The Zika outbreak of the 21st century

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ABSTRACT

The Zika virus outbreak has captivated the attention of the global audience and information has spread rapidly and wildly through the internet and other media channels. This virus was first identified in 1947, when it was isolated from a sentinel rhesus monkey placed by British scientists working at the Yellow Fever Research Laboratory located in the Zika forest area of Uganda, hence its name, and is transmitted primarily by the mosquito vector, *Aedes aegypti*. The fact that the rhesus macaque is an Asian species being placed in an African forest brings to mind the possibility of rapid adaptation of the virus from an African to Asian species, an issue that has not been considered. Whether such adaptation has played any role in acquiring pathogenicity due to cross species transmission remains to be identified. The first human infection was described in Nigeria in 1954, with only scattered reports of about a dozen human infections identified over a 50-year period. It was not until 2007 that Zika virus raised its ugly head with infections noted in three-quarters of the population on the tiny island of Yap located between the Philippines and Papua New Guinea in the western Pacific Ocean, followed by a major outbreak in French Polynesia in 2013. The virus remained confined to a narrow equatorial band in Africa and Asia until 2014 when it began to spread eastward, first toward Oceania and then to South America. Since then, millions of infected individuals have been identified in Brazil, Colombia, Venezuela, including 25 additional countries in the Americas. While the symptoms associated with Zika virus infection are generally mild, consisting of fever, maculopapular rash, arthralgia and conjunctivitis, there have been reports of more severe reactions that are associated with neurological complications. In pregnant women, fetal neurological complications include brain damage and microcephaly, while in adults there have been several cases of virus-associated Guillain-Barre syndrome. The virus was until recently believed to only be transmitted via mosquitoes. But when the Zika virus was isolated from the semen specimens from a patient in Texas, this provided the basis for the recent report of possible sexual transmission of the Zika virus. Due to the neurological complications, various vectors for infection as well as the rapid spread throughout the globe, it has prompted the World Health Organization to issue a global health emergency. Various governmental organizations have recommended that pregnant women do not travel to countries where the virus is epidemic, and within the countries affected by the virus, recommendations were provided for women of childbearing age to delay pregnancy. The overall public health impact of these above findings highlights the need for a rapid but specific diagnostic test for blood banks worldwide to identify those infected and for the counseling of women who are pregnant or contemplating pregnancy. As of this date, there are neither commercially licensed diagnostic tests nor a vaccine. Because cross-reactivity of the Zika virus with dengue and Chikungunya virus is common, it may pose difficulty in being able to quickly develop such tests and vaccines. So far the most effective public health measures include controlling the mosquito populations via insecticides and preventing humans from direct exposure to mosquitoes.

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1. Introduction

The Zika virus has found its way into the public consciousness, as it has recently been associated with brain damage in the offspring of infected pregnant women. The news outlets have

latched onto this hot item and have been bombarding the public with information that is both legitimate and misleading. The internet age has allowed rapid dissemination of such information, therefore causing rapid reactions or responses by both national and international agencies, regulatory bodies and professional organizations. The story of the impact the Zika virus has played upon humanity is an interesting and unique one, which has led to the government issuing non-traditional recommendations, in an effort to ensure the public's safety and health. Tracing the sequence of events from the discovery of the virus to the current state of affairs provides a useful learning tool that would allow effective responses during unexpected pandemics. Although there are over 325 published articles associated with the Zika virus, the majority coming out of Uganda, there is still little known about the potential of the virus [1].

The Zika virus was identified over 50 years ago in Africa where it is reasoned to have originated. It is known to have spread to various geographic areas of Asia and the Pacific Islands, with the most recent spread eastward to the Americas. Earlier, symptoms associated with infection included a mild response, which included fever and fatigue, until this year when it was reported that pregnant women, primarily Brazilian women, that tested positive with the Zika virus had fetuses born with brain defects. After Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory virus (MERV), the Ebola outbreak, and recurrent re-emergence of measles, influenza, dengue and cholera throughout the globe, the world now has a new enemy to contend with, serving as a reminder that human beings live in a complex relationship with other organisms in an ecosystem that is often unpredictable [2]. The continuous encroachment of otherwise forested and/or remote habitats by humans in search of new space for housing and adventure combined with the ever increasing speed of inter-continental air travel logarithmically increases the likelihood of the jumping of microbial species from its natural habitat to urban locations. This is a reality to be faced by those involved in public health safety and will remain a continuing challenge to health care providers worldwide.

2. History

Several viruses were reported to have been isolated by a team of British and American scientists that were in charge of the Yellow Fever Virus Research Institute housed in Entebbe, Uganda during the late 1930's to late 1940's primarily with the aim of isolating yellow fever viruses (YFV) [3]. In efforts to isolate the YFV, these scientists placed Asian species rhesus macaques (*Macaca mulatta*) in sentinel cages atop forest canopy level platforms in the Zika Forest area located near Entebbe, Uganda, presumably in a location where they could serve as potential hosts for the local mosquito population known to be vectors of the YFV. Blood samples isolated from one of these "sentinel" rhesus macaques (monkey 766) when injected into the brains of Swiss albino mice led to sickness of these mice. Homogenates of brain tissues from these sick mice led to the isolation of several filterable viruses, amongst one that was called the Zika virus766 because of the forest and the number of the monkey (hence, its name) [4,5]. These viruses were also isolated from mosquito populations from the same location and thus reasoned to be circulating and being kept endemic by virus host interactions between the indigenous monkey species (that include 13 species with the colobus species monkey being predominant) and mosquitoes. The fact that the virus was isolated from "sentinel" Asian rhesus macaques implies that there must have been an adaptation of the virus from the African monkey species to the Asian monkey species (jumping of species). Whether such adaptation was instrumental in further transmission of this virus and whether such adaptation led to increased pathogenicity remains

unknown. It should be noted that sera collected from blood samples of several local individuals residing in the same area during the same time period were found to contain antibodies against the Zika virus presumably suggesting that the virus was already circulating in the human population. However, there were no reports of any disease. What is not clear is the specificity of the antibody test that was utilized at the time since there is considerable cross reactivity among the flaviviruses. It was first isolated from a human in 1969 in Nigeria, though the illness caused by the virus, Zika fever or Zika disease has been known to infect humans since the early 1950s [6]. A time line of the history of the Zika virus is shown in Fig. 1; the countries involved are shown in Table 1.

Early reports of the Zika virus identified very rare cases of human disease. Lanciotti stated in a 2008 review that "Historically, Zika has rarely been associated with human disease" [7]. Early reports of epidemics were reported primarily in the South Pacific and in Southeast Asia. The Yap Island reported 185 cases of probably Zika infection and a seropositivity rate of greater than 74% of the population in 2007.

3. Biology of the Zika virus

3.1. Classification

The Zika virus belongs to the family Flaviviridae and the genus Flavivirus. Other Flaviviruses include yellow fever, dengue, West Nile and Japanese encephalitis viruses [8,9]. Flaviviruses belong to a group of viruses labeled as "arborviruses", which is a descriptive term that refers to hundreds of RNA viruses which rely on arthropods such as mosquitoes or ticks for transmission. Arboviruses (arthropod borne viruses) cause some of the most devastating diseases in humans and animals worldwide. The families of RNA arboviruses include Bunyaviridae, Flaviviridae, Reoviridae, Rhabdoviridae, and Togoviridae. The arboviruses are acquired orally by their hematogenous vectors in the form of a blood meal of an infected vertebrate host. These viruses are non-pathogenic to the vector but have to be able to survive in a live form in the vector which then transmits via saliva deposition into a new vertebrate host. This cycle is important to remember since the details of how the virus survives in the vector host and whether it replicates in the vector host, and if it replicates, the cell types that it infects in the vector and whether the virus changes in any form in the vector host (such as glycosylation of its envelope for example) are all important issues that influence the ability of the vector to transmit infection including the Zika virus. Evidence for recombination of the virus has already been documented to have potentially occurred by transmission of the virus via a different species of mosquitoes [10]. The Zika virus is closely related to the *Spondweni* virus. There are a total of seven groups of mosquito-borne flaviviruses, according to the International Committee on Taxonomy of Viruses (ICTV). The groups are categorized based on antigenic and genetic considerations. The genus Flavivirus consists of 39 different mosquito-borne viruses [11].

3.2. Structure of the Zika virus

The Zika virus is composed of a positive sense, single strand RNA genome. It is an enveloped, icosahedral virus that is a member of the Spondweni clade. The Zika virus is a positive polarity RNA virus with a genomic size of about 11 kb [12]. The single open reading frame sequence of its RNA genome encodes a polyprotein which constitutes the structural architecture of the virus [13]. This polyprotein contains 3 components, including a capsid (105 aa), membrane and premembrane portion (187 aa) termed C, M and P, respectively. There is also an envelope protein (E, 505 aa) and an

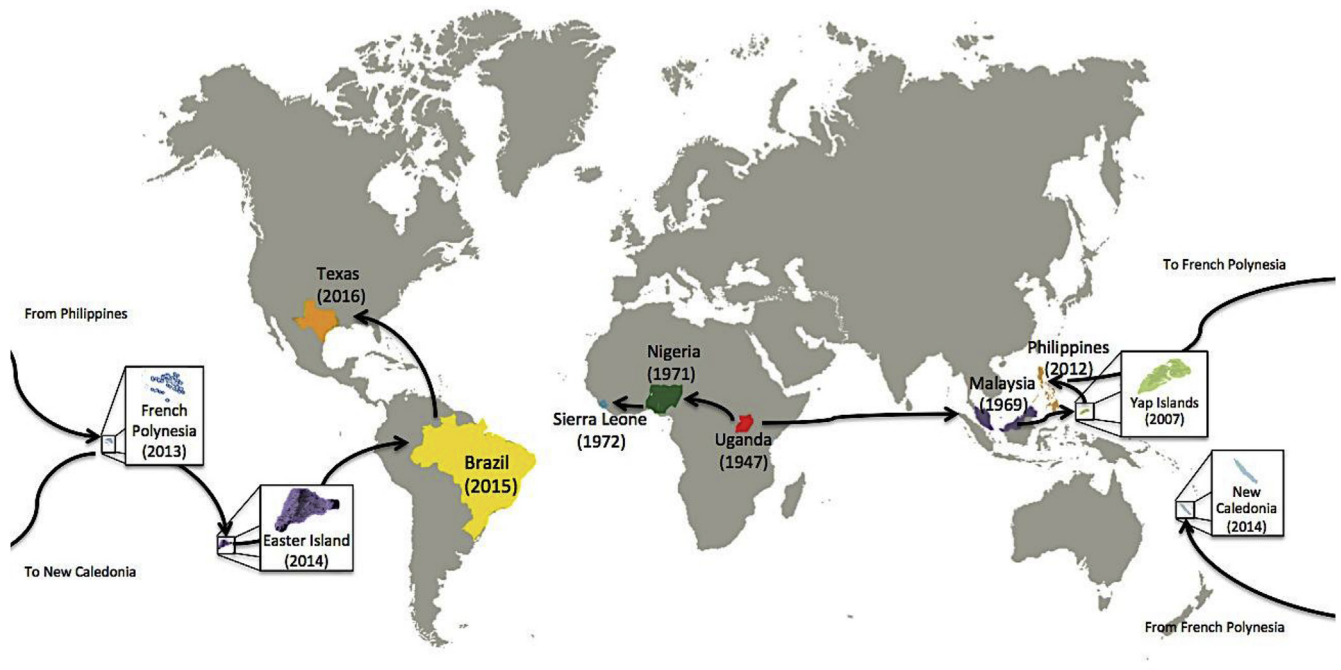# Potential Pathways of Global Zika Virus Spread

Fig. 1. Zika virus spread patterns and Aedes mosquito global distribution.

addition 7 components that are non-structural (NS). These seven proteins are designated NS1 (352 aa), NS2a (217 aa), ND2b (139 aa), NS3 (619 aa), NS4a (127 aa), NS4b (255 aa) and NS5 904 aa) [14]. The non-structural proteins NS2B/NS3 includes a serine protease that along with host proteases co-translationally and post-translation cleaves the polyprotein into its components. The envelope protein is the primary flavivirus antigenic site and dictates attachment of the virion and penetration into the host cell. Folding of the E protein is controlled by the premembrane protein, which is cleaved by furin to form the membrane protein prior to mature virion release from the cell [15]. The function of the remaining non-structural proteins remain unknown, but may have specific essential roles in various replication stages. For example, NS5, the most highly conserved of the flavivirus NS proteins functions as a RNA-dependent RNA polymerase [16].

3.3. Zika virus genome

There are two known lineages of the Zika virus, an African lineage and an Asian lineage [17]. These are distinguishable by detailed genetic analysis of the RNA sequence [18]. The primary variability when comparing strains appears to be related to differences in the availability of potential glycosylation sites. The complete coding sequence of the virus obtained from a patient from French Polynesia who was hospitalized after falling ill in metropolitan France revealed an Asian lineage, with 99.9% nucleotide and amino acid homology with isolates that circulated during the 2000s in Southern Asia and the Pacific Islands. It is believed that the Asian lineage was introduced to Asia from Africa as early as 1945 [14]. The Zika virus isolated from another two patients hospitalized in French Polynesia was also subject to phylogenetic analysis and found to be of Asian lineage [19].

Five patients with mild symptoms of Zika virus infection were identified from Suriname in October 2015. The sera of 4 of the viremic patients were submitted for genomic sequencing [20]. The complete coding sequence was obtained from one patient and the genome of the envelope protein was determined from the other three. The genome sequence from the Suriname patients is considered to be of the Asian genotype, as opposed to the African lineage. As with most epidemiological studies, the complete sequencing of the Zika virus genome has been rarely done, but the Suriname genotype shows over 99% protein and gene homology with the strain isolated from French Polynesia in 2013.

Sequencing of a 976 base pair region of the NS5 gene in 51 patients from the Easter Island outbreak revealed that phylogenetically, the strains from Easter Island were very closely related to the strains from French Polynesia, Cambodia and Micronesia, all from the Asian lineage [21]. In contrast, there were three Zika virus isolates from Sylvatic mosquitoes in the Central African Republic which demonstrated 99.9% and 100% nucleic acid sequence and amino acid sequence homology with each other respectively, but which differed from other Zika strains. This led the authors of this study to conclude that while these virus strains evolved from an African lineage, there exists a different West African Zika virus subtype in Central Africa from the African strains found in other countries such as Gabon [22].

It is unclear why Zika has suddenly evolved into a global pandemic. There is an extra 12 nucleotide sequence in the envelope gene discovered in the virus that led to the Yap Island epidemic in 2007, which was not present in the prototype MR766 Zika virus [7]. This 4 amino acid sequence corresponds to the 154 glycosylation motif in the envelope protein that may play a role in virulence. More research is necessary to clarify the role of glycosylation in the pathogenesis of Zika virus infection.

4. Geographical spread and distribution of the Zika virus

It is generally accepted that Zika virus originated in Africa and then likely spread in two directions. Thus, there were cases reported in various parts of Africa that included one in Nigeria in

Table 1
Countries with reported Zika virus infection (as of Feb 2016).

Country	Continent	First infection reported	Number of cases	Associated illnesses	References
Australia	Australia	2015	Case report following monkey bite, import from Cook Island, case report after travel to Indonesia	Unknown	[111−113]
Brazil	South America	2015	440,000−1,300,000	4000 microcephaly cases	[114]
Cambodia	Asia	2010	Case report	None	[115]
Canada	North America	2013	Case report of transmission from Thailand	Unknown	[40]
Colombia	South America	2015	578 RT-PCR confirmed cases as of Nov 28, 2015		[116]
Easter Island	South America	2014	89+ samples	Unknown	[21]
El Salvador	Central America				
Federated States of Micronesia	Asia	2007	70% of population		[7,17,114]
French Polynesia		2013−14			[71,117]
Germany	Europe	2013, 2014	Transmission in traveler from Malaysian Borneo, and in a traveler from Thailand	Unknown	[44,118]
Indonesia	Asia	1977	7 cases	Unknown	[7,119]
Italy	Europe	2015	Case report of patient from Brazil and French Polynesia − not autochthonous	Unknown	[120,121]
Jamaica	Central America				
Japan	Asia	2013−2014	2 cases imported from French Polynesia	Unknown	[122]
Maldives	Asia	June 2015	Case report		[123]
New Caledonia	Asia	2014	2 patients with co-infection with dengue	Unknown	[73]
Nigeria	Africa	1954	First human infection case, additional cases in 1971−5	Unknown	[3,124]
Norway	Europe	2013	Following travel to Tahiti	Unknown	[125]
Philippines	Asia	2012	Case report of a 15 year old boy	Unknown	[126]
Solomon Islands					
Suriname	South America				
Thailand	Asia	2012−2014	7 cases	Unknown	[127]
USA	North America	2015	Cases in New York and Texas, now 31 confirmed cases		[128,129]
Vanautu	Asia	2014	2 cases	Unknown	[73]
Venezuela	South America	2015			[130]

1971, one in Sierra Leone in 1972 and more recently in the Cape Verde Island. The other route of spread was Eastward to the Pacific Islands and Asia. Multiple arbovirus infections were reported in the late 1950s and early 1960s in East Africa, including Zika, yellow fever, Banzi, Wesselsbron, West Nile and dengue 1 (group-B arboviruses), chickungunya, o'nyong-yong and Sinbis (group-A arboviruses), and Bunyamwera virus [23]. Although known since 1947, extensive spread of the Zika virus to Asia probably only occurred as recently as 2012−2014 [24]. It is unknown what triggered the worldwide emergence of a disease confined to a narrow tropical band. RT-PCR based studies on the Zika virus suggest that genetic changes in protein glycosylation through recombination events may have led to a higher efficiency in transmission via mosquito vectors, thus facilitating changes in geographical spread patterns [10].

Health emergencies in the Pacific region are dealt with by the Pacific Public Health Surveillance Network (PPHSN), a voluntary network of 22 Pacific Island countries which are home to 10.6 million people. The network noted that beginning in January 2012, there was a sudden increase in mosquito-borne diseases including dengue, chickungunya and Zika virus. The network identified over the next 2 years a total of 28 separate outbreaks and a circulation of several mosquito-borne diseases. By 2014, a total of 120,000 people were infected, which was believed to be a conservative estimate due to underreporting [19,25].

The first reports of Zika virus in Brazil appeared in May of 2015 [26,27]. Prior to April of 2015, Zika virus was non-existent in Brazil. The first case of autochthonous transmission of the virus in Brazil

was reported in March of 2015 [28]. The increased incidence of microcephaly in infants born in Brazil led to the realization that there is an extremely likely association between brain damage and Zika virus infection, though even to this day this has not yet been definitively confirmed [8,29]. In barely eight months, there have been over 4000 confirmed cases of microcephaly in Brazil, according to a report in the British Medical Journal published on the 26th of January, 2016 [30]. The virus is widespread in multiple regions and states in Brazil [31−33], and the reports of microcephaly coincide temporally with the appearance of the Zika virus in Brazil. Other Latin American countries have since reported cases of Zika virus infection [34,35]. Tracing the route of spread, one theory is that the beginning of the disease in Brazil was caused by transmission from the outbreak in French Polynesia in 2014, which coincided with the World Cup football competition [36,37]. In addition, it has been proposed that climate changes may have facilitated the spread of Zika virus to the Americas by virtue of the warmer temperatures and drought conditions observed in the second half of 2015 in South America. Drier conditions may have enhanced vector dispersal [38].

A recent case of Zika virus was diagnosed in Japan in a patient who had traveled from Thailand. In this case, Zika virus infection was diagnosed by RT-PCR in the urine. The sera from this patient also had IgM cross reactivity with the dengue virus. This illustrates the need to be able to distinguish Zika virus from dengue and other flaviviruses [39].

One case report of a patient from Canada was found in the literature [40]. This report describes a patient who acquired the

infection in Thailand, so this was not a primary infection occurring in North America [41]. The *Aedes aegypti* is not widely found in Canada, so it has been reasoned that Canada is not at risk for an epidemic, although the recent reports of sexual transmission may alter this viewpoint.

There is a suggestion that the Zika virus may follow the migration patterns of the Chikungunya virus, an alpha virus which is also transmitted by mosquitoes [42]. Chikungunya virus belongs to the group IV Togaviridae arbovirus family. All alphaviruses are single strand positive sense RNA viruses that possess a nucleocapsid. Interestingly, the mosquito vectors for both these viruses have adapted to be active during the daytime and thrive in urban areas.

It is believed that since 1947, when the virus was first identified, it remained obscure and confined to an equatorial belt in Africa and Asia. The spread of the Zika virus was analyzed in a communication published in the Lancet, based on local and regional travel patterns and climate considerations. The spread to neighboring 13 countries was consistent with travel volume and may provide important information related to the upcoming Olympic Games in Rio de Janiero [43].

Numerous cases of Zika virus detectable in travelers returning from epidemic areas have been reported. It has been detected in multiple travelers in Europe, including Germany, France and Italy. Serological studies from German tourist travelers showed both IgG and IgM positivity to the ZIka virus upon return from Thailand [44].

In addition, some have speculated that El Nino has played an important role in the spread of Zika in Latin American countries as the phenomenon could help create the ideal conditions for the proliferation of mosquitoes. "El Nino", characterized by a warming of the central and eastern Pacific Ocean near the equator, is known to change rainfall patterns around the world, including Latin America.

5. Transmission of the Zika virus

5.1. General comment

The transmission of arboviruses by definition involve an arthopod borne vector (mainly species of mosquitoes and ticks) and a vertebrate host. It is reasoned that most of these arboviruses remain endemic in tropical and sub-tropical areas of the world that promote the life cycle of the arthropod. In most, if not all such cases, the virus in question does not cause any disease in its natural host. Thus, the infectious virus has to be present in sufficient quantity in the blood/tissues of the vertebrate host so that it is picked up by the arthropod when it bites the natural host. The infectious virus then has to survive in the arthropod and in some cases replicate in a specific tissue of the arthropod and once again be in sufficient infectious amounts to be delivered by the arthropod to another natural host to continue the cycle. Mosquitoes generally feed on plant nectars and it is primarily the female mosquito that feeds on vertebrate blood that is needed for the maturation of the eggs it carries. The average life span of a male mosquito is only 10 days and 1–2 months for the female and the range of a mosquito is 1–3 miles. Thus, female mosquitoes have to be transmission competent within this framework for the life cycle of the arbovirus to be complete. One study reported survival of the Zika virus for a period of two weeks in female mosquitoes, suggesting the stability of the virus in the vector host. As one may gather, these are rather stringent requirements for the life cycle of arboviruses and have evolved over millennia. It also seems clear that it is only when such viruses jump species (non-natural host), i.e. to humans, that we see evidence of disease often with serious pathology and, in a small number of cases, potential death of the host.

5.2. The Aedes mosquito

Arboviruses are obviously generally more prominent in tropical climates where the mosquito species thrive. They have the capability of undergoing explosive spread and a significant proportion of the population can be infected over weeks to months [45]. The Zika virus is spread by mosquitoes belonging to the Aedes species [46]. In the Uganda forest which is the namesake of the Zika virus, mosquito population analysis revealed a total of 58 species. The mosquito species diversity is changing, as mosquito populations are constantly being monitored, and 22 of the 58 species were considered new to the Zika forest, and 20 previously detected species were no longer found at the time of this study. Of the 58 species detected in 2014, the majority belonged to the genera *Aedes* and *Culex* [47]. Changing biodiversity is influenced by human activity (building roads, communities, hunting, agricultural incursions, and timber harvesting) and changing climate [48].

In particular, the *A. aegypti* mosquito is known to be the predominant carrier for Zika virus infection [49]. This is the same mosquito that spreads the yellow fever virus. This mosquito can breed in small quantities of water, and is known to be active and aggressive during the daytime hours, but especially during dusk or dawn in cloudy weather, and often indoors. This mosquito originated in Africa, but now can be found in almost all subtropical and tropical climates throughout the globe. Zika virus was isolated from the *A. aegypti* mosquito in Malaysia in 1969 [50]. In North America, the *A. aegypti* species are found primarily in Florida, but they have also been detected as far north as Washington, DC. The *A. aegypti* mosquito lives for 2–4 weeks, but its eggs can survive for long periods of time in a dry state, and are known to reintroduce large numbers of the mosquito after a cold, dry winter. Other diseases that are transmitted by the *A. aegypti* mosquito include dengue, yellow fever and chikungunya. *A. aegypti* has been identified to be the main vector for arboviruses in the Pacific region [51]. In Brazil, where recent Zika virus cases have exploded, the *A. aegypti* mosquito had been known for its ability to transmit related virus such as dengue or Chikungunya [52].

Another mosquito that has been known to transmit the Zika virus is *Aedes albopictus*, the Asian tiger mosquito [53]. *A. albopictus* was believed to play a role in the Zika virus epidemic in Gabon in 2007 [54]. Because *A. albopictus* is present in Southern Europe, the WHO issued a warning to European countries to be on the lookout for potential cases of Zika infection [55]. An analysis of the transmission potential for the Zika virus by various species of the *Aedes* mosquito was conducted [56]. *A. aegypti, A. albopictus, Aedes unilineatus* and *Aedes vittatus* were all found to be susceptible to Zika virus infection. Interestingly, in this study performed in Senegal, *A. aegypti* was found to have low potential for transmission of the Zika virus, which was discordant with other findings from other studies and field observations [57]. Studies designed to define the range of vector species employed the use of murine skin explants that served as a barrier when placed on Zika virus heparinized human blood and the candidate mosquito species allowed to feed on such an in vitro culture system. The *Aedes spp* of mosquitoes were found to be easily infectable with the Zika virus using this technique [58]. Zika virus has also been isolated from *Aedes africanus* in the Lunyo forest on the shore of the Entebbe peninsula as early as 1948 [59], and was again isolated from this strain of Aedes mosquitos caught from various heights of a 120 m tower in the Zika forest in Uganda in the early 1960s [60].

An early outbreak of the Zika virus in the Pacific Ocean can be traced to the case of French Polynesians who attended a cultural event on Easter Island, Chile. This may represent the path by which the Zika virus was introduced into the Americas from Asia [36]. The initial spread was slow, but once it appeared in South America, its

spread has hastened dramatically to affect almost all regions of the Americas [61].

In 2011, a large number of mosquito pools collected in Senegal between April and December were tested for Zika virus infection. Of a total of 1700 of these mosquito pools, 31 were positive for the Zika virus. The mosquitoes species found included *A. aegypti, Aedes furcifer, Aedes luteocephalus, A. vittatus, A. africanus, Aedes dalzieli, Aedes hirsutus, Aedes taylori, Aedes metallicus, Aedes unilinaetus*, and three other non-Aedes mosquito strains [57]. The *A. furcifer* males and *A. vittatus* were two species that were found to be infected in one of the villages and are most likely the vectors in this geographical locale. On the other side of the globe, a Zika epidemic was reported on the Cape Verde islands in the Atlantic Ocean, with 4744 reported cases as of December 6th, 2015 [62]. The *Aedes hensilli* species of mosquitoes was shown to be the likely vectors of Zika infection in this outbreak [63].

It has to be kept in mind that the transmission potential of the vector being evaluated is not the only factor in determining the role such a vector plays in the extent of a viral outbreak. This concept is highlighted by the finding of high density but low transmission potential of *A. aegypti* serving as a successful vector for the spread of yellow fever in Nigeria [64].

5.3. Non-mosquito transmission

The natural transmission cycle of arboviruses, in general, have been thought to involve mosquitoes. However, there have been reports of perinatal transmission of the Zika virus [65], and potential risk for transfusion related transmission of Zika virus has also been documented [66]. In addition, Foy et al. [67] has reported sexual transmission of the Zika virus to the wife of a patient who got infected in Senegal after his return to the US. The transmission was reasoned to be via semen but the semen was not tested. In this regard, it is important to note that Flaviviruses have been detected in urine samples of individuals infected with the West Nile virus with suggestive evidence that the virus was in fact replicating in these patients and being shed and thus thought to be infectious [68]. Similarly dengue virus RNA and non-structural protein of the dengue virus (NS1) has also been reported in urine samples of infected patients, but in vitro culture of the urine sample failed to show any evidence of replication competent virus. Finally, yellow fever virus RNA was shown to be present in the urine of a vaccinated individual [69]. More recently a case of sexual transmission of the Zika virus in Texas has been reported raising considerable concern among the public health officials in the state and at the CDC, Atlanta, GA. This was based on the isolation of Zika virus in the blood of a non-traveler who had sexual contact with a partner who had recently returned from Venezuela.

As with most infectious diseases, once the etiological agent has been identified in a given region, the agencies of the government such as the FDA and the CDC rapidly step in to include a requirement for testing of blood, organ and tissue donors for the suspected agent within the region. This was certainly the case with the discovery of cases of the West Nile virus in patients that had received blood transfusions from a specific blood center in New York during 2002. The CDC rapidly required a study of the blood donors involved and upon finding positive links between blood donors and transmission of WNV, have implemented PCR based tests to keep the blood supply safe [70]. There have been a series of studies conducted by blood donor centers including the Red Cross to determine the potential of setting up screening assays for viruses such as dengue, particularly in geographic areas that are eyed with a high degree of suspicion for the presence of dengue, such as Puerto Rico. These studies continue to this date and should result in critical recommendations (Table 2).

There are now a series of guidelines issued by the FDA that requires blood banks to follow strict rules for declaring a blood sample safe for transfusion. Examples of studies of screening of blood donors for Zika virus include the detection by PCR based assays of 42 positive samples out of a total of 1505 asymptomatic blood donors in French Polynesia [66]. Other reports of non-arthropod transmission have surfaced as well, in the form of contaminated semen in a patient in Tahiti [71], and one case in Colorado [67,72], or through blood transfusions in French Polynesia [66].

5.4. Vertical transmission of the Zika virus

Due to the potential link between microcephaly and Zika virus in pregnant women, the ability of Zika virus to be transmitted via the perinatal route is of significant interest and critical concern. A study in 2014 evaluated Zika virus in the serum of mother and newborns during delivery. Two such cases from French Polynesia were studied, and it was found that Zika virus RNA could be detected in the serum of the mother up to 5 days post-partum and in the newborn up to 6 days. Zika virus was also detected in the breast milk of the mothers. This finding needs to be confirmed in larger studies, but opens the possibility of transmission via the perinatal or post-delivery through nursing [65].

Overall though, with millions of cases of dengue and West Nile virus infections and other arthropod borne illnesses, there is very limited evidence for sexual transmission of arboviruses and thus it seems unusual to observe sexual transmission of the Zika virus. If borne out via larger studies, it would indicate that either the levels of viremia are significantly higher for Zika virus than the other arboviruses so that it can spill over to many body fluids including semen or the target cell population(s) of the Zika virus is distinct from the other arboviruses. The precise cell lineages targeted by arboviruses in vivo has been a subject of considerable debate over the last four decades. Further studies are clearly required to address these issues.

6. Clinical manifestation of Zika virus disease

Most infections caused by the Zika virus are asymptomatic (approximately 80%). The most common symptoms are fever, rash, arthralgias and non-purulent conjunctivitis. In 2007, a Zika outbreak in Yap Island in the Federated States of Micronesia, myalgia, edema and vomiting were also reported [17]. The incubation period of Zika virus is unknown but if similar to other flavivirus infections, is estimated to be between 3 and 7 days. Dengue and Chikungunya often have a similar but not identical presentation, and co-infection with these viruses has also been described in at least two case reports [73,74].

While there are similarities between dengue and Zika virus infection, there are some notable differences [75]. First of all, dengue infection is caused by four different serological types of dengue virus termed DEN 1, 2, 3 and 4. Infection with one type leads to life long immunity against the same type. However, exposure of the individual to a second type of dengue virus, in a small number of cases leads to a very severe form of illness resulting in shock and hemorrhage termed dengue shock syndrome and dengue hemorrhagic fever. This has not so far been reported for the Zika virus infections. The fever in dengue virus tends to be higher (>40 °C versus <38.5 °C). Nausea and vomiting can be a significant symptom in dengue virus. Headaches, retro-orbital pain and joint symptoms may occur in both infections. As indicated, in most cases, particularly in endemic countries, dengue infection is benign and limited to fever during the acute phase followed by a gradual return to normal. In the few cases with exposure to a

Table 2
Public health measures taken since the spread of the Zika virus.

Content of release	Country/Agency or release	Type of release	Content	Reference	Comments
Take precautions against mosquito bites	WHO			[30]	
Postpone pregnancy	Jamaica, El Salvador, Brazil	Advisory	Avoid pregnancy for 6–12 months, or up to 2 years	[30,131]	
Pregnant women should postpone travel to countries with Zika virus infection	United States, United Kingdom	Advisory	Travel advisory to Brazil and other areas	[30,132]	

secondary serological type of dengue, there are three distinct phases of disease: an acute febrile phase, a critical (plasma leak) phase where hematologic abnormalities, shock and death can occur, and a recovery phase. In Zika infections, the course is about 2–7 days and it is self-limiting. Secondary dengue infection often requires hospitalization and 2.5% of infected individuals will develop a lethal illness, but in Zika virus infections, most cases are managed on an outpatient basis. Non-steroidal anti-inflammatory drugs (NSAIDS) can increase risk of bleeding in dengue, but are acceptable to use in Zika virus infection as long as dengue has been ruled out. Long term sequellae may persist in dengue infection for up to 2 years, while none have been associated with Zika virus infection in the primary host (i.e. not including fetal losses and complications in offspring). There may be an association with Guillain-Barre syndrome in Zika infection [76]; this association has also been observed in dengue infection [77]. Laboratory findings in Zika virus infection include leukopenia, thrombocytopenia, elevated serum lactate dehydrogenase, gamma-glutamyl transferase and increased levels of inflammatory markers such as C-reactive protein, fibrinogen and ferritin [3,44].

6.1. Pregnancy, microcephaly and the Zika virus

The detection of Zika virus is one of the keys to formally define a definite link between Zika virus infection and microcephaly. Thus, previously serological tests have been performed for the detection of antibodies. However, it is generally known that there is considerable cross reactivity between the various flaviviruses and thus the specificity of the test becomes an issue. There have also been plaque reduction neutralization (PRNT) tests performed but the problem with such assays is that the presence of antibodies in the newborn could be due to passively transferred neutralizing antibodies from the mother, making it difficult to distinguish infection of the baby/fetus. The more recently developed PCR assay can obviously be performed and yield valuable information. However, if the fetus was infected in utero with neurological sequellae including microcephaly, the period of viremia may have passed and thus negative findings may not be informative. Thus, there are clear challenges. These challenges are highlighted by the findings of the recent outbreak in Brazil. The latest data from the Ministry of Health, Brazil, shows that there were 4180 reported cases of microcephaly associated with Zika virus infection. Of these 4180 cases, 732 (17.5%) have been investigated and classified. Of the 732 classified cases 270 were confirmed to have neurological manifestations but only six of these 270 cases were found to be positive for Zika virus. These findings have to be interpreted with caution as outlined above. There have been no reported cases of microcephaly from Southeast Asia (could be due to lack of clinical awareness) but the outbreak in French Polynesia in 2013 and earlier in Brazil both did report associations of Zika virus infections with neurological complications such as Guillain Barre syndrome (GBS). It is clear therefore considerable challenges lie ahead in establishing a cause and effect and the mechanism of Zika virus infection and microcephaly. A case control study would be one answer.

7. GBS and the Zika virus

The National IHR Focal Point of El Salvador reported a spike in the number of cases of GBS between the 1st of December 2015 and the 6th of January 2016. Normally, there are an average of 169 cases per annum in El Salvador, but in this short period there were 46 cases and 2 deaths reported. Zika-virus associated GBS, as stated above, has also been reported in French Polynesia [78]. The first case observed in December 2013 was published in March 2014 [76]. Subsequently, additional cases of GBS were identified, and the incidence was 20-fold higher than expected during the time coinciding with the Zika virus epidemic in French Polynesia [79]. GBS has been of significant interest to immunologists for decades because of the potential for disease induction via molecular mimicry; molecular mimicry has been a theme for induction of other autoimmune diseases as well [80–82].

8. Diagnosis of Zika virus infection

The diagnosis of Zika virus infections has been a challenge. Murine monoclonal antibodies which are specific to the viral envelope glycoprotein and NS1 glycoprotein from West Nile virus and yellow fever virus were tested against the C1008 cell line experimentally infected with the Zika and Langat viruses. The Mab541 and Mab109 clones against the viral envelope glycoprotein and NS1 glycoprotein, respectively, were shown to stain the nucleus of Zika virus infected cells and the nucleoli of Langat virus infected cells, respectively but not the cytoplasm [83]. These findings first of all highlight the problems of cross reactivity between the various flaviviruses and secondly, that nuclear and nucleoli localization are difficult to interpret. Early development of testing for flavivirus infection involved the use of solid-phase immunosorbent techniques and hemagglutination-inhibition assays to detect the IgM isotype against specific viruses, especially during primary acute infection [84]. There have also been plaque reduction neutralization assays (PRNT) performed. However, much like the problems with the diagnosis of other flaviviruses such as dengue and yellow fever, the issue of specificity and cross reactivity remain and need to be addressed. Besides these serological assays, the assay of choice at present is the Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). False positives due to cross-reactivity represent a major challenge. The presence of nucleic acids in body fluids may not be persistent enough to accurately diagnose patients with active infection. There are no commercially available tests, and Zika testing is currently only performed at the CDC laboratory and at some state and regional health departments [85].

8.1. RT-PCR

The entire Zika ss(+) RNA genome consists of 10,794 nucleotides. Full length genomic sequencing of the Zika virus was accomplished in 2006 [86]. RT-PCR methods for the rapid detection and identification of flaviviruses, including Zika virus, were established as early as 1994 [87]. Original primers were derived from

nucleotide sequences from the NS5 gene or from the 3'non-coding region. In 2008, an RT-PCR protocol utilizing sequences encoding the envelope protein region was developed. The detection limit was 7.7 pfu/reaction and the methodology was determined to be 100% sensitive in serum and specific by its failure to amplify genomes of 19 other flaviviruses [88]. In subsequent studies, polymerase chain reaction assays for Zika virus have mostly targeted the NS5 gene. The NS5 gene is chosen for primer development because this region displays a high degree of conservation among flaviviruses, while envelope protein genes may vary within a single flavivirus and thus present false negative data [89]. In a separate study, real time reverse transcriptase PCR (rRT-PCR) offers a quick and quantitative method to detect and evaluate the presence of Zika virus in mosquitoes. A method utilizing the NS5 protein coding regions was found to show high sensitivity for 37 Zika virus strains and specificity against other flavivirus strains. At the time of the study, this methodology had been tested on field mosquitoes and not yet applied for clinical diagnostic usage [90].

It is currently recommended that RT-PCR testing be done within the first 6 days of the onset of illness. It is also believed that in the early phases of the disease, the virus is present in its highest concentrations in the saliva, but that presence can be more persistent in the urine [91], thus testing should be done from one of these two specimens. In a study of 855 patients presenting with symptoms of Zika virus infection, investigators in French Polynesia analyzed 1067 samples from saliva, blood or both sources. In 182 patients from whom both urine and saliva samples were available, there were 35 that were saliva positive, blood negative and 16 of which were saliva negative, blood positive. The timing of obtaining the samples revealed no pattern with regard to saliva versus urine positivity [92]. A separate study found the presence of Zika virus in the urine of 6 patients greater than 10 days after onset of symptoms [91].

8.2. Enzyme linked immunosorbent assay (ELISA)

IgG and IgM titers to Zika virus can be obtained. False positive rates are higher in patients who live in countries in which flavivirus such as dengue is prevalent denoting exposure to a flavivirus. Plaque reduction neutralization testing (PRNT) is useful to differentiate between cross-reacting antibodies. One study from investigators in French Polynesia and France investigated the prevalence of seropositivity to various related arboviruses between 2011 and 2013. Overall, samples from 593 donors were obtained and were analyzed for IgG antibodies against Zika, Japanese encephalitis virus (JEV), West Nile virus (WNV), and four dengue viruses. Recombinant antigens composed of domain III of the envelope glycoprotein was used in these ELISA tests. Overall seropositivity rates were 0.8% for the Zika virus, 1.3% for JEV, 1.5% for WNV and 80.3% for at least one of the four dengue virus serotypes [93].

9. Pathogenesis of Zika virus infections

The pathogenic mechanisms that lead to potential fetal abnormalities by Zika virus infections is unknown. It has been postulated that Zika related arboviruses such as the dengue virus or the Chikungunya virus may act through the induction of an autophagic response in infected cells [52]. Autophagosomes were shown to contain poliovirus particles in patients infected by the polio virus. The mechanisms by which viruses use autophagy to infect cells varies amongst viruses. For example, the dengue virus is able to reorganize membranes to form autophagosomes which can fuse with endosomes to form amphisomes. The dengue virus then is able to replicate inside these double membrane vesicles that are

induced by the virus. A similar event is thought to occur with Zika viruses, but this has not been well characterized [94]. Flaviviruses including the Zika virus have been found to activate the unfolded protein response (UPR), which may play a role in autophagy which is linked to alterations in the function or degradation of endoplasmic reticulum architecture or composition [95].

To further characterize the role of Zika virus in microcephaly, it has been shown that microcephaly may be related to the abnormal function of centrosomes. Centrosomes are organelles that serve as the primary microtubule organizing centers of an animal cell. They have a role in mitosis and vesicle migration, polarization and trafficking. It has been demonstrated that increased centrosome number has been associated with microcephaly. It is not known if the Zika virus plays a direct role in centrosome development, but certain proteins have roles in both autophagy and centrosome development [96]. An increase in centrosome number can cause a delay in mitosis and an increase in apoptosis. This can lead to abnormalities in neural cell development and a consequence of this may be a reduction in brain matter formation or microcephaly [97].

Animal studies have shown that Zika virus leads to enlargement of astroglial cells and destruction of pyriform cells of Ammon's horn in newborn and 5-week old mice [98]. The virus is able to replicate in neuronal cells and is found within the endoplasmic reticulum. The significance of this finding on Zika virus associated microcephaly in human newborns remains unknown. The early studies on the effect of Zika virus in mice also demonstrated their presence in the central nervous system, but not in other tissues at the onset of illness. Intracerebral inoculation of guinea pigs, rabbits, and cotton-rats failed to induce clinical infection. Physical signs of infection (pyrexia) was inducible in Rhesus monkeys following subcutaneous inoculation with Zika virus [4,99].

9.1. Cytokine profiles in Zika infection

An analysis of cytokine levels was performed on plasma samples from a very small set of patients with active and convalescent Zika infection from Southeast Asia, Brazil or Polynesia. The subjects had a history of travel to Thailand, Tahiti, Malaysia or Brazil. Levels of pro-inflammatory cytokines, IL-1β and IL6 were elevated in samples from the acute phase, as were levels of IL-2, IL-4, IL-9, IL-10, IL-13 and IL-17. Increases in the levels of most of these cytokines persisted into the recovery phase, but granulocyte-macrophage colony stimulating factor (GM-CSF) and fibroblast growth factor (FGF), and IL-8 were also elevated during this recovery phase. Interferon-γ levels increased from the acute to the recovery phase, although the difference was not statistically significant. These results clearly need to be confirmed and expanded on a larger scale [100].

9.2. The role of skin in infection

The majority of Zika virus infection is transmitted when mosquitoes bite the skin of humans. The role of the skin in the pathogenesis of infection carries potential significance as an entry point into the body. Adhesion factors including AXL, Tyro3, and DC-SIGN were found to facilitate Zika virus entry into human skin cells. The phosphatidylserine receptor AXL was in particular found to be an entry receptor for the Zika virus. Dermal fibroblasts showed Zika permissiveness using specific RNA silencing and a neutralizing antibody. Transcription factors involved in the synthesis of the innate immune response molecules RIG-I, MDA5 and Toll like receptor (TLR)-3 were each enhanced following Zika virus infection. The fact that Axl tyrosine kinase is expressed by glioma cells provides a clue for its role in the pathology characterized to be associated with Zika virus infection. More recently, a potent inhibitor

for Axl (BMS-777607) has been identified [101], making this an interesting candidate for the study of therapeutic options. Interferon-stimulated genes were also enhanced. Autophagosomes induced by Zika infection of human skin fibroblasts was associated with increased viral replication using the autophagy inducer, Torin 1 and the autophagy inhibitor 3-methyladenine [94].

10. Prevention and treatment of Zika virus infections

10.1. Control measures

10.1.1. Insect bite precautions

Those who live in areas where *Aedes spp* mosquitoes exist should implement control measures such as the use of insect repellents such as DEET, use of mosquito nets at night and wearing light colored clothing and staying covered. The use of air-conditioning is also potentially effective in reducing risk for mosquito bites.

10.1.2. Vector source control

Vector source control measures include removing stagnant water and plants that contain water that are breeding grounds for mosquitoes, spraying of areas where larvae are detected and removal of yard debris [102]. In particular, automobile tires that tend to accumulate stagnant water and are dark seem to be a preferred breeding ground for mosquitoes, and should be removed. Climate changes should also be taken into consideration when discussing vector abundance issues. Global warming is a factor that drives vector abundance, as may changes in humidity or rainfall. In past studies of arbovirus circulation dynamics, rainfall negatively predicted dengue virus isolation, but positively predicted Zika virus isolation. Temperature had opposite effects on dengue versus yellow fever virus isolates. This is an illustration that while we can use other arbovirus in predictive models of Zika global spread, there are subtle nuances that may have to be considered for each viral species [103].

Education of the public regarding both insect bite precautions and mosquito management is important as a control measure. However, there are significant challenges in implementing comprehensive control measures because of the need for access to private property. Engaging homeowners to contribute to reducing container habitats for mosquitoes on their own property plays a significant role in the success of these control measures [104].

10.2. Public health measures

10.2.1. Travel precautions

Due to the pandemic nature of the current Zika disease phenomenon, governments, including those of the United States and the United Kingdom [105], have advised their pregnant citizens to avoid travel to countries with documented or suspected Zika virus epidemics.

10.2.2. Global government and regulatory reports and recommendations regarding the Zika virus − a timeline

Since the recognition of brain defects in three offspring of pregnant women inflicted by the Zika virus, a series of ongoing reports and recommendations have been proposed by various governments. On November 27th, 2015, the World Health Organization (WHO) reported 739 cases of microcephaly in Brazil. On November 30th, 2015, PAHO reported 1248 cases in microcephaly in Brazil.[1] On December 1st, the Pan American Health Organization (PAHO) updated its guidelines.

On December 15th, the WHO reported that on December 8th, 2015, the ministry of health in Brazil provided the PAHO and WHO with an update on the increased cases of microcephaly in Brazil.[2] By January 2nd, 2016, the number of cases of microcephaly in newborns in Brazil had risen to 3174.[3] On Friday, January 15th, 2016, the CDC issued a travel alert (Level 2 − practice enhanced precautions) advising pregnant women to postpone travel to Brazil and other countries due to reports of microcephaly and poor pregnancy outcomes. There are 3 levels of travel advisory, Level 1 recommends practice usual precautions and level 3 recommends the avoidance of non-essential travel.[4] On January 19th, 2016, the CDC issued interim guidelines for pregnant females which included recommendations directed at the travel and screening of pregnant women to countries where Zika virus infection has been identified. The guidelines outlined symptoms such as fever, rash, joint pain and conjunctivitis that occur within 2 weeks of travel should lead to an evaluation of the fetus by ultrasound to identify the presence or absence of fetal microcephaly or other abnormalities such as intracranial calcifications [106]. The guidelines include an algorithm for the management of these cases. Updated guidelines are expected.[5] On January 22nd, 2016, El Salvador issues a recommendation for women of childbearing age to delay pregnancy until 2018. This came after the release of a report of 492 cases found in El Salvador. The impact of this on population demographics and social structure can only be imagined.[6]

Colombia has also issued warnings for women of child bearing age to not get pregnant until more information is known about spread and control.[7]

The CDC issued interim guidelines for testing of infants with congenital Zika virus infection. These measures include fetal ultrasound review and maternal testing for Zika virus infection. Zika virus testing is recommended for "1) infants with microcephaly or intracranial calcifications born to women who traveled to or reside in an area with Zika virus transmission while pregnant or 2) infants born to mothers with positive or inconclusive test results for Zika virus infection" [85]. The CDC recommends additional health evaluation for infants with laboratory evidence of a possible congenital Zika virus infection. More comprehensive guidelines are available at the CDC website and in CDC releases. On 1st February, 2016, the World Health Organization declared Zika virus a Global Health Emergency.[8] On 3rd February, 2016 in the United States, Florida declares a health emergency after the identification of 5 patients with Zika virus infection On 5th February, 2016, Colombia's National Health Institute (INS) reported 3 deaths resulting from presumed Zika virus-associated Guillain-Barre syndrome.[9] On 5th February, 2016, the WHO declared 33 countries with autochthonous Zika virus infection and an additional 6 countries with local transmission. A total of 7 countries have now reported an increased incidence of microcephaly and/or Guillain-Barre syndrome.[10]

Due to the rapid developments in the global Zika virus pandemic, new guidelines and recommendations are issued almost

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[1] http://www.paho.org/hq/index.php?option=com_content&view=article&id=11585&Itemid=41688&lang=en.

[2] http://www.who.int/csr/don/15-december-2015-microcephaly-brazil/en/.

[3] http://www.who.int/csr/don/8-january-2016-brazil-microcephaly/en/.

[4] http://www.cdc.gov/media/releases/2016/s0315-zika-virus-travel.html.

[5] http://www.cdc.gov/mmwr/volumes/65/wr/mm6502e1.htm.

[6] http://www.independent.co.uk/news/world/americas/zika-virus-el-salvador-tells-women-not-to-get-pregnant-until-2018-to-avoid-birth-defects-a6826421.html.

[7] http://www.bbc.com/news/world-latin-america-35388842.

[8] http://terrance.who.int/mediacentre/presser/WHO-RUSH_Zika_virus_Emergency_committee_presser_01FEB2016.mp3?ua=1.

[9] http://news.yahoo.com/un-prods-latin-america-abortion-zika-spreads-185202353.html.

[10] http://www.who.int/emergencies/zika-virus/situation-report/en/.

weekly, if not daily. The reader should be cognizant that the recommendations provided in the paragraphs above are only valid as of the date of submission of this manuscript. Updated recommendations are available from well known resources such as the WHO and the CDC, as well as local or regional health departments.

10.3. Pharmacological intervention

10.3.1. The search for a vaccine

Previous vaccination against the yellow fever virus was not sufficient to protect laboratory workers against a Zika virus infection in a case report in 1973 [107]. At the present time, no vaccine for the Zika virus has been developed. There are plans, however, both by the NIAID, NIH and the Brazilian government to produce an effective vaccine but logistics dictate that it will take up to 10 years to have such a vaccine ready for use.

10.3.2. Using genetic modifications to reduce mosquito populations

In 1991, a study was published related to the genetic selection of mosquito strains that are resistant to flavivirus infection [108]. This study specifically addressed the common yellow fever and the Zika virus mosquito, *A. aegypti*. Identification of resistant versus susceptible phenotypes of the mosquito can lead to the isolation of genetic differences. The artificial introduction of strains that are not only resistant to infection but also do not transmit the virus into mosquito breeding areas is a potential method to mitigate the spread of infection. While this is only in concept phase, genetic modification of wild type *Aedes spp*. may yet prove to be an effective way to help curb the global spread of Zika virus.

Another strategy that has been proposed is to introduce genetically modified male mosquitoes which do not have the ability to produce offspring into mosquito infested areas. The idea is that these mosquitoes will compete with indigent mosquitoes and the resultant offspring will not be viable, thereby reducing mosquito populations.

10.3.3. Medications

The use of amotosalen combined with ultraviolet light A has been shown to be able to inactivate Zika virus in vitro. Prior to inactivation, Zika titers and RNA loads from the plasma spiked with Zika virus were 6.57 log TCID50/ml and 10.25 log copies/ml respectively. After inactivation, the RNA load was 9.51 log copies/ml, but the inactivated plasma was unable to render cell cultures infective. In addition, the inactivation procedure was able to decrease the production of replicative virus after a single passage, providing some guidance to be utilized for the discovery of antiviral drugs effective against Zika virus [109].

11. Challenges in the control of the Zika virus pandemic

11.1. Eliminate or minimize circulation of virus

One of the challenges of controlling the spread of Zika virus is to control the mosquito population. Methods to be utilized have to be environmentally safe but retain effectiveness. These include the use of insecticides in spray form and its use in areas considered optimal for breeding of mosquitoes such as stagnant water, ponds, and areas where rain water collects etc. The use of insect repellents such as DEET and the education of residents plays a major role in curbing mosquito populations. There are also efforts being made to introduce genetically resistant strains of mosquitoes. Ironically due to the mild nature of most Zika virus infections, the infection often goes undetected and those capable of spreading the disease may not appear clinically ill, and would be difficult to identify, which provides a challenge.

11.2. Diagnostic challenges

The only reliable diagnostic assay for Zika virus that has high specificity and sensitivity is the CDC-Atlanta, USA based RT-PCR assay. The limitation of this assay is that it is obviously only useful for detection of acute illness when viremia is present. Unfortunately, serologic testing for Zika IgM and IgG is confounded by the high cross-reactivity with other flaviviruses including dengue virus and Chikungunya. Plaque reduction neutralization tests often are unable to resolve this cross reactivity.

11.3. The 2016 Rio Olympic games

Timing of the appearance of Zika virus infections in Brazil coincided with a major global event that attracted millions of tourists and increased travel to the host country, as the FIFA World Cup [110]. One hypothesis that has been proposed is that it was during the World Cup that Zika virus was brought from Africa and the Pacific Islands into Brazil [37]. This view has provided considerable concern to the organizers of the Olympic Games scheduled to be held in Rio de Janiero in 2016. Officially, there have not been any discussions to move or cancel the Rio Olympics, although this has been suggested in the press, and at least one Olympic athlete has threatened to boycott the Games.

12. Conclusions

The current Zika pandemic is a rapidly changing phenomenon that is affecting more and more countries. Previously associated with only mild clinical, subclinical or even asymptomatic disease, the virus is now associated with multiple cases of neurological damage in neonates born to Zika infected mothers, as well as cases of GBS in areas of Zika virus outbreaks. Previously believed to be spread only by mosquitoes, thus the classification as an arbovirus, it is now known to be potentially transmissible by sexual contact or blood transfusion. The fact that it is mostly a mild disease may actually hinder control measures, as infected but asymptomatic individuals may be free to spread the disease to others without being identified.

The pathogenesis of Zika virus infection, in particular the more severe complications, is unknown. Several cellular mechanisms may be in play, including autophagy, cytokine balance and molecules such as adhesion receptors that facilitate viral entry. Interferon inhibitors have been effective against Zika virus infections in vitro, providing one alternative [94]. Since the skin is the entry point for Zika virus infection, immune dynamics in the skin clearly plays a role in viral entry and infection. As the Zika virus spreads, the need for a vaccine becomes increasingly urgent. A better understanding of the pathogenesis will facilitate development of an effective and safe vaccine. Other control measures must be implemented in the meantime, including vector control and prevention of mosquito bites. These control measures are outlined in Table 3.

Challenges for Zika virus research.

1. To clearly establish whether a link exists between the neurologic sequelae and Zika virus infection, and if so, to determine the risk factors (i.e., genetics and/or environmental) associated with such a complication.
2. To define assays that can identify Zika virus infection of the fetus during pregnancy.
3. Studies need to be performed to determine the molecular signatures of the virus that are associated with virulence and/or a link/mechanism with the neurologic complications and the association with GBS.
4. Determine if the Zika virus isolated from its natural host in Africa is different from the isolates from the current Zika virus.

Table 3
Management and Control measures for the Zika virus vector.

Prevention of mosquito bites
Use of house screens, window screens and mosquito nets
Use of air-conditioning
Wear light colored clothing, keep skin covered with long sleeve garments and long pants
Use insect repellent when appropriate (may not be possible for children under 3 months of age)
Source and spread control measures
Removal of stagnant water
Removal of yard debris and mosquito breeding sites
Spraying of sites where larvae present
Release of insects carrying dominant lethal genes (RIDL) [133]
Inhibiting replication via Wolbachia [133]
Disease management
Establish surveillance systems to detect the prevalence of the infection as well as potential complications [134]
Engage social media to increase awareness
Public education regarding mosquito management [104]
Accelerate research and development [134]
Regulatory reports and recommendations
Public health statements
Travel advisories

5. Define the vertebrate host range of Zika virus in more detail to limit its spread.
6. Define methods to prevent spread of the virus via blood transfusions or sexual contact.
7. Define the precise cell lineage that is targeted by the virus in vivo in humans and in its natural host.
8. Determine if differences in glycosylation patterns of the envelope of the virus are different between different isolates.
9. Establish a pro-active Global surveillance strategy to prevent or minimize the occurrence of an outbreak.
10. To define a more specific diagnostic test that does not require sophisticated methodologies.
11. Produce an effective vaccine against Zika (see Table 4).

Table 4
Challenges in the control of the Zika virus epidemic [135].

1. Lack of a specific diagnostic test
2. Lack of a vaccine
3. Rapid spread
4. Population demographic issues − overcrowding, poverty, poor hygiene
5. Evolution of the arboviruses
6. Increased freedom of travel
7. Mass gatherings and events (e.g. Olympics)
8. High rate of asymptomatic infected individuals (tends to mask potential carriers of the virus)

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