

REVIEW ARTICLE

ZIKA VIRUS – A REVIEW

Vanda Bostikova^{1,3} ✉, Kamil Kuca³, Pavel Blazek⁴, Radek Sleha¹, Marketa Pasdiorova¹, Jan Marek¹, Hana Stritecka², Vladimir Hytych⁵, Pavel Bostik¹

¹ Department of Epidemiology, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

² Department of Military Internal Medicine and Military Hygiene, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

³ Faculty of Informatics and Management, University of Hradec Kralove, Hradec Kralove, Czech Republic

⁴ Department of Military Medical Service Organisation and Management, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

⁵ Thomayer Hospital, Prague, Czech Republic

Received 18th March 2016.

Revised 30th June 2016.

Published 27th September 2016.

Summary

In February 2016, the World Health Organization (WHO) declared the current Zika outbreak a Public Health Emergency of international concern. The Zika virus disease is caused by a virus (family *Flaviviridae*) transmitted by *Aedes* spp. mosquitoes. People with the Zika virus disease usually have symptoms that can include a low-grade fever, maculopapular rash, conjunctivitis, arthralgia, malaise, headache and retro-ocular headaches. Neurological and autoimmune complications have been described during the outbreaks in Polynesia and recently in Brazil. There are no licensed medical countermeasures - vaccines, therapies or preventive drugs available for the Zika virus infection and disease.

Key words: Zika virus; infection; travel; Guillan-Barre syndrome; Aedes spp.; microcephaly; mosquito; prevention; vaccine

INTRODUCTION

The past decades have seen exotic arboviruses and other arthropod-borne infections emerging into epidemic diseases of global concern. The expanding geographical distribution of these diseases is affected by climate changes and importation of invasive arthropod species (mostly *Aedes* spp. mosquitoes) into

temperate countries of Europe and North America. Zika virus - an emerging mosquito-borne virus from the family *Flaviviridae* - is the latest addition to a list of arbovirus epidemics that emerged in the past two decades [1 – 3].

There are currently four genera in *Flaviviridae*: *Flavivirus* (53 species), *Hepacivirus* (one species, the hepatitis C virus), *Pegivirus* (two species), and *Pestivirus* (four species). Flaviviruses are responsible for yellow fever, Zika fever and dengue, all of which are major human diseases found in tropical regions of the globe. They are zoonoses with a transmission cycle that involves primates as reservoirs and mosquitoes as vectors. Flaviviruses appear significantly more pathogenic when introduced into new niches

✉ University of Defence, Faculty of Military Health Sciences, Department of Epidemiology, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic

vanda.bostikova@unob.cz

(420) 973 253 205

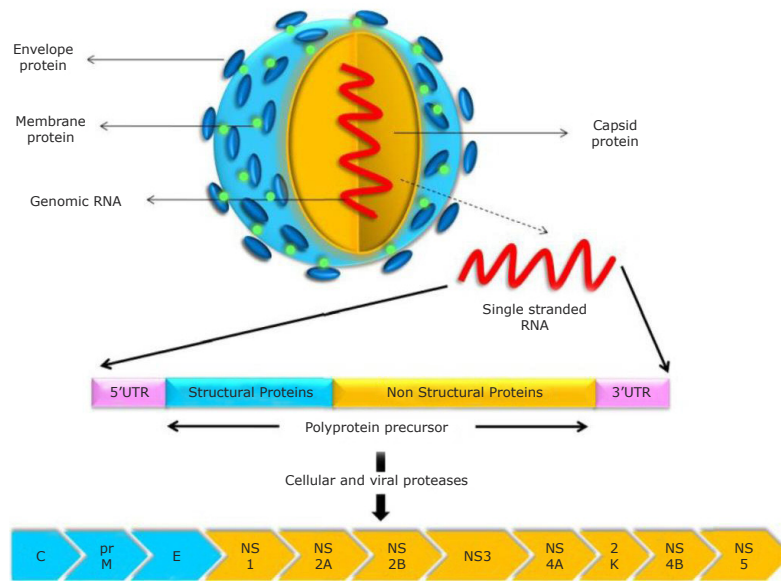


Figure 1. The organization of Zika virus genome [13].

and populations, but as a new virus becomes established, herd immunity effects often attenuate the apparent virulence. For example the West Nile virus in birds shifted from a relatively benign profile in the traditional endemic African host range to the very high mortality upon introduction in North America. This change was associated with specific mutations that increased the viral reproductive fitness in avian hosts and the North American environment. Another case: the rapid spread of chikungunya virus into India, was the result of adaptation to a different mosquito vector involving a single nucleotide change. Interestingly, a recent study on the molecular evolution of Zika virus during its emergence in the 20th century, showed that the virus may have experienced several adaptive genetic changes (though uncommon among flaviviruses), including protein glycosylation patterns, which could be related to the lack of any clear preference for host and vector species [3, 4].

Zika virus was first identified in 1947 in the Zika Forest (Uganda). It was discovered in a *Rhesus* monkey that had been placed in a cage on a sentinel platform in the forest by the Virus Research Institute. Imperato P.J., 2016 [6] described his visit at the Institute and the Zika Forest in 1961. During that time researcher's work was underway to identify mosquito species at various levels of the tree canopy. This was done through the placement of traps at vari-

ous levels of a 120-foot-high steel tower. At that time, researchers isolated 12 strains of Zika virus from *Aedes africanus* from traps on the tower [6, 7].

In 1954, the first three cases of human infection were reported during an epidemic of jaundice in Nigeria. Over the next decades, the virus spread to other parts of Africa, and eventually appeared in Southeast Asia. By 1981, only 14 cases of illness had been reported as being caused by the Zika virus. Since most infections with this virus were either mild or asymptomatic, its true geographic spread was not fully appreciated [8].

Zika virus

Zika virus is related to yellow fever, dengue, West Nile, Japanese encephalitis viruses, and most closely to Spondweni virus. Studies in *Rhesus* monkeys suggest that adaptive immune responses to Zika infection interfere with, but do not fully protect against, yellow fever infection and disease. Serologic cross-reactivity, including non-neutralizing antibodies, is observed with other closely related flaviviruses and flavivirus vaccines [9, 10].

Zika virus is an enveloped, icosahedral positive single stranded (ss) RNA arbovirus of the family *Flaviviridae*, genus *Flavivirus*. The Zika virus reference genome [11] comprises a noncoding region

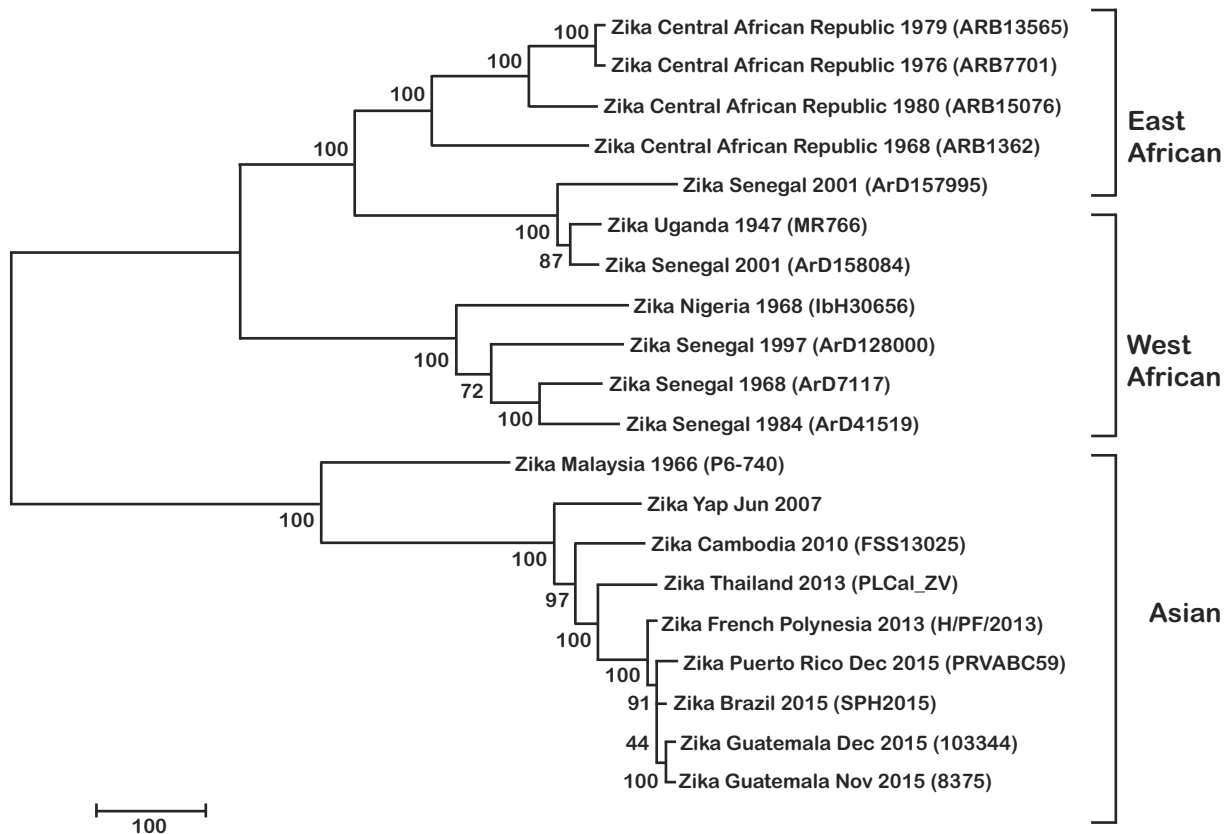


Figure 2. Phylogenetic tree of Zika virus isolates compared with reference isolates obtained from GenBank [14].

and sequences coding for a 3419 amino acid polyprotein [12]. The viral genome is approximately 11 kb long. The virus's RNA includes its complete open reading frame (ORF) sequence. The ORF encodes a polyprotein with three structural components: capsid, membrane and envelope and the seven nonstructural proteins NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 (Fig. 1).

Currently, three distinct genotypes are recognized: West African/Nigerian cluster, East African/MR766 prototype cluster, and Asian cluster (Fig.2). It has been postulated that the virus originated in East Africa and then spread into both West Africa and Asia, approximately 50 to 100 years ago. Asian genotype viruses have been evolving and spreading geographically throughout Asia and the Pacific Islands since at least 1966. Malaysia 1966 Zika virus is representative of an ancestral genotype. The percent nucleotide identity among all the Western Hemisphere ZIKVs is >99%, and as a group, these Western Hemisphere viruses are ~89% identical to viruses of the East African and West African genotypes [13, 19, 20].

MussoD. et al,2016 [14] reported that the phylogeny and movement of Zika and chikungunya viruses are strikingly similar. Each virus is grouped into 3 genotypes of very similar geographic distribution: East Africa, West Africa, and Asia. For both viruses, it also seems that viruses from East Africa moved into Asia 50–100 years ago and evolved into a unique Asian genotype. Probably similar ecologic and human social factors might be responsible for the movement of chikungunya and Zika viruses into the New World at approximately the same time.

Zika virus is passed on to humans through the bites of an infective female *Aedes* spp. mosquito, which mainly acquires the virus while feeding on the blood of an infected person.

Aedes aegypti is an invasive species of mosquito in the Western Hemisphere that has adapted well to densely-populated urban environments. Flight range studies suggest that most female *Ae. aegypti* may spend their lifetime in or around the houses where they emerge as adults and they usually fly an average of 400 metres. This means that people,

rather than mosquitoes, rapidly move the virus within and between communities and places. *Ae. aegypti* breed indoors and are capable of biting anyone throughout the day. The indoor habitat is less susceptible to climatic variations and increases the mosquitoes' longevity [15].

Ae. albopictus is able to survive cooler temperatures and has high ecological plasticity. *Ae. albopictus*, is distributed through the northern United States, southern Brazil, northern China, and southern Europe, as well as Africa, Central America, and Australia and is rapidly colonizing new regions. This territory expansion is aided by temperature changes, globalization and urbanization, all factors which are also associated with an increased risk of autochthonous Zika virus transmission [15].

The potential involvement of other insect vectors including *Culex* sp. mosquitoes are currently being examined. In the outbreak on Yap island (Micronesia), 12 mosquito species belonging to four genera were identified as potential vectors [16].

Current circulation of Zika virus

The current Zika epidemic began on Yap island in Pacific in 2007. This was the first known presence of the Zika virus outside of Africa and Southeast Asia. Nearly 75 % of the population was infected [17].

In 2013, the virus spread to French Polynesia where an estimated 28,000 cases occurred in a population of 270,000. During that year and afterwards, microcephaly and other congenital abnormalities were observed in the infants of women who were pregnant when they contracted the virus [18].

On March 2014, Chile notified WHO of autochthonous transmission of Zika virus on the Easter Island, where the virus continued to be detected until June 2014 [21].

In May 2015, Brazil confirmed the transmission of Zika virus in the country's northeast. The Ministry of Health has estimated that between 440,000 and 1,300,000 cases of Zika virus infection may have occurred in the country during 2015.

Zika virus infection was associated with microcephaly in the infants of some women who were pregnant when they contracted the disease. Primary microcephaly (usually defined as head circumference ≤ 3 standard deviations below the mean at birth) is a rare multifactorial condition with incidence of from 1,3 to 150/100,000 live births. Microcephaly is variously attributed to genetic factors, intrauterine infection (including rubella, toxoplasmosis, or cytomegalovirus), maternal malnutrition, and toxin exposure during gestation. Symptoms include hearing loss, mental retardation, development delay,



Figure 3. Transmission of Zika virus worldwide [15]

seizure disorders, and cerebral palsy. There is no specific treatment beyond supportive care. The reported annual incidence rate of microcephaly in all of Brazil was from 139 to 175 between 2010 and 2014. The 3,530 cases of Zika-associated primary microcephaly reported in Brazil during 2015, indicated a twenty-fold increase in a single year [22, 23].

Cases of the Guillain-Barré syndrome (GBS) were also found to be associated with Zika virus infection. GBS is a clinical syndrome of multiple autoimmune etiologies, which involve idiopathic peripheral neuropathy leading to acute flaccid paralysis. This process can be initiated by an infection with various viruses or bacteria. A treatment consists of intravenous immunoglobulin and/or plasma exchange with a supportive care for patients with respiratory compromise. The clinical course varies; 25% of patients require artificial ventilation (days to months), 20% of patients remain non-ambulatory (not able to walk around) at 6 months and 3–10% of patients die despite standard of care treatment. Globally, annual GBS incidence is estimated at 1.1 to 1.8/100,000/year, of which approximately 70% appear associated with an antecedent infectious disease. Such infections are typically gastrointestinal or respiratory, but include dengue infection. A retrospective seroneutralization analysis of GBS cases which were suspected of being associated with Zika during the 2013–2014 outbreak in French Polynesia, has demonstrated that 42 cases were positive for both dengue and Zika virus infection, yielding a ratio of 1 case of Zika-associated GBS for every 208 suspect cases of Zika virus infection. However, the concomitant regional increase in dengue and chikungunya infections suggests that the increased GBS incidence may be attributable to these risk factors and/or to Zika infection [24, 25].

Since October 2015, other countries and territories of the Americas have reported the presence of the virus (Fig.3).

In February 2016, the World Health Organization declared the current Zika outbreak a Public Health Emergency of international concern.

Transmission of Zika virus

The virus has been detected in blood donors in areas where Zika is circulating. Transmission of related viruses (dengue, chikungunya and West Nile virus) by blood transfusion has been documented, and thus transmission of Zika virus is

also possible this way. Brazilian health authorities have reported 2 cases of possible transmission of the virus by blood transfusion. Studies are needed to assess the prevalence of the virus and of transmission through blood transfusion and blood products to better understand the risk that Zika presents. Specific measures need to be recommended to prevent Zika infection. Ideally the blood supply during a regional outbreak of Zika should be maintained by increasing blood collections in non-affected areas where consideration may be given to deferring potential donors who have recently visited areas with ongoing transmission of Zika virus infection for 28 days after their departure from these areas [26–28].

The most common form of Zika transmission is through mosquito bites, but the virus has been isolated in semen, and cases of sexual transmission have been observed. Currently the available evidence is being analysed to better understand the public health impact of sexual transmission of Zika [29].

Pregnant women should be advised not to travel to areas of ongoing Zika virus outbreaks; pregnant women whose sexual partners live in or travel to areas with Zika virus outbreaks should ensure safe sexual practices or abstain from sex for the duration of their pregnancy. Women who have had unprotected sex and do not wish to become pregnant because of concern with infection with Zika virus should also have a ready access to emergency contraceptive services [30].

Research is currently under way on possible mother-to-child transmission of the virus and its effects on babies.

Zika virus disease

Anyone not previously exposed to the virus and who lives in an area where the mosquito is present, and where imported or local cases have been reported, may be infected.

Historically, adult human infection with Zika virus has presented with mild, non-life threatening symptoms in 20% of infected patients, with 80% being clinically asymptomatic during initial infection. Typical acute symptoms persist from days to one week, and include fever (37.9°C or below), maculopapular rash (average duration 6 days), arthralgia (average duration 3.5 days, range 1 to 14 days) and/or conjunctivitis, myalgia, headache, retro-orbital pain and emesis. The incubation period of Zika virus

disease is not clear, but it is likely to be a few days. Based on blood bank screens in French Polynesia, it appears that viremia can begin up to 10 days before onset of symptoms, suggesting it may be longer than for some other arboviruses [31, 32].

Death after Zika virus infection of an otherwise healthy patient with sickle cell disease has also been reported, indicating increased risk to otherwise medically compromised individuals [32].

Dengue or chikungunya are transmitted by the same type of mosquito and present similar symptoms as Zika disease. But certain symptoms can be useful for differential diagnosis: dengue usually presents with higher fever and more severe muscle pain. There can be complications when the fever breaks: attention should be paid to warning signs such as bleeding. Chikungunya presents with higher fever and more intense joint pain, affecting the hands, feet, knees, and back. It can disable people, bending them over so that they cannot walk or perform simple actions such as opening a water bottle. Zika does not have clearly characteristic features, but most patients have skin rashes and some have conjunctivitis [33].

Confirmation of Zika virus disease

Diagnosis is based on clinical symptoms and epidemiological circumstances. Infection with Zika virus may be suspected based on symptoms and recent history (e.g. residence or travel to an area where Zika virus is known to be present). Zika virus diagnosis can only be confirmed by laboratory testing for the presence of Zika virus RNA in the blood or other body fluids. Reverse-transcriptase PCR (RT-PCR) can be used to detect the Zika virus during the first 1 week (in blood) to 4 weeks (in urine) of the illness. Zika virus RT-PCR can also be performed on amniotic fluid although it is not currently known how sensitive or specific this test is for the congenital infection.

Serology is less reliable due to a potential cross reaction with antibodies against other similar viruses. For individuals presenting 4 to 7 days after onset of symptoms with negative Zika virus RT-PCR, Zika virus serologic testing should be performed. Measuring virus-specific neutralizing antibodies is useful for discriminating between cross-reacting antibodies from other flavivirus infections; testing is considered inconclusive if Zika virus neutralizing antibody titers are <4-fold higher than dengue virus neutralizing antibody titers. Acute and convalescent

sera should be obtained to detect an increased antibody titer in paired samples with an interval of two to three weeks. Serologic testing for dengue virus infection and chikungunya virus infection should also be pursued. All serologic results should be interpreted with caution since there can be cross-reactivity with other flaviviruses (including dengue virus and West Nile virus). Cross-reactivity may also be observed in individuals who have been vaccinated against yellow fever or Japanese encephalitis.

For individuals presenting 8 to 14 days after the onset of symptoms, diagnostic testing for Zika virus infection should include urine RT-PCR for detection of Zika virus RNA as well as Zika virus serologic testing.

For individuals presenting ≥ 15 days after onset of symptoms, diagnostic testing for Zika virus infection should consist of Zika virus serologic testing.

This makes it difficult to differentiate Zika virus infection using antibody testing alone. For this reason, Zika virus serology is not recommended at this time as part of the algorithm for assessing pregnant women with a history of travel to areas with an active Zika virus transmission [34 – 37].

Treatment of Zika virus disease

The treatment consists of relieving the pain, fever, and any other symptoms that causes inconvenience the patient. To prevent dehydration, it is recommended to control the fever, rest, and drink plenty of water. There is no vaccine or specific drug for this virus [38].

Control strategies

Pregnant women are discouraged from travelling to Zika-endemic areas. In addition to bite avoidance measures, non-pregnant, sexually active women of reproductive age residing in endemic areas should consider the issues of family planning and contraception.

At present, the only flaviviral vaccines available for the human use are the yellow fever (live attenuated), Japanese encephalitis (inactivated, live attenuated, and chimeric), tick-borne encephalitis (inactivated) vaccines, and the newly marketed dengue vaccine (live attenuated, recombinant, tetravalent; marketed since 2015). Claims were made by an Indian biotechnology company that two Zika virus vaccine candidates (recombinant and inactivated)

can be tested soon; however, no details on the vaccine preparations are currently available in the scientific literature.

Avoiding mosquitoes bites can be done by using insect repellent regularly; wearing clothes (preferably light-coloured) that cover as much of the body as possible; using physical barriers such as window screens, closed doors and windows; and if needed, additional personal protection, such as sleeping under mosquito nets during the day. It is extremely important to empty, clean or cover containers regularly that can accumulate water, such as buckets, drums, pots, etc. Other mosquito breeding sites should be cleaned or removed including flower pots, used tyres and roof gutters. Also cover domestic water tanks so that mosquitoes cannot get in. Communities must support the efforts of the local government to reduce the density of mosquitoes in their locality.

DEET remains the gold standard insect repellent. It was developed and patented by the US Army in 1946 and commercialized since 1957. It is generally applied to the skin in the form of liquids, aerosols, or lotions, and can be used to impregnate clothings if necessary.

DEET formulations range from 4% to 100% in concentration. It is a common misconception that higher concentrations provide “more powerful” protection against arthropods. Higher concentrations merely prolong the duration of protection. At a concentration of 15%, DEET protects against *Ae. aegypti* and *Ae. albopictus* bites for about 7 to 8 hours.

Product label instructions should be strictly followed. Special attention and help should be given to those who may not be able to protect themselves adequately, such as young children, the sick or elderly.

DEET is not oncogenic, teratogenic, or genotoxic in animals. On the other hand there are reports of toxicity in humans, which mainly relate to neurotoxicity, especially seizures in children. However, such reports remained rare and in many of the reported cases, a definitive causal relationship between DEET and neurotoxicity cannot be established. Detectable levels of DEET can be found in cord blood of infants born to mothers using DEET during the second and third trimesters of pregnancy, but no adverse outcomes of pregnancy have been found in a double-blind, randomized trial. Hence, when



Figure 4. A health worker fumigates school of Lima, Peru, as part of measures to prevent the spread of the Zika virus

used appropriately according to recommendations, DEET is still considered to be safe in children older than 2 to 6 months of age, as well as in pregnant and lactating women. A DEET concentration of 20% to 30% is generally recommended for adult use.

Various botanical compounds – essential oils extracted from plants, have been advocated as natural and harmless insect repellents. While many of these oils do possess repellent activities, most of them are too volatile to offer lasting protections (less than 1 hour) and even these natural products may cause adverse reactions, especially skin irritation.

Insect repellent-treated wristbands, garlic, oral vitamin B, and electronic buzzers (which claim to produce ultrasound to repel insects) are completely ineffective as bite avoidance measures [39 – 42].

Transmission control activities should target *Ae. aegypti* (or any of the other vectors depending on the evidence of transmission) in its immature (egg, larva, and pupa) and adult stages in the household and immediate vicinity. This includes other settings where human–vector contact occurs, such as schools, hospitals and workplaces (Fig. 4).

There is an urgent need to develop vector control tools for sustained control of *Aedes* populations such as ovitraps or oviposition traps, which collect the eggs laid by the mosquitoes which develop into larva, pupa and adult mosquitoes. Ovitrap are often used for surveillance of *Aedes* vectors and can be modified to render it lethal to immature or adult populations of *Ae. aegypti*. Lethal ovitraps (which incorporate an insecticide on the oviposition substrate), autocidal ovitraps (which allow oviposition but prevent adult emergence), and sticky ovitraps (which trap the mosquito when it lands) have been used on a limited basis. Studies have shown that population densities can be reduced with sufficiently large numbers of frequently-serviced traps.

An emerging tool for mosquito control is represented by genetically modified mosquitoes. Currently there are two methods of reducing the disease transmission by vectors using genetics: the population suppression and population replacement.

Population suppression means to reduce the mosquito population to such levels that it would not be able to sustain the pathogen transmission. This includes sterility, reduced adult longevity, or decrease of larva/pupa survival.

The population replacement aims to reduce the inherent ability to transmit the pathogen. The effects of the genetic modification can be self-limiting or self-sustaining. Self-limiting technologies are not capable of persisting in the environment and in the wild genome pool. Self-sustaining genetic transfer will be able to transfer genes across generations and is approached with caution to avoid any other issues [43 – 47].

CONCLUSION

Zika virus is likely a harbinger of future diseases driven by the ecosystem change and global interconnectedness. Many questions about the Zika virus epidemiology and transmission remain, but among the most pressing questions are whether the change in the disease phenotype correlates to changes in viral genotype, and if current clinical disease is influenced by the viral entry into a new population with the indigenous confounding or effect modification.

The development of a general use prophylactic vaccine for Zika virus-induced disease will require considerable time and careful evaluation of the safety, effectiveness, and risk/benefit ratio for the population at large. This is particularly true for a vaccine designed to protect against a virus apparently associated with both neurologic teratogenic effects and neurologic autoimmune disease, and which belongs to a genus notorious for antibody-mediated enhancement of infection. For example, during 2002 it was announced that a vaccine for the closely related West Nile Virus was in preparation with licensure anticipated within three years. While an equine vaccine for West Nile Virus has been licensed, there are currently no vaccines licensed for preventing West Nile Virus disease in humans. With any prophylactic vaccine intended for human use, the requirement for careful evaluation of safety (including a potential for eliciting an autoimmune disease) and efficacy require large and sustained clinical development efforts. In Brazil, Institute Butantan has announced an expedited Zika vaccine development effort projected for completion in three to five years after an initial year of non-human primate testing, which may involve collaboration with the NIH. Experience suggests that this is an optimistic timeline for a development and licensure of a flavivirus vaccine, which may require up to twenty years of clinical development and testing.

REFERENCES

1. Medlock, J.M; Hansford, K.M., Versteirt, B., et al. An entomological review of invasive mosquitoes in Europe. *Bull Entomol Res.* **2015**, 105, 637–663
2. Zammarchi, D.; Tappe, C.; Fortuna, M.E.; et al. Zika virus infection in a traveller returning to Europe from Brazil, March 2015. *Euro Surveill.* **2015**, 23, 21153
3. Roth, A.; Mercier, C.; Lepers, D.; et al. Concurrent outbreaks of dengue, chikungunya and Zika virus infections—an unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012–2014. *Euro Surveill.* **2014**, 19, pii: 20929
4. Zmurko, J.; Neyts, K.; Dallmeier F. NS4b, chameleon and jack-in-the-box roles in viral replication and pathogenesis, and a molecular target for antiviral intervention. *Rev Med Virol.* **2015**, 25, 205–23
5. Choumet V., Despres P. Dengue and other flavivirus infections. *Rev Sci Tech.* **2015**, 34(2), 473–72
6. Imperato P.J. The convergence of a virus, mosquitoes, and human travel in globalizing the Zika virus. *J Community Health.* **2016**, 33, 78–86
7. Haddow, M.C.; Williams, J.P.; Woodall. Twelve isolations of Zika virus from *Aedes* (*Stegomyia*) *africanus* (Theobald) taken in and above a Uganda forest. *Bull World Health Organ.* **1964**, 34, 57–69
8. E.B. Hayes. Zika virus outside Africa. *Emerg Infect Dis.* **2009**, 15, 1347–50
9. S. Idrees, U.A. Ashfaq. RNAi: antiviral therapy against dengue virus. *Asian Pac J Trop Biomed.* **2013**, 13, 232–36
10. Wong, S.S., et al: Zika virus – the next wave after dengue? *J Formosan Medical Assoc.* **2016**, 3, 56–67
11. <http://www.ncbi.nlm.nih.gov/protein/226377834>
12. http://www.ncbi.nlm.nih.gov/nucleotide/NC_012532.1
13. Enfissi, J.; Codrington, J.; Roosblad, M. Zika virus genome from the Americas. *Lancet.* **2016**, 387, 227–8
14. Musso D., Baud D. Zika virus time to move from case reports to case control. *Lancet Infect Dis.* **2016**, 1, 33–6
15. Hamel, O.; Dejarnac, S.; Wichit, P.; et al. Biology of Zika virus infection in human skin cells. *J Virol.* **2015**, 89, 8880–96
16. Mogi, M. Overwintering strategies of mosquitoes (Diptera: Culicidae) on warmer islands may predict impact of global warming on Kyushu, Japan. *J Med Entomol.* **1996**, 33, 438–44
17. Duffy, T.H.; Chen, W.T.; Hancock, A.M.; et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med.* **2009**, 360, 2536–43
18. Kutsuna, Y.; Kato, T.; Takasaki, M.; et al. Two cases of Zika fever imported from French Polynesia to Japan, December 2013 to January 2014. *Euro Surveill.* **2014**, 19, pii: 20683
19. Haddow, A.J.; Schuh, C.Y.; Yasuda, M.R.; et al. Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. *PLoS Negl Trop Dis.* **2012**, 6, e1477
20. Faye, C.C.; Freire, A.; Iamarino, O. Faye, J.V.; et al. Molecular evolution of Zika virus during its emergence in the 20th century. *PLoS Negl Trop Dis.* **2014**, 8, e2636
21. Tognarelli, S.; Ulloa, E.; Villagra, et al. A report on the outbreak of Zika virus on Easter Island, South Pacific, 2014. *Arch Virol.* **2015**, 41, 65–8
22. Gaunt, A.A.; Sall, X.; de Lamballerie, A.K.; et al. Phylogenetic relationships of flaviviruses correlate with their epidemiology, disease association and biogeography. *J Gen Virol.* **2001**, 81, 1867–1876
23. Mlakar, M.; Korva, N.; Tul, M.; et al. Zika virus associated with microcephaly. *N Engl J Med.* **2016**, 33, 54–8
24. Endy, K.B.; Anderson, A.; Nisalak, I.K.; et al. Determinants of inapparent and symptomatic dengue infection in a prospective study of primary school children in Kamphaeng Phet, Thailand. *PLoS Negl Trop Dis.* **2011**, 5, e975
25. Marcondes, M.F. Zika virus in Brazil and the danger of infestation by *Aedes* (*Stegomyia*) mosquitoes. *Rev Soc Bras Med Trop.* **2015**, 3, 45–6
26. Stramer SL, Hollinger FB, Katz LM. Emerging Infectious Disease agents and their potential threat to transfusion safety. *Transfusion.* **2009**, 49, 59–60
27. Straner SL, Hollinger FB, Katz LM. Emerging Infectious Disease agents and their potential threat to transfusion safety. *Transfusion.* **2009**, 49, 67–69
28. Stramer SL, Hollinger FB, Katz LM. Emerging Infectious Disease agents and their potential threat to transfusion safety. *Transfusion.* **2009**, 49, 1–29S.
29. Cao-Lormeau, C.; Roche, A.; Teissier, E., et al. Zika virus, French polynesia, South Pacific, 2013. *Emerg Infect Dis.* **2014**, 1085–86
30. Musso D, Roche C, Robin E. Potential sexual transmission of Zika virus. *Emerg Inf Dis.* **2015**, 21: 359–61.

31. Stramer SL. Current perspectives in transfusion transmitted Infectious diseases: emerging and reemerging infections. *ISBT Sci Ser.* **2014**, 9:30-6
32. www.who.net
33. Kutsuna, Y.; Kato, M.L.; Moi, A., et al. Autochthonous dengue fever, Tokyo, Japan, 2014. *Emerg Infect Dis.* **2015**, 21, 517–20
34. Ioos, H.P.; Mallet, I.; Leparç Goffart, V. Current Zika virus epidemiology and recent epidemics. *Med Mal Infect.* **2014**, 302–7
35. Faye, O.; Faye, A. One-step RT-PCR for detection of Zika virus. *J Clin Virol.* **2008**, 43, 96–101
36. Faye, O.; Faye, D.; Diallo, M. Quantitative real-time PCR detection of Zika virus and evaluation with field-caught mosquitoes. *Virol J.* **2013**, 10, 311
37. Balm, C.K.; Lee, H.K.; Lee, L. A diagnostic polymerase chain reaction assay for Zika virus. *J Med Virol.* **2012**, 84, 1501–5
38. eCDC rapid risk assessment. **2014**, 19: 20687
39. Thome RCS, Yang HM, Esteva L. Optimal control Ae.aegypti mosquitoes by sterile insect technique and insecticide. *Mathem Biosc.* **2010**, 223
40. Oster, J.T.; Brooks, J.E.; Stryker, R.E., et al. Interim guidelines for prevention of sexual transmission of Zika virus—United States, 2016. *MMWR Morb Mortal Wkly Rep.* **2016**, 65, 120-1
41. Sanchez, V.; Vanlerberghe, L.; Alfonso, C.; et al. Aedes aegypti larval indices and risk for dengue epidemics. *Emerg Infect Dis.* **2006**, 12, 800–6
42. Faull, C.R. Intraspecific variation in desiccation survival time of Aedes aegypti (L.) mosquito eggs of Australian origin. *J Vector Ecol.* **2015**, 40, 292–300
43. Carroll, D.C. Pre-travel vaccination and medical prophylaxis in the pregnant traveler. *Travel Med Infect Dis.* **2008**, 6, 259–75
44. Baldacchino, B.; Caputo, F. Chandre, A.; et al. Control methods against invasive Aedes mosquitoes in Europe: a review. *Pest Manag Sci.* **2015**, 71, 1471–85
45. PPAV Working Groups. Personal protection against biting insects and ticks. *Parasite.* **2011**, 18, 93–111
46. Antwi, L.M.; Shama, R.K. Risk assessments for the insect repellents DEET and picaridin. *Regul Toxicol Pharmacol.* **2008**, 51, 31–6
47. Koren, D.; Matsui, B. DEET-based insect repellents: safety implications for children and pregnant and lactating women. *CMAJ.* **2003**, 169, 209–12