Chapter 18
Zika Virus

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Core Message Although Zika virus is generally considered a relatively benign flavivirus, it is hypothesized that the study of this virus is useful as an indicator of other more virulent viruses. The increased spread and prevalence of Zika virus thus may be indicative of similar changes in more virulent viruses. It is also hypothesized that Zika may mutate into a more virulent form than what has hitherto occurred.

1 Introduction

Among many public health alerts, the global spread of arboviruses is of concern and alarm. The hypothesis in this chapter is that the inclusion of Zika virus in arbovirus monitoring is a well-justified expense because its spread may be diagnostic for the spread of flaviviruses, its spread is largely unexplained, and the virus has the potential to mutate into strains that are more virulent. Moreover, such evolutionary studies are of importance in and of themselves for the same reasons.

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2 Epidemiology: Temporal and Geographical Associations

Zika virus is a flavivirus and is related to other arboviruses such as yellow fever virus, Japanese encephalitis virus, dengue virus, and West Nile virus [1]. The Eighth Report of the International Committee on Taxonomy of Viruses describes several neglected species of mosquito-borne flavivirus, some of which are without apparent pathogenicity in animals, but which are pathogenic in humans. This group includes Zika virus, lineage-2 West Nile virus, and Usutu virus [2].

In 1947, Zika virus was originally isolated from a febrile sentinel rhesus monkey and from a pool of *Aedes africanus* mosquitoes in the Zika forest in Uganda during a yellow fever study [3]. Zika virus was first detected in humans 5 years later in 1952 using neutralizing antibody testing in sera from East Africa [3–5]. Zika virus was first isolated from a human in Uganda [6].

In specimens from humans in Nigeria, from 1964 to 1970, there were 15 types of arboviruses among 171 isolations. The majority of isolations were from children below 4; however, isolations were made from all age groups. Zika virus was isolated at low frequency in comparison to yellow fever, Chikungunya, dengue types 1 and 3, and Tataguine (endemic in Ibadan). Additional viruses isolated were Bwamba, and Bunyamwera group viruses that were isolated from humans for the first time. Isolation rates varied from peaks in 1969 to lows in 1965 and 1967. Zika virus isolation rates also varied by season: peaks in rainy seasons (June to August) and lows in dry seasons (January to February). Zika virus was also detected in a study in Ibadan, Nigeria, in 1975 [5].

In 1954, Zika virus was detected during viral serological survey studies. In 38 localities in 6 states in India 15 arboviruses were studied including yellow fever, Bwamba fever, Bunyamwera Ilhéus Semliki Forest virus, St. Louis encephalitis virus, West Nile virus, dengue types 1 and 2, West Nile virus, Uganda S, Nitaya, Japanese B, Murray Valley virus, and Russian spring-summer encephalitis virus [7].

Between 1977 and 1978, in Malaysia and Indonesia, there were clusters of Zika virus infection towards the end of the rainy season when *Aedes aegypti* flourish. Thirty patients had serological tests for alpha and flavivirus infections including Zika—MR766, Japanese encephalitis—Nakayama, dengue type 2—New Guinea C, Tembusu—MM1775, and Murray Valley encephalitis—original [8].

Three strains of Zika virus were isolated as part of yellow fever studies in the Ivory Coast in 1999. Amaril, yellow fever, and dengue viruses were prevalent among vector and human populations in this study [9]. The first known isolation of yellow fever from *Aedes africanus* mosquitoes was in Africa, in Touba, the Ivory Coast.

In Sabah, Malaysia, Zika virus infection was shown in some of 60 semi-captive and 84 free-ranging orangutans (*Pongo pygmaeus pygmaeus*). Both groups showed evidence of exposure to 10 of 46 additional viruses including Japanese encephalitis virus [10].

This confirmed earlier studies in North Bornean forests where arbovirus transmission in wild orangutans was studied and included three virus families (Flaviviridae, Alphaviridae, and Bunyaviridae). Viruses detected included Zika, Japanese encephalitis, dengue 2, Langat, Sindbis, Tembusu, Batai, and Chikungunya viruses [11].

How far had the virus spread? Surprisingly, Zika virus spread further, beyond the confines of Africa and Asia as it was detected on Yap Island in Micronesia in 2007 in the southwestern Pacific Ocean [1]. In June 2007, Zika virus appeared in Southeast Asia and Federated States of Micronesia, in Yap state, more than 60 years since its detection in 1947 in Uganda [12]. The illness was characterized by conjunctivitis, arthralgia, and rash affecting 100 individuals whereas prior studies had detected only 14 infected individuals. No deaths, hospitalizations, or hemorrhagic manifestations were found. It was estimated that 74 % (95 % CI, 68–77 %) of residents of Yap, 3 years and older, were recently virus infected. The predominant mosquito species vector was *Aedes hensilli*. Public health officials and clinicians were alerted as Zika virus had expanded outside Africa and Asia. In this outbreak, no dengue virus or other arboviral RNAs were detected [13].

Zika virus is one of the arboviruses that have spread in Southeast Asia including dengue, Japanese encephalitis, and Kunjin as well as alpha viruses such as Chikungunya, Sindbis, and Getah. The increase and spread of these viruses has been overall exponential since 2009 and generally is linked to several complex factors (as discussed below) [12].

Zika virus infections were also detected among people in Nigeria, Uganda, Egypt, India, Pakistan, North Vietnam, Thailand, Malaysia, Indonesia, and the Philippines. Sentinel animal and mosquito studies also supported the endemic presence of Zika virus in Africa and Southeast Asia. In addition, Zika virus was detected north and west of the Wallace line (a biogeographical line situated between Borneo and Java demarcating a syzygy of species between Australia and South East Asia) [14].

Occupational infection of Zika virus was reported in a scientist who contracted the virus in the laboratory after having had yellow fever virus vaccination. This individual demonstrated an anamnestic response related to yellow fever virus that complicated virus identification. The problem was resolved after isolation of Zika virus during the acute phase of disease [15]. An Australian traveler upon returning from Indonesia had been infected with Zika virus [16]. Another publication reported that two American scientists contracted Zika virus when working in Senegal in 2008. One of them upon returning to the USA transmitted the arbovirus to his wife. Clinical and serologic evidence helped identify Zika virus. The route of transmission from the scientist to his wife was concluded to be sexual [17].

Table 18.1 provides a brief outline of the chronology and geography of Zika virus studies.

3 Vectors and Reservoirs

3.1 Mosquitoes

Aedes mosquitos play a significant role in Zika virus transmission. In 1972 in Sierra Leone during an entomological and serologic survey, sera from children up to 14 years were analyzed for 12 antigens from viruses including Zika, Chikungunya, West Nile, and yellow fever using HI and CF testing. The prevalence was much

Table 18.1 Chronology and geography of Zika virus studies

Study year	Study locations	References
1947	Zika forest, Uganda, Nigeria, and East Africa	[1, 3–5]
1954	India	[4]
1970	Nigeria	[5]
1978	Malaysia, Indonesia	[8]
1999	Ivory Coast	[9]
2001	Sabah, Malaysia	[10, 11]
2007	New World, Easter Islands, Nepal, Argentina, Hawaii, Scandinavia, Saudi Arabia	[18]
2007	Micronesia	[13]
2008	Southeast Asia, Australia	[19]
2009	Southeast Asia	[12]
2008–2011	Senegal, Nigeria, Uganda, Egypt, India, Pakistan, North Vietnam, Malaysia, Indonesia, the Philippines, Borneo/Java, Micronesia, USA	[1, 14, 17]
2012–2013	Indonesia, Singapore, Australia, Tahiti, Germany	[16, 20–24]

greater than the incidence for arboviruses but varied among different geographical sites. In most areas, Zika virus abounded and was active whereas, for example, Chikungunya virus was active in Northeast savannas and plateaus. The entomological survey indicated that pools of water and *Ae. aegypti* larvae were present in greater abundance in urban dwellings near mines than in rural areas. It was concluded that conditions were ripe for epidemic outbreaks within a few years [25].

There are potential additional mechanisms of spread of Zika virus because of its relatedness to other viruses. In Central and West Africa, arbovirus survival was studied during interepidemic periods. Several yellow fever strains were isolated from *Aedes africanus*, *Aedes furcifer-fylori*, *Aedes opol*, and *Aedes luteocephalus*. Savannas (including savannas without forest and differentiated savannas with forests) that have abundant *Isoberlinia doka* (a hardwood tree) were associated with sylvatic yellow fever circulation. The primary endemic areas of yellow fever include equatorial moist forests, termed the emergence zone. The sylvatic yellow fever circulation in this forest zone was concluded a major threat and source of yellow fever for humans via penetrating epizootics into the savannas. A case in point supporting this model was the 1978 Gambia outbreaks. Several additional observations further support this approach and model for the spread of such arboviruses. Transovarial transmission (TOT) was demonstrated for mosquitos including *Aedes aegypti* and *Aedes furcifer-fylori*, and explained an emergent zone for survival of virus in the dry season. The size of monkey populations appears to further influence the degree of virus propagation. In addition, tick eggs and adults were sources of yellow fever virus. This reservoir acts as a tributary adding to the vertebrate-mosquito cycle promoting arbovirus survival [26].

TOT of arboviruses is a serious concern and was demonstrated for Culex flavivirus (CxFV). CxFV was detected using reverse transcription-polymerase chain reaction

(RT-PCR) in *Culex pipiens* (L) mosquitos captured in the field. Their progeny were viral infected as well and viral RNA was detected in several progeny tissues (salivary glands, ovaries, testes, head, fat bodies, and midgut) [27]. Previously, TOT had been proposed as a mechanism contributing to the spread of Zika and yellow fever viruses in Uganda [28]. Thus, TOT should be studied in greater detail and therapies developed to combat the fail-safe mechanism that allows seasonal survival of arboviruses.

There was also an interesting dynamic of Zika virus spread by *Aedes* (*stegomyia*) *africanus* (*theobald*) arboreal mosquitoes in the Zika forest in Uganda. Between November 1961 and June 1963, on a 120-ft (36.5-m) tower, twelve Zika virus strains and one strain of a different Group B arbovirus were isolated. Pools of mosquitoes were used for the virus isolations. Serum antibodies from Zika virus forest small animals did not show any reactivity with Zika virus. It was stated that some other virus reservoir was responsible for the infected mosquitos and that convection currents above the forest canopy could spread virus-infected mosquitoes during the first few hours after sunset [29].

Yellow fever virus-resistant and susceptible phenotype inbred (isofemale) *Aedes aegypti* mosquito lines were produced. Resistance was due to a block in the virus life cycle that prevented virus passage beyond the mosquito midgut during its life cycle. In addition to yellow fever virus, other flaviviruses that were restricted included Zika, dengue 1–4, and Uganda S viruses. Further mosquito genetics studies indicated that the midgut resistance phenomenon is due to a group of genes that includes a major gene and several minor genes or several genes in a group that are linked [30].

A recent Chikungunya virus pandemic in the Singapore area prompted further study of mosquito vectors. More than 20 arboviruses are transmitted by sylvatic *Aedes albopictus* mosquitoes in and around Singapore. Further studies demonstrated that *Aedes albopictus* mosquitoes were capable of transmitting Zika virus. In Singapore and environs, the same *Aedes* mosquito vector is shared among dengue and Chikungunya viruses as well as Zika virus. Existing programs in Singapore to control dengue and Chikungunya viruses may help control Zika virus as well [20, 21, 31].

Several methods in use to control mosquito vectors that transmit arboviruses include insecticides, genetically modified sterile insects, and draining swamps [32]. A recent novel approach was described by Darbro et al. [33], which utilizes the fungus, *Beauveria bassiana*. In laboratory conditions, this fungus reduces *Aedes aegypti* longevity and fecundity, whereas egg batch size and viability were unaffected. In semi-field conditions in northern Queensland, Australia, mosquito survival was reduced in cages of various sizes and there was some reduction in blood feeding [33]. This approach requires some caveats as fungi can mutate, immune-compromised humans may be susceptible to fungal infections, and fungal infected mosquitos may develop resistance with unpredicted consequences. Identifying the effective molecules that affect the mosquito life cycle may be most specific and effective in applying this method of control towards arbovirus control.

3.2 Monkeys

In 1947 Zika virus was originally isolated from a febrile sentinel monkey in Uganda during a yellow fever study [3]. In Uganda, monkeys serve as two types of sylvan hosts for yellow fever (YF). (1) As an enzootic state in the Zika forest in Western Uganda (Bwamba County) and (2) as epizootics in central Uganda zone of forest savannas. However, an epizootic for Zika virus occurred in two episodes in the Zika forest near Entebbe: (1) in 1969, post-epizootic of 1962–1963, with consequent accumulation of nonimmune monkeys and (2) in 1970, when biting densities increased for *Aedes africanus*. Eighteen months after that, an intensive epizootic for YF developed. This contradicted the hypothesis that subsequent YF epizootics would be subdued by Zika virus infections in nature for red-tail monkeys. Two factors important for further study of flavivirus mosquito are transovarial and phlebotomine sand-fly transmission [28].

During the Zika forest yellow fever epizootic in 1972, several other arbovirus antibodies were discovered as well in monkeys near Entebbe, Uganda. The viruses in addition to yellow fever included Zika, West Nile (WN) O'nyong-nyong (ONN), Chikungunya (CHIK), and Wesselsbron (WESS). That these viruses are immunologically cross-reactive was known at the time of the study. In addition, it was found that although YF virus is deadly for humans it is mild in monkeys in their sylvan natural habitat [34, 35].

3.3 Additional Species

Many species have been under the radar with unsuspected potential flavivirus infections. However, several different species are implicated in their susceptibility to viruses related to Zika virus and function as potential reservoirs: West Nile virus—cat, dog, horse, alligator, deer, primate, rodent, rabbit, reptile, opossum, bird, and raccoon; Japanese encephalitis virus—bird, pig, cow, horse, monkey, and rodent; St. Louis encephalitis virus—bird, armadillo, rodent, opossum, raccoon, and squirrel; yellow fever virus—monkey, opossum, rodent, kinkajou (an arboreal raccoon-like mammal with a prehensile tail but NOT a primate), bat, hedgehog, wild dog, mongoose, wild bird, anteater, and squirrel; dengue virus—bat, chipmunk, rabbit, guinea pig, mouse, Yucatan miniature pig, and horse [36].

Recent work indicates that snakes are a reservoir for EEEV in North America. This adds to the list of animal reservoirs for EEEV and possibly related viruses and may have an impact on our understanding of additional reservoirs for flaviviruses and Toga viruses [37].

Thus, the jury is still out related to Zika virus reservoirs as well.

3.4 Cell Culture Susceptibility Profile

A study of several strains of dengue virus infectivity of cell cultures derived from various species demonstrated a wide ability of these strains to infect various cell types, although no correlation from in vitro and in vivo situations could be made. However, it was proposed that dengue virus strains might be more prevalent in bats than had been hitherto considered. The virus strains were DENV-1 Hawaii, DENV-2 New Guinea C, DENV-3H87, DENV-4H241, DENV-1 BC-89/94, DENV-2 BC-100/98, DENV-3 BC-14-97, DENV-1 WestPac-74, DENV-2s16803, DENV-3 CH5548904500, and DENV-4 341750. The species were free-tailed bat, chicken, cottontail rabbit, human, domestic cat, horse, grey fox, raccoon, North American mule deer, Virginia opossum, sheep, nine-banded armadillo, domestic pig, rhesus monkey, cow, domestic dog, and eastern woodchuck [38].

4 Conditions for Spread

4.1 Ecology and Geography

During the last 20 years, human actions have been pinpointed as profound influential variables in virology and vector-driven diseases of viral origin. This has become most evident in Oceania and in Southeast Asia. Viruses, their vectors, their geographical distributions, increased demographic and ecologic dysgenesis, and increased travel and trade are contributory factors. Barboza et al. [19] also review emergent viruses in the Pacific and Southeast Asia including Zika virus, dengue, Chikungunya, and Japanese encephalitis viruses. Likewise, the steady annual increase in Ross River and Barmah viruses in Australia, the Nipah virus deadly epidemics in Southeast Asia, and lyssavirus including Kunjin and Murray Valley viruses are examples of consequences of ecologic and geographic alterations [19].

Many flaviviruses cause diseases in humans, livestock, and wildlife. Vector-borne flaviviruses have spread globally at increased rate during the last two decades. This occurred outside the bounds of the traditional geographical ranges of these viruses. For example, there are increased cases of introduction of West Nile virus into the New World and Easter Islands in the Pacific; outbreaks of dengue in Nepal, Argentina, and Hawaii; Usutu virus into Europe; tick-borne encephalitis in Scandinavia; and tick-borne Alkhurma Kyasanur Forest disease virus in Saudi Arabia [18]. One may well ask whether these events are due to global warming. See below for a discussion in regard to the impact of global warming and viral spread.

4.2 Global Warming

Zika virus as an emergent virus is reflective of the emergence and spread of other viruses as mentioned. It is thus important to describe the global setting, as it exists for the spread of Zika and related viruses and this setting is global warming. The following is a brief description of the effects of global warming on the spread of infectious diseases.

The Copenhagen United Nations Climate Change Conference in 2009 produced important information related to global climate change. This conference took place to continue the work of the Kyoto protocol that went into effect in 2005 and expired in 2012. The Kyoto protocol, although ratified by 187 countries, was not ratified by the USA. Climate change is due to an imbalance of inbound vs. outbound terrestrial radiation energy. However, unfortunately, the conference ended without a resolution addressing global climate change and vector-borne and waterborne infectious diseases [39].

The spread of mosquito-borne viruses into geographical zones that have had temperate climates (e.g., Usutu virus in Central Europe) appears to be associated with global warming. Moreover, a rise in international trade and travel further facilitated permanent establishment of mosquito-borne viruses. This is occurring in industrialized countries worldwide and facilitates spread from competent mosquito vectors to less competent vectors [2].

4.3 Social Change and Urbanization

The main arbovirus vectors are *Aedes aegypti* and *Aedes albopictus* mosquitos. Their spread is due to human behaviors including the slave trade from the fifteenth to nineteenth centuries, economic enterprises and expansion, more recent globalization of trade and economics, urbanization of Latin America and Asia, increased concentrations of human populations, and concomitant sanitary issues that promote the spread of mosquito vectors. Arboviruses reflect this vector spread and across 100 countries, for example, there is a pandemic of 50 million dengue infections annually with spread continuing [40].

The term climate change is a euphemism for global warming. The damage that is occurring and projected to occur is actually due to the increased temperature. This process results in increased vector activity and disease transmission. For example, the Anopheles mosquito that transmits malaria needs temperatures of just 16 °C and above to complete its life cycle. As global warming increases, so do deleterious results increase as well, including vector-borne disease, diarrheal disease, malnutrition, and injury from natural disasters. A major consequence of these effects is an increase of premature deaths. Premature deaths and disability are measured in disability-adjusted life-years (DALYs) per million population as follows: Africa 3,071.5; Eastern Mediterranean 1,586.5; Latin America and Caribbean 188.5;

Southeast Asia 1,703.5; Western Pacific 111.4; developed countries 8.9; and global average 1,111.7 [39].

The golden toad (*Bufo periglenes*) and Monteverde harlequin frog (*Atelopus sp.*) became extinct 23 years ago in Costa Rica. In addition, it is estimated that 67 % of approximately 110 or so species of *Atelopus* that are endemic to the American tropics are also extinct. A pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*) is primarily to blame. This ecologic "writing on the wall" is due to global warming [41]. Thus, the spread of infectious disease can have profound and unanticipated effects on ecology, biology, and evolution.

The distribution, transmission, and abundance of vectors that bear and transmit diseases are being enhanced by global warming. Encephalitic viruses as well as dengue, malaria, and plague are increasing due to the infestation of such vectors and their cognate arthropods into geographical regions that were hitherto too cool for their presence. Likewise, south of the Southwest US-Mexican border, Mexican states show a 500-fold increase in dengue disease. West Nile virus has taken up residence in the USA near stagnant water, golf courses, waterways, swamps, and ponds. St. Louis and Equine encephalitis viruses are spreading as well. The increased spread of typhoid and cholera into Zimbabwe and Ethiopia also exemplifies and supports the contention of such deterioration—due to hygiene and water quality deterioration in conjunction with global warming [42].

The mean global temperature increased approximately by 1° centigrade during the last several hundred years. However, during the next 20 years it is anticipated to increase by 2–3° centigrade. Consequently, it is expected, for example, that the global malaria risk population will increase from 3 to 5 % and diarrheal diseases will increase by 10 %. The increased prevalence of malaria will be due to the vector-borne spread of this disease into geographical areas including the East African highlands where it has not yet been endemic [39]. Thus, warnings are dire and expectations grim.

5 Clinical

5.1 Clinical Findings and Neurological Disease

Zika virus is not as benign as it is sometimes considered to be and may be one of the most commonly reported human illnesses where it occurs. Most of the cases are described with relatively mild disease. Initial clinical descriptions indicate fever, headache, body pains, and rash as the manifestations of Zika virus infection. It can present with a syndrome reminiscent of influenza infection and thus may be underreported. Symptoms include lymphadenopathy, edema, retro-orbital pain, and diarrhea. In addition, common presentations accompanying the febrile illness include maculopapular rash, arthralgia, and conjunctivitis and are frequently confused with dengue virus infection that may also result in underreporting Zika virus infection.

Other symptoms range from fever and headache to fatigue, malaise, stomachache, dizziness, anorexia, hematospermia, prostatitis, dizziness, and lightheadedness [1, 5, 6, 8, 18, 43–45]. Although severe neurologic manifestations have not as yet been reported, misdiagnosis or underdiagnosis cannot be excluded.

In a mouse model, newborn and 5-week-old mice were inoculated intracerebrally with Zika virus. Astrocytes became enlarged and there was some destruction of pyriform cells of the hippocampus. Virions were produced within the endoplasmic reticulum in both neurons and astrocytes [46].

There are indications that the Zika-related viruses do indeed cause invasive neurological infections and disease including encephalitis due to Japanese encephalitis virus and encephalitis/meningitis due to West Nile virus and Chikungunya virus [47]. Whether Zika virus causes or has the ability to cause neurologic disease in humans is still unclear. More work needs to be done to investigate the effect of Zika and other related viruses on the central nervous system (CNS).

5.2 Diagnosis

There is still some difficulty in clinically diagnosing Zika virus infection because it is easily mistaken for other arbovirus infections including Chikungunya and dengue fever [44].

Differential diagnosis of Zika virus infection includes other arboviral diseases causing fever, headache, rash, and arthralgia; in addition tick-borne encephalitis (TBE) flavivirus infection causes severe hemorrhagic fevers, meningitis, and encephalitis. Molecular and immunological methods are important concerning the question of specificity of diagnosis. PCR and serologic studies have been used to make diagnosis of Zika virus infection. For example, in 2010, Zika virus infection of a child was confirmed in Cambodia using PCR in addition to immunological methods in specimens taken in the field. Furthermore, dengue, West Nile virus, and yellow fever virus infections were excluded [44]. Zika virus infection has similar manifestations to other arboviral infections. Clinicians should be aware of this and utilize additional confirmatory tests to make the diagnosis in patients who live in or have recently visited Zika virus endemic areas. See also Sect. 5, below.

6 Molecular biology

6.1 Viral Molecular Pathogenesis

Complete genome sequences were produced for the first time for Zika virus (as well as Bagaza and Kedougou viruses). Open reading frames (ORFs) were characterized including protein cleavage sites, gene sizes, distribution of cysteine residues, potential glycosylation sites, and unique motifs. Genetic relatedness was studied

using alignment procedures for full-length ORFs of the viruses vs. selected reference viruses and other African flaviviruses. Specific conserved organizational patterns were found for 3′-terminal noncoding regions that correlated with virus grouping. Zika virus is representative of the Spondweni virus group. Kedougou virus is only slightly more distantly related to Zika and Spondweni. Bagaza virus is interrelated to West Nile virus in several segments of its genome and representative of another distinct group (Ntaya).

Of the sequenced mosquito-borne flaviviruses, Zika virus has a 3′-noncoding region (NCR) with conserved sequences (CS) organized in a CS1-CS2-CS3 pattern. This is new for the Spondweni virus group. Kedougou and Bagaza viruses have also been sequenced, with GenBank accession numbers AY632540 and AY632545, respectively. Based on the partial sequence of the NS5 protein, Bagaza virus is 98 % identical to Israel turkey virus (ITV) GenBank EU303198; both viruses show a high degree of immune (neutralization) similarity [18].

Highly conserved universal primers from sequences in the 3′-coding region of the NS5 gene were used in reverse transcription/polymerase chain reaction (RT/PCR) for the rapid detection of mosquito-borne flaviviruses (Zika, West Nile, Japanese encephalitis, yellow fever, dengue 1, dengue 2, dengue 3, and dengue 4 viruses). This region of the NS5 gene showed less amino acid identity (20–36 %) across viruses than sequences in the C-terminus of the NS5 gene (56–76 % amino acid identity). In addition, recombinant plasmids containing flavivirus cDNA (derived from RNA from experimentally infected mosquitoes) were used in dot-blot membrane and digoxigenin detection methods. Zika virus classification was confirmed using serology [48].

Zika virus is a single-stranded positive-sense RNA virus and is approximately 11,000 nucleotides in length. Its relationship to Spondweni, Kedougou, and Bagaza viruses is illustrated in the phylogenetic tree, Fig. 18.1. There are 5′- and 3′-untranslated regions on either side of one ORF encoding a polyprotein in the genome. Phylogenetic analysis places Zika virus in three groups, West African (three strains analyzed), East African (two strains analyzed), and Asian (three strains analyzed) [14, 18]. A PCR amplicon 100 bp fragment from a 2010 Cambodian child patient had 100 % identity to accession number EU545988 Zika virus NS5 gene. The phylogenetic position of the patient's Zika virus was not stated [44].

Severe hemorrhagic fevers, meningitis, and encephalitis can be caused by tick-borne encephalitis (TBE) flaviviruses. The viruses that cause these diseases are pathogenic with a high mortality rate and are pathogenic due to inhibition of the interferon (IFN) response in the infected individual. Langat virus (LGTV) is a member of this group and is highly sensitive to the effects of IFN. A luciferase reporter gene driven by each of IFN-α/β and -γ-responsive promoters was inhibited by this virus in infected cells via the IFN-mediated JAK-STAT (Janus kinase-signal transducer and activator of transcription) signal transduction pathway. Several mechanisms of inhibition were IFN-α signaling blocks of Jak1 and Tyk2 Janus kinases and IFN-γ stimulation-associated Jak1 phosphorylation. Of all viral nonstructural (NS) proteins, NS5 alone inhibited IFN-γ-induced STAT1 phosphorylation. Moreover, NS5 forms complexes with IFN-α/β and -γ receptors. These observations were

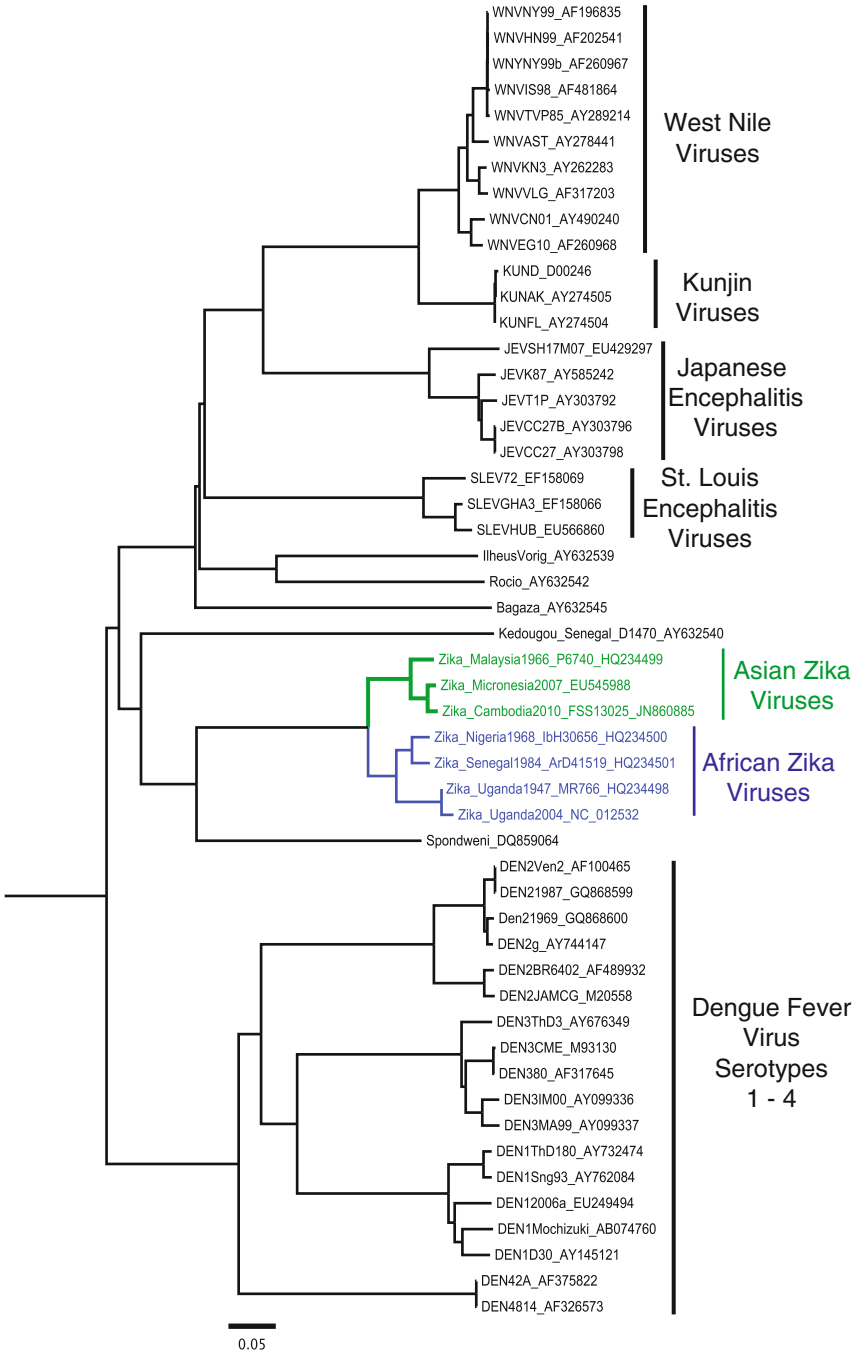

Fig. 18.1 Phylogenetic tree of Zika virus with related flaviviruses. Sequences were obtained from GenBank. Complete viral genome sequences were aligned with MAFFT, and a maximum likelihood phylogenetic tree was constructed from the DNA alignment using DNAML [49]

confirmed in LGTV-infected human monocyte-derived dendritic cells [50]. It should be noted that LGTV and Zika virus share additional molecular novelties in terms of their replication pathways.

6.2 Life-Cycle Observations

Unexpectedly, the detection of virus-specific antigen in the nuclei including nucleoli of Zika and Langat virus-infected cells brought into question flavivirus replication and the role of the nucleus. Overall, MAb 541 was specific for flavivirus ENV (envelope proteins) and MAB 109 specific for flavivirus NS1 (nonstructural glycoproteins). The detection of Zika virus proteins with the nucleus and nucleolus o virus-infected cells may be due to early transient transport of the polyprotein from cytoplasm into the nucleus followed by protein processing and transport back into the cytoplasm where viral maturation occurs later. Very careful experiments were done to rule out artifacts and viral contamination in these studies, thus providing greater credence that Zika virus may have some different viral properties compared to other flavi-arboviruses including WNV (West Nile virus), YFV (yellow fever virus), Bussuquara virus, and Ntaya virus [51].

6.3 Molecular Epidemiology, Evolution, and Phylogenetic Analyses

Zika arbovirus has been known since the 1950s to be dispersed in Asia as well as Africa. Based on phylogenetic analysis of complete genomes, two genetic lineages exist for Zika virus that correspond to African and Asian geographical regions. Genetic relationships and sources of Zika strains that occurred in the Federated States of Micronesia (Yap Island) in 2007 and in Cambodia (a pediatric case) in 2010 were investigated. Between 1947 and 2010, isolates had been accumulated and stored from Nigeria, Senegal, Uganda, Cambodia, and Malaysia.

The complete genome sequences of these isolates and additional published sequences were used for phylogenetic analysis. Two main Zika virus lineages were identified, African and Asian. It was concluded that the Cambodian case and Yap outbreak were Southeast Asian in origin. The virus proteins appear to lose glycosylation sites over time. It may be inferred that Zika virus made its way from Africa where it was first discovered to Southeast Asia whence it spread further including Yap [45].

Table 18.2 summarizes the Zika virus complete genomes that have been sequenced. The accession numbers are provided from GenBank. Figure 18.1 shows a phylogenetic tree of the complete Zika virus sequences and related flaviviruses. The phylogenetic tree in Fig. 18.1 indicates that the Zika viruses circulating in Asia (Malaysia, Micronesia, and Cambodia) are distinct from those circulating in Africa

Table 18.2 Genetic sequences of Zika viruses (as of July 2013) [49, 52]

[a]Country	Year	Isolate	GenBank accession number
Uganda	1947	MR766[a]	HQ234498
Nigeria	1968	IbH_30656	HQ234500
Senegal	1984	ArD_41519	HQ234501
Uganda	2004	–	NC_012532
Micronesia	2007	–	EU545988
Malaysia	1966	P6-740	HQ234499
Cambodia	2010	FSS13025	JN860885

[a]It should be noted that several additional viral passages of the same Ugandan isolate, MR766, were sequenced and produced identical sequences (AY632535, EU303241, EU074027, AY326412, and AF372422). – = no isolate name

(Nigeria, Senegal, and Uganda), but the two populations are no more diverse than those found within a single serotype of dengue fever viruses. The sequences (cf. Table 18.2) of the 1947 and 2004 Uganda isolates are 99.89 % identical to each other, indicating that Zika virus evolves rather slowly over time and that the African and Asian populations have been evolving for many decades. Similarity plots across the genomes (not shown) exhibited no evidence of recombination within or between isolate genotypes. However, recombination between dengue viruses has recently been detected [52].

Sequencing of many additional isolates of Zika virus would be needed, in order to estimate a true date of divergence between the African and Asian lineages, but a comparison can be made to dengue viruses, which have been more heavily sampled and sequenced.

6.4 Serology

Lanciotti et al. [14] describe genetic and serologic properties of Zika virus during the Yap state (Micronesia) epidemic.

The fundamental immunological finding in this study of the Yap state outbreak is that IgM antibodies had cross-relativities against other arbovirus flaviviruses. One interpretation of these findings is that Original Antigen Sin is being exhibited. This would suggest that the immune response is under some restriction and implies that there could be developing and spreading evolutionary changes of Zika virus that could lead to states of greater pathogenicity.

An epidemic of arthralgia, rash, and conjunctivitis was described by physicians in Yap state, Federated States of Micronesia in April 2007. Dengue virus was indicated as the cause using rapid ELISA. However, specimens sent to the Arbovirus Diagnostic Laboratory at the Centers for Disease Control and Prevention (CDC, Fort Collins, CO, USA) for confirmatory testing in June 2007, using IgM capture

dengue antigen ELISA, confirmed recent flavivirus infection. However, Zika virus reverse transcription-PCR (RT-PCR) assays followed by DNA sequencing supported 90 % nucleotide identity with Zika virus. Therefore, the Yap epidemic was due to Zika virus [14, 53, 54].

Zika, yellow fever, Chikungunya, and dengue type 2 viruses were identified as arboviruses with highest prevalence in humans in Nigeria. Antibodies to six arboviruses were surveyed in 267 human sera from the Kainji Lake region of Nigeria. One hundred and fifty-eight (59 %) had flavivirus hemagglutination-inhibiting (HI) antibody and 139 (52 %) had alphavirus HI antibody. The prevalence of antibodies was Zika—56 %, dengue type 2—46 %, yellow fever—31 %, Chikungunya—45 %, Semliki Forest—25 %, and Sindbis—33 % [55].

Sera randomly selected from 446 individuals across various age groups in Nigeria were tested for flavivirus IgM antibodies using hemagglutination inhibition (HI). Sixty-nine percent (314 sera) tested positive for three or more flaviviruses including Zika, West Nile, Potiskum, Uganda S, and yellow fever viruses. The prevalence was greater in younger than in older individuals [56].

Earlier studies using serological techniques (cross-hemagglutination inhibition and cross-complement fixation) and reactions did not find many antigenic differences distinguishing Zika viruses vs. flaviviruses such as Uganda S, Potiskum, Banzi, dengue type 1, and dengue type 2 viruses. However, differences were observed comparing Zika, Banzi, and Uganda S viruses vs. yellow fever, Wesselsbron, and Potiskum viruses in these studies [57].

The detection of serum IgM antibodies against Zika virus (using ELISA) is indicative of infection 2–5 months previously. This methodology was used in addition to viral isolation from mosquitoes for comparisons across several villages in southeastern Senegal 1988–1990. Human infections with Zika virus occurred in 1990 and epizootic outbreaks occurred annually. In addition, dengue 2 virus was isolated from mosquitoes and humans over the years of the study. However, other flaviviruses were isolated including Wesselsbron, Ked Kedougou, Westle, Chikungunya, Crimean-Congo hemorrhagic fever, and Rift Valley fever viruses as well as viruses that were not considered of public health concern during that period [58].

Serology studies (hemagglutination inhibition and immunofluorescence tests) in the Karamoja district, Uganda, using sera from 132 resident adults collected in 1984 detected 47 % positive for Chikungunya virus (and Semliki Forest alpha viruses (Togaviridae)) and 16 % positive for flaviviruses. It is stated that the latter were most likely mainly due to West Nile virus and included Zika and Wesselsbron viruses. A few individuals had antibodies against Marburg, Ebola-Zaïre, Ebola-Sudan viruses (*Filoviridae*), Lassa virus (Arenaviridae), and Crimean-Congo hemorrhagic fever virus. Yellow fever and dengue type 2 viruses were absent as were Ilesha, Tahyna, Sicilian sand fly fever phlebovirus, and Bunyamwera (Bunyaviridae) [59].

In Southeast Gabon, 197 adult human sera, 28 paired sera of mothers and their newborns, and 34 simian sera were surveyed for arbovirus HI and CF antibodies. Eighty-eight percent of the human sera had yellow fever virus due to vaccination,

58 % against Orungo virus, and 20 % against Chikungunya virus (that was a recent infection demonstrated by CF). Zika, Chikungunya, yellow fever, Uganda S, and Orungo viruses were transmitted transplacentally. Zika virus and Chikungunya viruses were detected as well in simian sera [60].

In April 1979, a survey of human sera was done in southeast Central African Republic. HI was studied in 459 sera and CF in 50 sera. Eighty-nine percent of the tested population had antibodies to Zika, yellow fever, West Nile, Chikungunya, Semliki forest, Sindbis, Uganda S, Bunyamwera, and Zinga viruses. Zika and Chikungunya viruses were active primarily in adults. CF assay detected Orungo virus antigens in 88 % sera. Ilesha, Bwamba, CHF-Congo, Dugbe, Bhanja, Tataguine, Nyando, and Bangui antigens were not detected. CF assay also detected antibodies for CHF-Congo and Bhanja viruses [61].

A study in Pakistan detected complement-fixation antigen for eight Toga viruses in 372 serum samples (43 humans, 172 domestic animals, and 157 rodents). The prevalence rates were Zika 2.4 %, West Nile (WN) 7.8 %, Japanese encephalitis (JE) 3.2 %, and Sindbis (SIN), Chikungunya (CHIK), Uganda S (UGS), and Royal Farm (RF) viruses 1.6–1.3 %. Dengue 1 (DEN) virus antigen was present in serum of one human patient. In human sera, antibodies were detected to all viruses except for RF that was detected in domestic animals and rodent sera. Studies in the epidemiology of Zika, JE, and WN viruses should include the role of rodents [62].

Between October 1977 and December 1977, in North-West Ivory Coast, at the end of the rainy season, an unexpected high number of deaths occurred among 100 patients with febrile hemorrhagic jaundice. This is an area with a high prevalence of yellow fever. Serological and epidemiological surveys indicated that during this period, vectors that could potentially carry yellow fever were detected. However, no viruses had been isolated and neutralization, complement fixation, and hemagglutination inhibition tests were then performed using antigens from six flaviviruses, i.e., Zika, yellow fever, West Nile, Uganda S, Wesselsbron, and Ntaya. These analyses were performed on two to three sera from 49 school children and 29 adults who had a recent history of jaundice, some with hemorrhagic symptoms. For comparison, sera were analyzed from 402 inhabitants of surrounding villages as well as 53 young rural workers. Twenty-one cases definitely had yellow fever, 20 cases probably had yellow fever, 15 cases were inconclusive, and 476 individuals definitely did not have yellow fever [63].

During 1985–1987, at the Dan refugee camp near Hargeysa, Somalia, malaria-like illness epidemics affected a few thousand residents. In some patients, headache, back and joint pains, fever, chills, and sweats were described, lasting up to 10 days. Malaria was not detected in blood smears from acutely ill patients. Zika, Chikungunya, Rift Valley fever, Crimean-Congo hemorrhagic fever, yellow fever, and Sindbis viruses were all absent in 10 convalescent and 28 acute sera using indirect fluorescent antibody (IFA) and hemagglutination inhibition (HI) tests. However, IFA and HI tests demonstrated dengue 2 antibody in 39 % (15/38) and 11 of 29 (38 %) sera, respectively. In 60 % (17/28) and 14 % (4/28) of the sera, using enzyme immunoassay (EIA), IgG and IgM antibody to dengue 2, respectively, was detected [64].

6.5 Antibody Enhancement of Viral Infection

In a serological study, suboptimal concentrations of several antibodies that did not neutralize their viral targets enhanced viral infection in murine macrophage cell culture. In addition, some heterologous combinations of antibodies and viruses showed such infection enhancement as well. Homologous enhancement was greater than heterologous enhancement. These effects were observed for Zika, West Nile, Wesselsbron, and Uganda S viruses, Dakar yellow fever, Potiskum, and dengue 2 viruses. Potiskum virus antibody showed the widest ability for heterologous virus infection enhancement [65, 66].

It was initially hypothesized that in regions of Nigeria where Zika virus is endemic other flaviviruses are less prevalent because of some serological cross-reactivity among these viruses, and thus some cross-resistance [67]. However, as the current hypothesis indicates, cross-reactive antibodies may enhance viral infection.

6.6 Multiple Viruses Present Contemporaneously

The presence of multiple related viruses with varying degrees of pathogenicity is more than a clinical diagnostic problem as this is also a problem in pathology and treatment. For example, many additional arboviruses have been found in conjunction with Zika virus in Lombok, Indonesia [68], adding to the danger of heterologous antibody-enhanced infections. The arboviruses that infect humans included in the study were Zika, Japanese encephalitis (JE), MVE, Tembusu (TMU), LGT, KUN, SEP, dengue type 2 (DEN-2), CHIK, RR, GET, SIN, BUN, BAT New Guinea C, Murray Valley encephalitis (MVE), MM 1775, and BAK. Testing was also done for infections in ducks, chickens, wild birds, bats, cattle, horses, goats, and rats. Infections of domestic animals included JE, MVE, KUN and SEP, BAT, and BUN [68]. Thus, there is a danger posed by the multiple virus infections, immunity, and cross-reactivity. Moreover, the utilization of vaccines in this context adds to the complexity. Due to cross-reactivity and antibody-virus infection enhancement, Zika virus vaccination would help or hinder a virus vaccination program.

Another study further supported the occurrence of contemporaneous multiple virus infections. Between 1971 and 1975, virology and seroepidemiology of Zika, yellow fever, dengue, West Nile, and Wesselsbron viruses were studied in four locales in Oyo State, Nigeria. Zika virus was isolated from two human cases with mild febrile illness. Percentage positive sera (measured by hemagglutination inhibition tests) were 31 % Zika, 50 % yellow fever, 46 % West Nile, and 59 % Wesselsbron. Forty percent Nigerians tested had neutralizing antipodes to Zika virus. Fifty percent individuals positive for Zika virus were positive for Zika virus alone or Zika virus and one other flavivirus, 40 % were positive for Zika virus and two other viruses, and 10 % were positive for Zika virus and at least three other flaviviruses. Of the other

viruses in these Zika antibody-positive individuals, 81 % were positive for dengue type 1, 58 % were positive for yellow fever, 7 % were positive for Wesselsbron, 6 % were positive for West Nile, and 3 % were positive for Uganda S [67].

Numerous additional studies support the occurrence of Zika virus with a high prevalence in several regions in Africa (approximately 40–50 %) contemporaneous among several additional flaviviruses. For example, in south-eastern Gabon these additional viruses have included yellow fever, Chikungunya, Koutango, and Wesselsbron viruses [69]; in Upper Casamance and in Eastern Senegal these included yellow fever, Chikungunya, West Nile, Bunyamwera, and Sindbis viruses (and related to where migrating birds rest) [70]; and in Igbo-Ora, Nigeria, dengue types 1 and 2, yellow fever, Chikungunya, West Nile, and Wesselsbron [71].

6.7 Vaccination and Superinfection, Multiple Infections

It had been hypothesized prior to 1980 that 17D yellow fever vaccine did not induce complement-fixing antibodies and that wild yellow fever virus infection did induce complement-fixing antibodies. However, the following studies demonstrated that this vaccine does produce such antibodies in specific situations and permitted distinction between natural yellow fever infection and vaccination. Yellow fever virus seroepidemiological studies were being done during a yellow fever vaccination campaign using 17D strain yellow fever in Gambia, West Africa, in 1979, during which a yellow fever epidemic ensued. Fifty-eight vaccinated participants were studied in three groups (see Table 18.3): group 1 had participants with pre-vaccination yellow fever-neutralizing antibodies; group 2 had participants without any pre-vaccination yellow fever-neutralizing antibody or hemagglutination-inhibiting antibodies to heterologous flaviviruses (including Zika, Uganda S, Ntaya, West Nile, dengue 1, or Spondweni); and group 3 had participants who lacked pre-vaccination yellow fever-neutralizing antibodies. However, group 3 participants

Table 18.3 Flavivirus vaccination outcomes [72]

Group	Prior exposure to wild yellow fever virus	Prior exposure to Zika, Uganda S, Ntaya, West Nile, dengue 1, or Spondweni viruses	Yellow fever vaccination outcome
Group 1	Pre-vaccination neutralizing antibodies	No prior exposure to HI antibodies	No response. But 24 % had complement-fixing (CF) antibodies
Group 2	No prior exposure to neutralizing antibodies	No prior exposure to HI antibodies	Seroconverted. Produced neutralizing antibodies and/or HIV but no CF antibodies
Group 3	No prior exposure to neutralizing antibodies	With prior exposure to HI antibodies	46 % produced homologous CF antibodies. Nine patterns of HI and homologous and heterologous CF antibodies

nonetheless had heterologous flaviviral hemagglutination-inhibiting antibodies. The findings were that group 1 participants sustained no vaccination response except for production of complement-fixing antibodies in 24 % of this group of vaccines. Group 2 participants upon vaccination seroconverted and sustained yellow fever-neutralizing and/or hemagglutination-inhibiting but no complement-fixing antibodies. Group 3 participants (46 %) produced homologous CF antibodies. There were nine patterns of HI and homologous and heterologous CF antibodies [72].

These studies demonstrate a high prevalence of flavivirus exposure (including Zika virus) that obscures what might have been expected to be a clear response to vaccinations on a wide scale. Moreover, the yellow fever vaccine anamnestic response did result in CF antibodies that were indistinguishable from natural yellow fever virus infection-induced CF antibodies [72].

6.8 RT-PCR

Zika fever diagnosis entails serology and virus isolation that are time-consuming procedures. In addition, serology frequently shows cross-reactivity and is not specific. Thus, tests that are more specific are required. A single-step reverse transcriptase polymerase chain reaction (RT-PCR) procedure was thus developed for the detection of Zika virus RNA. The assay targeted the envelope protein-coding region and was evaluated for sensitivity, specificity, and reproducibility. Additionally, the test was evaluated for its ability to detect Zika virus isolates preserved during the prior 40 years from a variety of hosts from several African countries. The RT-PCR test can be clinically helpful to detect Zika virus infection in regions where other clinically related arboviruses including dengue and Chikungunya viruses co-circulate. The first-generation RT-PCR test detected 7.7 pfus per reaction. The test was 100 % reproducible in patient serum and in cell cultures. Moreover, 19 other flaviviruses were undetected [43]. The same investigators developed a rapid single-step RT-PCR test that can be performed in less than 3 h using sequences from the NS5 protein-coding region of African Zika virus based on representative sequences from GenBank [49]. This assay is able to detect 37 Zika virus isolates from mosquitoes. It remains to validate the test for clinical use [73].

In Singapore, an initial analysis of 88 specimens of patient plasma from patients who had dengue-like disease but not Chikungunya did not show any Zika virus RNA. The newly devised PCR assay had a sensitivity of 140 copies of synthetic RNA per reaction. Further testing on known Zika virus plasma aliquots is needed. In addition, a wider and larger sample of patients needs to be studied in the community [31]. In this regard, it should be noted that Zika virus infection could be masked and not easily confirmed. Past circulation of arboviruses that had been silent (undetected) is the case in the Cameroons (Fako Division). In related studies, although virus isolation was not accomplished, serological studies detected Zika virus antibodies [74].

7 Conclusions

Prior to the 1980s, it was generally considered that the world had entered a new era of increased health and reduced infectious disease because of the defeat of smallpox, TB, and polio. What is the magnitude of the novel contemporaneous changes in arbovirus and vector evolution that appear to be occurring? Remarkable changes have occurred since the 1990s in arbovirus evolution (flaviviruses, mosquito-borne). Since 1999, West Nile virus completely occupied the Americas and since 1995, Japanese encephalitis virus extended its grip to Australia (northeastern). Subsequently, since 2001, Usutu virus occupied Europe; since 2010, Tembusu virus invaded duck farms in China; Bagaza virus caused encephalitis in India and reached Spain (southern). It is also remarked that new vertebrate hosts and mosquito species have become involved in the complex recent proliferation [75]. Similarly, Zika virus has been spreading as remarked in Table 18.1.

The hypothesis based on the data presented in this work is that detection and emergence of Zika virus has been associated with an upsurge of other more pathogenic flavivirus infections including for example dengue 1 and 2, JEV, and yellow fever. Some of these pathogenic viruses are proliferating and not yet fully controlled. The findings reported here of emergent viruses increasingly detected raise many health concerns as to what havoc emergent viruses may play in the future given what has happened with the spread of HIV and HCV, since the 1980s.

Furthermore, in Africa and tropical America, due to flavivirus superinfections including the high prevalence of Zika virus, with high backgrounds of flavivirus immune responses, seroepidemiological studies need to be conducted prior to institution of vaccination programs. An additional caveat is that such tropical risk regions are expanding due to global warming [72].

Clearly, much molecular and epidemiological work needs to be done. Unfortunately, contemporaneous economics and social structures are unable to support properly the work that is needed to understand and rectify current global health issues.

Postscript Consistent with the hypotheses presented in this chapter, as of 1-5-2014, Zika virus has demonstrated continuous spread. On 12-20-2013, the CDC issued a health advisory in regard to visiting French Polynesia (Tahiti) due to Zika virus. Zika virus spread to the islands of Arutua, Bora Bora, Fakarava, Hao, Hiva Oa, Huahine, Moorea, Nuku Hiva, Raiatea, Rangiroa, Tahaa, Tahiti, Takaroa Ahe, Tikehau, and Ua Pou. There were 35,000 suspected cases and 99 confirmed cases reported [22]. In addition, Zika virus was detected in an individual in Germany who had traveled in Thailand [23, 24]. Recent studies further support the need for continued surveillance due to the persistent spread of Zika virus under the radar and concomitant with other flaviviruses. Moreover, the possibility is raised that the prevalence of this virus is under-reported. Moreover, a range of symptoms is denoted, from mild to more severe disease including Guillain-Barre syndrome [76, 77].

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