 billion in supplemental funding to respond to the Zika epidemic, all of which is requested as emergency discretionary appropriations and therefore effectively exempt from spending limits per the Budget Control Act of 2011 (BCA, P.L. 112-25). The emergency request includes \$1.509 billion for the Department of Health and Human Services (HHS), \$335 million for the U.S. Agency for International Development (USAID), and \$41 million for the Department of State. The request also seeks authority to transfer some of those supplemental emergency appropriations across other federal agencies such as the Department of Defense, the Environmental Protection Agency, and the U.S. Department of Agriculture, to allow greater flexibility as circumstances change. It also would provide HHS, the Department of State, and USAID with broad authority for direct hiring, not be limited to positions related to Zika response efforts, and would authorize personal services contracting by HHS, State and USAID, for the Zika virus, but not limited to Zika virus efforts.

On April 6, 2016, the White House Office of Management and Budget (OMB) and the Secretary of HHS announced that they had identified \$589 million—\$510 million of it from “existing Ebola resources within the Department of Health and Human Services and Department of State/USAID”—that can quickly be redirected and spent on immediate efforts to control and respond to the spread of the Zika virus in the Americas.

* This is an edited, reformatted and augmented version of a Congressional Research Service publication R44460, prepared for Members and Committees of Congress, dated June 2, 2016.

On April 8, 2016, the Administration notified Congress of a transfer of \$295 million out of the \$510 million from FY2015 unobligated Ebola Economic Support Funds (ESF) to be used for the Zika response efforts. Of that amount, USAID is providing \$158 million to CDC including \$78 million for Zika response and \$80 million for Ebola response. The remaining \$137 million also from FY2015 ESF is funding various USAID activities for its Zika response efforts.

As of mid-May 2016, congressional action on supplemental appropriations for Zika-related purposes has occurred in both the House and the Senate. The Senate action occurred with regard to an amendment to the combined FY2017 Military Construction-Veterans Affairs and Transportation-Housing and Urban Development appropriations bills (S.Amdt. 3900); that amendment provides \$1.1 billion, which would be available until September 30, 2017 (some until expended). House action has occurred with regard to a stand-alone supplemental appropriations bill (H.R. 5243); this bill provides \$622.1 million for Zika funding, which would be available until September 30, 2016. Nearly half of the funds are designated as emergency funding. Rescissions are included. CBO scored the total budgetary effects of these rescissions as reducing budget authority by \$622 million, and reducing outlays by \$12 million during FY2016. On net, CBO estimates the budgetary effects of the bill to be \$0 in budget authority and an increase of \$9 million in outlays for FY2016.

This report will identify the various Zika response funding options and track legislation in the 114th Congress.

INTRODUCTION

In its second session, the 114th Congress is considering whether and how to provide funds to control the spread of the Zika virus throughout the Americas. Zika infection, primarily spread by *Aedes* mosquitoes, has been linked to severe birth defects and other health concerns. Local transmission of the Zika virus has occurred in American Samoa, Puerto Rico, and the U.S. Virgin Islands, and transmission is expected on the U.S. mainland this summer, in areas where *Aedes* mosquitoes are present.¹

Federal efforts to address the outbreak include research on the infection and its effects, mosquito control measures, and efforts to develop a vaccine. The public health focus, both domestically and elsewhere in the Americas, is to protect pregnant women from infection and prevent birth defects. Administration officials and some in Congress are concerned about the resources needed to prevent widespread Zika infections as the Northern Hemisphere summer approaches.

This report presents the Administration's request for supplemental appropriations for the Zika response, supplemental appropriations measures that have received congressional action, and information about unobligated Ebola supplemental funds as of January 1, 2016 (the most recent publicly available).

ADMINISTRATION ACTIONS

On February 22, 2016, the Obama Administration submitted a request for more than \$1.89 billion in supplemental funding to respond to the Zika epidemic, all of which is requested as emergency FY2016 discretionary appropriations, and therefore effectively exempt from

spending limits in the Budget Control Act of 2011 (BCA, P.L. 112-25).² The Administration's request includes \$1.509 billion for the Department of Health and Human Services (HHS), \$335 million for the U.S. Agency for International Development (USAID), and \$41 million for the Department of State. The request also seeks authority to transfer some of those supplemental emergency appropriations across other federal agencies, such as the Department of Defense, the Environmental Protection Agency, and the U.S. Department of Agriculture, to allow greater flexibility as circumstances change. It also would provide HHS, the Department of State, and USAID with two personnel management authorities for, but not limited to, addressing the Zika outbreak; broad authority for direct hiring;³ and authority for personal services contracting.⁴

A major theme in congressional debates thus far has been over whether unobligated (uncommitted for expenditure) FY2015 funds that had been provided to respond to the Ebola virus outbreak should be used to fund part of the Zika response, either temporarily or permanently.⁵ On April 6, 2016, the White House Office of Management and Budget (OMB) and the Secretary of HHS announced that they had identified \$589 million—\$510 million of it from “existing Ebola resources within the Department of Health and Human Services and Department of State/USAID”—that could quickly be reprogrammed and spent on immediate efforts to control and respond to the spread of the Zika virus in the Americas.⁶

As part of the reprogramming, on April 8, 2016, USAID notified Congress of its intent to redirect \$295 million of the \$510 million from FY2015 unobligated Ebola Economic Support Funds (ESF) to be used for Zika response. Of that amount, USAID transferred \$158 million to CDC, including \$78 million for Zika response and \$80 million for Ebola response. The remaining \$137 million, also from FY2015 ESF, is to be redirected to fund various USAID activities for Zika response efforts.

CONGRESSIONAL ACTIONS

On May 16, 2016, the Chairman of the House Appropriations Committee introduced the Zika Response Appropriations Act, 2016 (H.R. 5243). The bill would provide \$622.1 million that would be available until September 30, 2016, for domestic and international Zika response efforts. Nearly half of the funds are designated as emergency funds. The bill also includes rescissions of certain Ebola-related appropriations and the HHS nonrecurring expenses fund.⁷ Provisions in the bill provide that its appropriations shall be subject to the same requirements for funds that applied to the Consolidated Appropriations Act, 2016 (P.L. 114-113). This would include any restrictions on the use of funds that were contained therein, such as the applicable prohibitions on the use of funds for abortions. The House passed the measure on May 18, 2016, without amendment.

Senate action has occurred in the context of the FY2017 Military Construction-Veterans Affairs and Transportation-Housing and Urban Development appropriations bills. The texts of these bills were combined for the purposes of initial consideration in the Senate, and offered as a substitute amendment to an unrelated measure (S.Amdt. 3896 to H.R. 2577). On May 12, 2016, Senator McConnell (on behalf of Senator Blunt) proposed an amendment to that substitute (S.Amdt. 3900 to S.Amdt. 3896) that would provide \$1.1 billion for Zika response and preparedness, which would be available until September 30, 2017 (with the exception of Global Health Funds, which would be available until expended). On May 19, 2016, the Senate adopted

S.Amdt. 3900,⁸ followed by the pair of appropriations measures (i.e., H.R. 2577) to which it was amended.

Table 1. Supplemental Funding Amounts for Zika Response for FY2016: Comparison of Administration Request with Senate and House Proposals
Budget Authority in \$ Millions

Agency	Administration Request	S.Amdt. 3900 as Passed in Senate	H.R. 5243 as Passed in House
CDC Subtotal	828.0	449.0 ^a	170.0 ^{b,c}
HRSA: Community Health Centers for territories (non-add)	0.0	(40.0)	0.0
HRSA: National Health Service Corps for territories (non-add)	0.0 ^d	(6.0)	0.0
HRSA: Maternal and Child Health Block Grant (non-add)	0.0 ^d	(5.0)	0.0 ^c
HRSA Subtotal	0.0	51.0	0.0
PHSSEF: Social Services Block Grant for territories (non-add)	0.0	(75.0)	0.0
PHSSEF: Other (non-add)	(295.0) ^d	(75.0) ^e	(103.0)
PHSSEF Subtotal	295.0	150.0	103.0
NIH/NIAID Subtotal	130.0	200.0	230.0
FDA Subtotal	10.0	0.0	0.0 ^f
CMS Subtotal (Medicaid federal matching rate)	246.0	no provision	no provision
HHS Total	1,509.0	850.0 ^g	503.0
State: Diplomatic and Consular Programs (D&CP)	14.6	14.6	9.1 ^h
State: Emergencies in the Diplomatic and Consular Service	4.0	4.0	0.0 ⁱ
State: Repatriation Loans	1.0	1.0	0.0
State: Nonproliferation, Anti-Terrorism, Demining and Related Programs (NADR)	8.0	4.0	0.0
State: International Organizations and Programs (IO&P)	13.5	13.5	0.0
USAID: Operating Expenses (OE)	10.0	10.0	10.0
USAID: Global Health Programs (GHP)	325.0	211.0	100.0 ^j
State / USAID Total	376.1	258.1	119.1
REQUEST OR BILL TOTAL	1,885.1	1,108.1	622.1

Source: CRS analysis of text of White House, Office of Management and Budget, "Estimate #1—FY 2016 Emergency Supplemental: Appropriations Request to Respond to the Zika Virus both Domestically and Internationally," February 22, 2016, https://www.whitehouse.gov/omb/budget_amendments; S.Amdt. 3900; and H.R. 5243 IH.

Notes: Numbers in parentheses are included in subtotals.

^a Of the CDC funds provided, \$88 million may be used to reimburse prior Zika response spending.

^b Of the CDC funds provided, up to \$500,000 each must be transferred to the HHS Office of Inspector General and the Comptroller General for oversight activities.

^c Up to \$50.0 million of the CDC funds provided may be transferred to HRSA MCH for specified activities.

^d Unspecified amounts from the PHSSEF may be transferred to HRSA for National Health Service Corps activities in the territories, and for the Maternal and Child Health Block Grant.

^e Other Public Health and Social Services Emergency Fund (PHSSEF) activities principally involve development and purchase of medical countermeasures.

^f The House-reported Agriculture and Related Agencies appropriation for FY2017 included \$10 million for FDA activities related to the response to Ebola, Zika, and other emerging threats. H.Rept. 114-531, p. 70.

^g Broad authority is provided for funds to be transferred to or merged with other CDC, PHSSEF, HRSA, and NIH appropriations accounts to fund the purposes specified in the measure. Such transfers can only occur following consultation with OMB with a 10-day notification in advance, among other requirements.

^h Up to \$1.35 million of funds for Diplomatic and Consular Services may be used for medical evacuation costs for any U.S. agency.

ⁱ Up to \$1.0 million of funds for Diplomatic and Consular Services may be transferred to Emergencies in the Diplomatic and Consular Service

^j Of the Global Health Program funds provided, up to \$500,000 each must be transferred to the USAID Office of Inspector General and the Comptroller General for oversight activities.

See *Table 1* above for a comparison of the Administration's supplemental request, S.Amdt. 3900, and House bill H.R. 5243. For a comparison of non-monetary provisions of these measures, see *Table 2*.

Table 2. Selected Supplemental Provisions for Zika Response for FY2016: Comparison of Administration Request with Senate and House Proposals

Provision	AdministrationRequest	Senate(S.Amdt. 3900)	House(H.R. 5243IH)
<i>Period of Availability of Funds</i>			
HHS Funds	Until expended.	Until Sept. 30, 2017.	Until Sept. 30, 2016.
State / USAID Funds: D&CP:Nonproliferation, Anti-Terrorism, Demining and Related Programs;International Organizations and Programs;Operating Expenses	Until Sept. 30, 2017.	Until Sept. 30, 2017.	Until Sept. 30, 2016.
State / USAID Funds: Emergencies in the Diplomatic and Consular Service; Repatriation Loans; Global Health Programs.	Until expended.	Until expended.	Until Sept. 30, 2016.
<i>Scope of Use of Funds</i>			
CDC Funds	To prevent, prepare for, and respond to Zikavirus, other vector-borne diseases, or other infectious diseases and related health outcomes, domestically and internationally.	To prevent, prepare for, and respond to Zika virus, other vector-borne diseases, and related health outcomes, domestically and internationally.	To prevent, prepare for, and respond to Zika virus, domestically and internationally.
HRSA Funds	(Scope for PHSEF funds would apply to any funds transferred to HRSA.)	To prevent, prepare for, and respond to Zika virus, other vector-borne diseases, and related health outcomes, domestically and internationally.	(Scope for CDC funds would apply to any funds transferred to HRSA.)
NIH Funds	To prevent, prepare for, and respond to Zika virus, other vector-borne diseases, or other infectious diseases and related health outcomes, domestically and internationally.	To prevent, prepare for, and respond to Zika virus, other vector-borne diseases, and related health outcomes, domestically and internationally.	For development of vaccines for the Zikavirus.
PHSEF Funds	To prevent, prepare for, and respond to Zika virus, other vector-borne diseases, or other infectious diseases and related health outcomes, domestically and internationally.	To prevent, prepare for, and respond to Zika virus, other vector-borne diseases, and related health outcomes, domestically and internationally.	To respond to Zika virus, domestically and internationally.
State Dept. Diplomatic and Consular Programs (D&CP)	To support response efforts related to the Zika virus and related health outcomes, other vector-borne diseases, or other infectious diseases.	To support response efforts related to the Zika virus and related health outcomes, other vector-borne diseases, or other infectious diseases.	To support cost of medical evacuations and other response efforts related to the Zika virus and health conditions directly associated with the Zika virus.

Table 2. (Continued)

Provision	AdministrationRequest	Senate(S.Amdt. 3900)	House(H.R. 5243IH)
<i>Scope of Use of Funds</i>			
State Dept. Emergencies in Diplomatic and Consular Service	To support response efforts related to the Zika virus and related health outcomes, other vector-borne diseases.	To support response efforts related to the Zika virus and related health outcomes, other vector-borne diseases.	No comparable provision.
Repatriation Loans Program	For direct loans to support response efforts related to the Zika virus and related health outcomes, other vector-borne diseases, or other infectious diseases.	For direct loans to support response efforts related to the Zika virus and related health outcomes, other vector-borne diseases, or other infectious diseases.	No comparable provision.
USAID Operating Expenses (OE)	To support response efforts related to the Zika virus and related health outcomes, other vector-borne diseases, or other infectious diseases.	To support response efforts related to the Zika virus and related health outcomes, other vector-borne diseases, or other infectious diseases.	Response efforts related to the Zika virus and health conditions directly associated with the Zika virus.
Global Health Programs (GHP)	For assistance or research to prevent, treat, or otherwise respond to the Zika virus and	For assistance or research to prevent, treat, or otherwise respond to the Zika	For vector control activities to prevent, prepare for, and respond to the Zika
	related health outcomes, other vector-borne diseases, or other infectious diseases.	virus and related health outcomes, other vector-borne diseases, or other infectious diseases.	virus internationally.
Dept. of State, Nonproliferation, Anti-terrorism, Demining and Related Programs (NADR)	To support response and research efforts related to the Zika virus and related health outcomes, other vector-borne diseases, or other infectious diseases.	To support response and research efforts related to the Zika virus and related health outcomes, other vector-borne diseases, or other infectious diseases.	No comparable provision.
International Organizations and Programs (IO&P)	To support response and research efforts related to the Zika virus and related health outcomes, other vector-borne diseases, or other infectious diseases.	To support response and research efforts related to the Zika virus and related health outcomes, other vector-borne diseases, or other infectious diseases	No comparable provision.
<i>Transfer Authority</i>			
HHS Funds	CDC funds may be transferred within CDC. NIH funds may be transferred within NIH. PHSSEF funds may be transferred to two stated HRSA accounts, as specified, to an HHS countermeasures injury compensation fund, and to any other HHS accounts.	Any HHS funds in the amendment may be transferred to accounts in CDC, HRSA, NIH, and PHSSEF. \$75 million in PHSSEF funds must be transferred to the HHS Social Services Block Grant.	CDC funds may be transferred within CDC, and to three stated HRSA accounts, as specified. NIH funds may be transferred within NIH. PHSSEF funds may be transferred to an HHS countermeasures injury compensation fund.

Provision	AdministrationRequest	Senate(S.Amdt. 3900)	House(H.R. 5243IH)
<i>Transfer Authority</i>			
International Affairs	Funds may be transferred between foreign affairs accounts within the same headings to carry out the purposes of this Act and are in addition to other transfer authority within this proposal.	Funds within certain foreign affairs accounts may be transferred between foreign affairs accounts within the same headings to carry out the purposes of this Act and are in addition to other transfer authority within this proposal.	Specified funds within D&CP may be transferred for medical evacuation, transferred for Emergencies in Diplomatic and Consular Services, and are in addition to any other transfer authority within this proposal.
<i>Notification, Reporting and Oversight</i>			
HHS Notification Requirement for Obligation	No comparable provision.	No comparable provision.	15 days in advance of obligation.
International Affairs Notification Requirement	No comparable provision.	15 days in advance of obligation.	15 days in advance of obligation.
HHS Reporting Requirement	No comparable provision.	Within 30 days of enactment the HHS Secretary must report to the Appropriations Committees with a spend plan, followed by quarterly reports on obligations until funds have been fully expended.	Within 30 days of enactment the HHS Secretary must report to the Appropriations Committees with a spend plan, which must be updated and resubmitted every 30 days until funds have been fully expended.
International Affairs Reporting Requirement	No comparable provision.	Within 45 days after enactment and prior to obligation of international funds, the USAID Administrator must submit spend plans to the Committees on Appropriations, update and resubmit to those committees every 90 days until September 30, 2017, and every 180 days thereafter until all funds are expended.	Within 30 days after enactment the Secretary of State and USAID Administrator must submit to Appropriations Committees a consolidated report and update and submitted to those committees every 30 days until all funds are expended.
HHS Oversight	No comparable provision.	No comparable provision.	\$500,000 of CDC funds must be made available to the HHS Office of the Inspector General for oversight; an additional \$500,000 from CDC must be made available for oversight by the Comptroller General of the United States.

Table 2. (Continued)

Provision	AdministrationRequest	Senate(S.Amdt. 3900)	House(H.R. 5243IH)
<i>Notification, Reporting and Oversight</i>			
International Affairs Oversight	No comparable provision.	\$500,000 made available within the International Affairs Chapter isto be made available for the Comptroller General of the United States for oversight activities and for consultation with the Secretary of State and the USAID Administrator prior to obligating such funds.	\$500,000 from GHP within this Title must be made available to USAID's Office of the Inspector General for oversight; an additional \$500,000 from GHP must be made available for oversight by the Comptroller General of the United States.

Source: CRS analysis of text of White House, Office of Management and Budget, "Estimate #1–FY 2016 Emergency Supplemental: Appropriations Request to Respond to the Zika Virus both Domestically and Internationally," February 22, 2016, https://www.whitehouse.gov/omb/budget_amendments; S.Amdt. 3900; and H.R. 5243 IH.

THE EMERGENCY SUPPLEMENTAL APPROPRIATIONS REQUEST FOR ZIKA RESPONSE EFFORTS

The following describes the Administration's February 2016 Zika emergency supplemental request components by agency.

It was reported on April 18 that the Administration submitted a revised Zika supplemental request to Congress, which would maintain departmental request totals, while redirecting some of the HHS funds requested for contingency use to vaccine research and development at the National Institutes of Health (NIH).⁹ Detailed information about this is not publicly available, and the following narrative does not reflect this revision.

Health and Human Services

The Administration's emergency supplemental appropriations request to respond to the Zika outbreak seeks for HHS a total of \$1.509 billion. Each HHS agency request includes the statement that funds would be "to prevent, prepare for, and respond to Zika virus, other vector-borne diseases, or other infectious diseases and related health outcomes, domestically and internationally...." Most of the requested funds would support research, surveillance, vaccine and test development, and various domestic preparedness activities. A portion would support international response activities. The request proposes that all supplemental appropriations to HHS be designated as emergency spending, and remain available until expended.

Centers for Disease Control and Prevention (CDC)

A total of \$828 million of the February 2016 request is for the CDC-Wide Activities and Program Support account. Proposed request language would, among other things, authorize the CDC Director to transfer funds between CDC accounts, and authorize funds to be used for real

property acquisition and improvements to non-federal facilities. Funds would be used as follows:

- *Grants and technical assistance to Puerto Rico and U.S. Territories*—\$225 million to, among other purposes, monitor pregnant women and establish a registry of women infected while pregnant; expand mosquito control activities; and enhance laboratory testing capacity.
- *Domestic Response*—\$453 million to provide grants to southern and other U.S. states with *Aedes* mosquitoes for surveillance, improved test methods and testing capacity, public education and outreach, mosquito control measures in areas at risk, and additional federal and state response activities.
- *International Response Activities*—\$150 million to expand the public health workforce, and enhance infectious disease surveillance and emergency response activities, in Zika-affected countries; and to support the laboratory network of the Pan American Health Organization (PAHO), the regional arm of the World Health Organization (WHO) for the Americas.

Public Health and Social Services Emergency Fund (PHSSEF)

The PHSSEF is a fund used by appropriators to provide the HHS Secretary with ongoing or onetime emergency funding, such as for the response to disease epidemics. The emergency supplemental request seeks \$295 million for the PHSSEF for the following:

- several maternal and child health and home visitation programs for low-income pregnant women at risk of Zika infection, and families that have children born with birth defects related to Zika infection;
- several health care workforce assistance programs for Puerto Rico and other territories; and
- compensation for persons harmed by the use of tests or vaccines used under emergency authority.¹⁰

The requested PHSSEF funds could, in consultation with OMB, be transferred to other agencies within HHS or across the federal government. The request stated that this transfer authority is to provide flexibility in response to changing needs. No congressional notification requirement is included.

National Institutes of Health (NIH)

The emergency supplemental request seeks \$130 million for the NIH National Institute of Allergy and Infectious Diseases (NIAID) to expand research efforts to characterize the progression and effects of Zika infection and other vector-borne diseases, and to develop vaccines against them.¹¹ Proposed request language would authorize the NIH Director to transfer funds between NIH accounts. No congressional notification requirement is included.

Food and Drug Administration (FDA)

The emergency supplemental request seeks \$10 million for FDA's role in reviewing the safety and effectiveness of medical countermeasures (such as test methods, vaccines, and

treatments), and post-market monitoring of such countermeasures if and when they become available.

Medicaid Funding for Territories¹²

The emergency supplemental request would temporarily increase the federal matching rate for Medicaid in the territories. The territories operate Medicaid programs under different rules from those that apply to the 50 states and the District of Columbia. Federal Medicaid funding to the states and the District of Columbia is open-ended, but the territories receive capped annual allotments (i.e., the maximum amount of federal funds available in a year). In addition, the Patient Protection and Affordable Care Act (ACA, P.L. 111-148, as amended) provides the territories with additional federal Medicaid funding to use by September 30, 2019. The territories have a federal medical assistance percentage (FMAP) rate (i.e., federal matching rate) for Medicaid of 55%.¹³

The supplemental request includes a provision that would increase the FMAP rate for the territories to 65% for one year beginning with the first day of the fiscal quarter following enactment. This increased FMAP rate would be available for all Medicaid expenditures, not limited to those provided to treat Zika infection. The federal funding for the increased FMAP rate would not count against the territories' annual federal spending caps or additional ACA funding. The Administration estimates this FMAP rate increase would cause federal Medicaid expenditures to grow by \$246 million.¹⁴

There is some question about how this provision would affect Puerto Rico if it were to exhaust its additional ACA funding prior to FY2019.¹⁵ Depending on the timing of enactment, Puerto Rico might not have access to its full annual Medicaid allotments or additional ACA funding for a portion of the time the provision would be in effect.¹⁶

Retroactive Reimbursement

The request proposes language that would allow funds provided in the act to be used to reimburse HHS accounts for Zika response expenses incurred prior to enactment.

Transfer Authority

The request proposes language that would allow funds appropriated to HHS in the act to be transferred to other federal accounts, including the Department of Defense, the Environmental Protection Agency, and the Department of Agriculture "to prevent, prepare for, and respond to Zika virus, other vector-borne diseases, or other infectious diseases and related health outcomes, domestically and internationally....," following consultation with OMB. No congressional notification requirement is included.

Expanded Definition of "Security Countermeasure"

The request proposes language that would allow the government to support the advanced development and procurement of medical countermeasures against Zika virus through Project BioShield. Currently, Project BioShield supports only countermeasures against specific chemical, biological, radiological, and nuclear terrorist threats.¹⁷ The proposed expansion is not limited to countermeasures against the Zika virus or vector-borne diseases, but rather is stated broadly as a "countermeasure to diagnose, mitigate, prevent, or treat harm from any infectious disease that may pose a threat to the public health."

International Assistance Programs

The Administration's February 2016 emergency supplemental appropriations request to respond to the Zika outbreak seeks for the Department of State and USAID a total of \$376.1 million. This includes funds for control of the disease, prevention, surveillance, evacuating U.S. employees and American citizens, vaccine development, and diagnostic research, among other things. Specifically within the International Assistance section of the request is a request for transfer authority (without a requirement for congressional notification) with certain limitations, reimbursement authority, and hiring of personal services contractors, as well as authorization to use unobligated Ebola balances to combat Zika and other infectious diseases. Also worth noting is that, unlike HHS, funds for international assistance programs have varying periods of availability, as specified below.

Department of State

A total of \$41.1 million is requested for the Department of State operations, multilateral assistance within International Organizations and Programs (IO&P), and international security assistance (nuclear research and techniques) as follows:

- *Diplomatic and Consular Programs account (D&CP)*—\$14.6 million to remain available until September 30, 2017. Of this amount.
- \$8.4 million to support the Office of Medical Services for medical support and possible evacuation under the Chief of Mission authority of at-risk U.S. employees in Zika-affected countries; and
- \$6.2 million to support regional coordination efforts and public diplomacy outreach, among other activities.
- *Emergencies in the Diplomatic and Consular Service*—\$4 million to remain available until expended to support response efforts, including potential evacuation of U.S. citizens.
- *Repatriation Loans Program*—\$1 million to remain available until expended to finance repatriation loans to U.S. citizens who may seek to leave Zika-affected areas or who have been exposed to or have contracted Zika.
- *Nonproliferation, Anti-Terrorism, Demining and Related Programs (NADR)*—\$8 million to remain available until September 30, 2017, for additional voluntary U.S. contributions to the International Atomic Energy Agency (IAEA), an autonomous intergovernmental organization related to the United Nations that promotes the safe, secure and peaceful use of nuclear technologies. Funds would support Zika research to develop and deploy nuclear techniques to help accelerate diagnosis, provide related specialized training, and to implement sterile insect projects to suppress mosquito populations.¹⁸
- *International Organizations and Programs (IO&P)*—\$13.5 million to remain available until September 30, 2017, to support Zika response actions taken by UNICEF, the Food and Agriculture Organization, the WHO, and PAHO.

USAID

For the U.S. Agency for International Development, the Administration is requesting \$335 million to cover USAID's health programs and implementation expenses:

- *USAID Operating Expenses (OE)*—\$10 million to remain available until September 30, 2017, to support Zika response efforts.
- *Global Health Programs (GHP)*—\$325 million to remain available until expended to prevent, treat, or respond to the Zika virus and related health concerns, other vector-borne diseases, or other infectious diseases. Multi-year funding commitments are requested to provide incentives for the development of global technologies such as vaccines, diagnostics equipment, and vector control innovations. Anticipated allocations include
 - \$100 million to implement vector management and control activities in Zika-affected countries;
 - \$100 million to stimulate private sector research and development of vaccines, diagnostics, and vector control innovations through public-private partnerships;
 - \$50 million for maternal and child health support in affected and at-risk countries, including training of health care workers; ensuring access to family planning information, services, and methods; providing support for children with microcephaly; and helping pregnant women and their partners have access to personal protection, including condoms and repellent to protect against mosquitoes;
 - \$25 million for public health communication and behavior change campaigns for affected communities and countries to take actions to protect themselves from Zika and other vector-borne diseases; and
 - \$50 million to issue Global Health Security Grand Challenges that would call for groundbreaking innovations in diagnostics, vector control, personal protection, community engagement and surveillance, and other tools to address Zika and other infectious diseases, as well as to develop public-private partnerships to accelerate development of innovative tools and practices.

Use of Ebola Balances for Other Infectious Diseases

Within the Department of State and Other International Programs General Provisions (in addition to the General Provisions for the entire request), the supplemental request would authorize the use of unobligated Ebola Funds (Title IX, Div. J, P.L. 113-235), stating: “[Unobligated Ebola funds] shall also be available to respond to the Zika virus and related health outcomes, other vector-borne diseases, or other infectious diseases.”

As of January 1, 2016, the Department of State/USAID's unobligated Ebola funds totaled nearly \$1.3 billion. Of that total, about \$600 million is available until September 30, 2016 (just a few months away), and about \$694 million is available until expended.¹⁹ (See the subsequent section, “HHS, State/USAID, and DOD Unobligated Ebola Response Funds.”)

Transfer Authority

The Department of State and Other International Programs General Provisions in the supplemental request would allow transfer of State Department-related funds in the request only among State Department-related accounts within the request and transfer of USAID-

related funds in the request only among USAID-related accounts. No congressional notification requirement is included.

Notwithstanding Authority

The supplemental's request for notwithstanding authority could allow funds from this or prior acts supporting the U.S. Zika virus response to be expended despite any previously enacted restrictions and conditions on U.S. foreign aid. For example, if enacted, this authority could allow foreign aid to be provided to states that are otherwise restricted by law: those designated as sponsors of terrorism, those with debt arrearage, human rights violators, or states that practice coercive family planning. The Department of State has indicated in the Global Health Program (GHP) section, however, that funds will provide support for "ensuring access to voluntary family planning information, services, and methods."

Direct Hiring Authority/Personal Services Contractors

The General Provisions Title in the request, and also the General Provisions Title for the Department of State and Other International Programs, allows for expedited hiring authority to directly hire staff during critical public health threats, such as Zika, and to enter into contracts with individuals who are experts in Zika-related fields. This measure does not limit direct hiring or personal services contractors only for Zika-related purposes. This authority for direct hiring and personal services contractors could be used in a broader set of public health circumstances than the Zika virus.

HHS, STATE/USAID, AND DOD UNOBLIGATED EBOLA RESPONSE FUNDS

In December 2014, the Consolidated and Further Continuing Appropriations Act, 2015 (P.L. 113- 235), provided \$5.4 billion in emergency supplemental appropriations to HHS, the Departments of State and Defense, and USAID to address the Ebola outbreak that began in West Africa in January 2014.²⁰ Because these funds were designated as emergency appropriations, they are effectively exempt from spending limits in the Budget Control Act of 2011 (BCA, P.L. 112-25).²¹

On April 6, 2016, the Obama Administration announced its plan to reprogram \$510 million of unobligated FY2015 Ebola funding to respond to the Zika virus.²² HHS Ebola funds may be reprogrammable without additional congressional action (subject to existing restrictions on reprogramming, including notification). This is because the relevant appropriations measures stated the funds are available for Ebola and other infectious diseases. Some have debated whether congressional action is necessary to provide the Department of State and USAID with the authority to reprogram the unobligated Ebola funds, as much of the funding was appropriated with specific language to be used to "prevent, prepare for, or respond to the Ebola disease outbreak."

Table 3 provides, by account, the original appropriated Ebola funds, remaining (unobligated) amounts, the period of funding availability, and purpose of the funds, based on quarterly reports to Congress as required by the law. As of January 1, 2016, unobligated Ebola funds totaled \$2.77 billion: \$1.46 billion for HHS, \$1.29 billion for State/USAID, and \$17.3

million for Defense. A portion of the total, \$652.9 million—most of which is USAID funding—expires September 30, 2016. Nearly all of the remaining unobligated funds expire September 30, 2019, or are available until expended. *Table 3* does not incorporate the Administration's announced plans to reprogram \$510 million of unobligated Ebola funds.

**Table 3. FY2015 Emergency Funds Appropriated
for Ebola Response and Related Activities, and Unobligated Balances.**
Amounts are U.S. dollars in millions. Unobligated amounts are as of January 1, 2016

Agency and Accountor Activity	P.L. 113-235 ^a	Unobligated Funds	Period of Availability	Purpose(s)
DEPARTMENT OF HEALTH AND HUMAN SERVICES				
CDC:International activities	603.0	369.4	Until Sept.30, 2019	Disease control assistance to affected and neighboring countries.
CDC:Global Health Security	597.0	525.2	Until Sept. 30, 2019	Implementation of Global Health Security Agenda (GHSA) activities. ^b
CDC:Public health emergency preparedness	165.0	11.3	Until Sept. 30, 2019	Domestic preparedness and response activities, including control in health care settings, and procurement for stockpile.
CDC:State and local	255.0	56.0	Until Sept. 30, 2019	Grants to state health departments for surveillance, testing, case management.
CDC:Worker training	0.0	—	—	\$10 million for this activity was transferred to NIH. See below.
CDC:Migration/quarantine	119.3	57.4	Until Sept. 30, 2019	Screening and management of entrants from affected countries/regions.
CDC:Other domestic activities	37.0	11.9	Until Sept. 30, 2019	Vaccine trials and other applied public health research.
CDC Subtotal	1,776.3	1,031.2	—	—
ASPR (PHSSEF):Hospital Preparedness Program	208.5	21.2	Until Sept. 30, 2019	Domestic training, PPE, and establishing regional Ebola Treatment Centers (ETCs).
ASPR (PHSSEF):Other prep. and response	352.2	347.4	Until Sept. 30, 2019	Not specified. Could include domestic treatment costs for affected individuals.
ASPR (PHSSEF):BARD A	157.0	3.7	Until Sept. 30, 2019	Research, development, and procurement of vaccines and treatments.
ASPR/PHSSEF Subtotal	717.7	372.3	—	—
NIH, NIAID	238.0	35.2	Until Sept.30, 2016	Research and clinical trials on investigational vaccines and treatments.
NIH, NIAID	10.0	9.0	Until Sept. 30, 2019	Ebola responder safety training, funds transferred from CDC.
FDA	25.0	13.7	Until expended	Development, review, and regulation of vaccines and treatments.
HHS Total	2,767.0	1,461.4	—	—
STATE DEPARTMENT/USAID				
USAID, Operating Expenses	19.0	15.2	Until Sept. 30, 2016	Operating costs to address Ebola outbreak in West Africa, including temporary staffing and technical support.

Agency and Accountor Activity	P.L. 113-235 ^a	Unobligated Funds	Period of Availability	Purpose(s)
USAID, Inspector General	5.6	3.4	Until expended	Oversight of Ebola response in West Africa.
USAID, International Disaster Assistance(IDA)	1,436.3	542.4	Until expended	Disaster assistance to address humanitarian needs for West Africa, such as rapid response, maintaining surveillance, screening, and contact tracing.
USAID, Global Health Programs	312.0	148.0	Until expended	Expanded USAID global health security activities to control infectious diseases and limit spread of Ebola, including surveillance and building lab capacity.
State/USAID, Economic Support Fund	711.7	583.0	Until Sept. 30, 2016	Training and program assistance to prevent economic and government instability during Ebola crisis, including reimbursement for earlier response. Activities include rehabilitation of the water infrastructure, strengthening health information systems, and developing technology to prevent the spread of Ebola.
State, Diplomatic, Consular Programs(D&CP)	36.4	2.2	Until Sept. 30, 2016	Medical support and evacuation capacity, repatriation assistance, and other needs.
State, Repatriation Loans Program	ns	ns	ns	Repatriation loans to U.S. citizens as necessary related to Ebola outbreak. Funding not specified in P.L. 113-235. Explicit transfer authority is provided for up to \$1 million from D&CP into this account. ^c
State, International Organizations and Programs(IO&P)	ns	ns	ns	Estimated U.S. contributions to UNMEER. Funding not specified in P.L. 113-235. Explicit transfer authority is provided for up to \$35.3 million from IDA for this account. ^c
State, Contributions to International Organizations (CIO)	ns	ns	ns	Funding not specified in P.L. 113-235. Explicit transfer authority is provided for up to \$35.3 million from IDA and \$50 million from Global Health Programs for this account. ^c
State, Nonproliferation, Anti-terrorism, Demining, and Related Programs	5.3	0.0	Until Sept. 30, 2016	Biosafety and hazardous materials training in affected countries, efforts to mitigate illicit acquisition of Ebola virus and to promote biosecurity practices associated with outbreak response efforts.
State/USAID Total	2,526.3	1,294.2	—	—

Table 3. (Continued)

Agency and Accountor Activity	P.L. 113-235 ^a	Unobligated Funds	Period of Availability	Purpose(s)
DEPARTMENT OF DEFENSE				
Defense/DARPA: Defense-wide research, development, testing, and evaluation (RDT&E)	45.0	0.8	Until Sept. 30, 2016	Developing medical countermeasures technologies (e.g., using antibodies from survivors) and shortening vaccine development time.
Defense/CBDP: RDT&E	50.0	16.4	Until Sept. 30, 2017	Research and Development, testing and evaluation.
Defense/CBDP: procurement	17.0	0.1	Until Sept. 30, 2017	Procurement of detection and diagnostics systems, mortuary supplies, and isolation transport units. ^d
Defense Total	112.0	17.3	—	—
TOTAL	5,405.3	2,772.9	—	—

Sources: Departmental spend plans and/or quarterly reports for HHS, State/USAID, and Defense, as required by P.L. 113-235, and obtained by CRS; and additional departmental communications.

Note: Amounts may not add due to rounding; “ns” means not specified.

Glossary: ASPR is HHS Assistant Secretary for Preparedness and Response; BARDA is HHS Biomedical Advanced Research and Development Authority; CBDP is Chemical Biological Defense Program; CDC is HHS Centers for Disease Control and Prevention; DARPA is Defense Advanced Research Projects Agency; FDA is HHS Food and Drug Administration; NIAID is NIH National Institute of Allergy and Infectious Diseases; NIH is HHS National Institutes of Health; PHSSEF is HHS Public Health and Social Services Emergency Fund, administered by the HHS Secretary; PPE is personal protective equipment; UNMEER is United Nations Mission for Ebola Emergency Response, and WHO is World Health Organization.

^a HHS amounts reflect transfers between the funded agencies, as permitted by the law. P.L. 113-235, 128 Stat. 2522, §604, December 16, 2014.

^b For more information, see CDC, Global Health Security Agenda, <http://www.cdc.gov/globalhealth/security/index.htm>.

^c P.L. 113-235, 128 Stat. 2694, §9001.

^d Explanatory statement accompanying H.R. 83, Congressional Record, vol. 160 (December 11, 2015), p. H9635.

END NOTES

¹ For more information, see CRS Report R44368, Zika Virus: Basics About the Disease; CRS In Focus IF10353, Mosquitoes, Zika Virus, and Transmission Ecology; CRS Insight IN10433, Zika Virus: Global Health Considerations; and CRS Report R44385, Zika Virus: CRS Experts.

² White House, Office of Management and Budget, Estimate #1 – FY 2016 Emergency Supplemental: Appropriations Request to Respond to the Zika Virus both Domestically and Internationally, February 22, 2016, https://www.whitehouse.gov/omb/budget_amendments. For more information on discretionary spending limits and Ebola funds designated for emergency requirements, see OMB Final Sequestration Report to the President and Congress for Fiscal Year 2015, January 20, 2015, at https://www.whitehouse.gov/sites/default/files/omb/assets/legislative_reports/sequestration/sequestration_final_january_2015_president.pdf.

³ For more detail, see <https://www.opm.gov/blogs/Director/direct-hire-authority/>.

⁴ As defined in regulation, “The Government is normally required to obtain its employees by direct hire under competitive appointment or other procedures required by the civil service laws. Obtaining personal services by contract, rather than by direct hire, circumvents those laws unless Congress has specifically authorized