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REVIEW ARTICLE

Zika virus – emergence, evolution, pathology, diagnosis, and control: current global scenario and future perspectives – a comprehensive review

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ABSTRACT

This review converses the Zika virus which has attained global concern due to its rapid pandemic potential and impact on humans. Though Zika virus was first isolated in 1947, till the recent large-scale outbreak which occurred in Micronesia, in 2007, the virus was placed into the innocuous pathogen category. The World Health Organization on 1 February 2016 declared it as a 'Public Health Emergency of International Concern.' Of the note, American as well as Pacific Island strains/isolates is relatively closer to Asian lineage strains. The African and American strains share more than 87.5% and 95% homologies with Asian strains/isolates, respectively. Asian strains form independent clusters, except those isolated from China, suggesting relatively more diversity than African strains. Prevention and control are mainly aimed at the vector population (mosquitoes) with *Aedes aegypti* being the main species. Surveys in Africa and Asia indicated seropositivity in various animal species. However, so far its natural reservoir is unknown. There is an urgent need to understand why Zika virus has shifted from being a virus that caused mild illness to unforeseen birth defects as well as autoimmune-neurological problems. Unfortunately, an effective vaccine is not available yet. Availability of cryo-electron microscopy based on 3.8 Å resolution revealing mature Zika virus structure and the probable virus attachment site to host cell would provide critical insights into the development of antiviral treatments and vaccines.

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1. Introduction

The geographic modulations, in terms of population and climate dynamics, have become a potential threat to the global community health in past, in particular due to uprising infection rates of mosquito-borne diseases such as Malaria, Chikungunya, Dengue, West Nile, and Zika (Chen & Wilson 2010; Dhiman et al. 2010; Dhama, Tiwari, et al. 2013; Lee et al. 2013; Hubálek et al. 2014; Carneiro & Travassos 2016; Fauci & Morens 2016). The animal and vector-borne (primarily ticks and mosquitoes) viral diseases are of major concern due to their easy and accelerated migration across the continents (Hubálek et al. 2014). In the past 60 years, several deadly disease outbreaks in man resulted from the emergence of viruses of animal origin such as Henipaviruses (Hendra, Nipah, and Cedar virus) in Australia (Ong & Wong 2015), Hantavirus in the United States of America (Knust & Rollin 2013), Ebola virus in Africa (Martínez et al. 2015; Nunes-Alves 2016), several influenza subtypes (bird flu, swine flu, etc.) (Kapoor and Dhama 2014), severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS). Recently, it was Ebola that threatened the whole world by its outrageous nature (Dhama, Malik, et al. 2015; Martínez et al. 2015; Nunes-Alves 2016) and now it is time for Zika virus which has already set the floor on fire due to its rapid and wider spread (Fauci & Morens 2016). The Zika outbreaks are more challenging, as they are unpredictable and rapidly spreading among the affected people (Hayes 2009; Gautret & Simon 2016; Grard et al. 2014; Gatherer & Kohl 2016; Gulland 2016a). Moreover, least is known about disease ecology and further lack of effective prevention and treatment options worsen the situation (Elachola et al. 2016; Petersen, Wilson, et al. 2016). Zika virus raised concern in the recent past because of its worldwide spread whereas earlier it was found limited

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only to two of its dwelling continents, Africa and Asia (Heang et al. 2012; Brown 2015; Lucey & Gostin 2016; WHO 2016).

The viruses of the *Flaviviridae* family found in arthropods (primarily in ticks and mosquitoes) can occasionally infect humans (Chastel 2012; Choumet & Desprès 2015). Members of this family belong to a single genus, *Flavivirus*, associated with considerable morbidity and mortality throughout the world. Some of the tick-transmitted viruses are responsible for encephalitis and hemorrhagic diseases viz. tick-borne encephalitis (TBE), Kyasanur Forest Disease (KFD), Alkhurma Disease, and Omsk Hemorrhagic Fever (OHF). Several other flaviviruses such as Japanese encephalitis, Yellow Fever, West Nile, Dengue, Chikungunya, and Zika virus are being transmitted by the mosquitoes (Daep et al. 2014; Hubálek et al. 2014; Medlock & Leach 2015; Parham et al. 2015). North America has been victimized by the emergence of several arthropod-borne diseases, including Jamestown Canyon, Powassan, Chikungunya, and Zika diseases recently (Pastula et al. 2016). Due to recent outbreaks, Zika virus (Fauci & Morens 2016) is being considered as an emerging infectious disease with the potential to spread to new areas where the *Aedes* mosquito vector is present (Hayes 2009; Chen & Wilson 2010; Cao-Lormeau et al. 2014; Carneiro & Travassos 2016; CDC 2016; Hennessey et al. 2016) and reports also document the involvement of other vectors like *Culex* spp. (Marcondes et al. 2016).

Zika virus possesses an un-segmented, single-stranded, positive-sense RNA genome of approximately 11 kb and its single open reading frame (ORF) (10272 bp) encodes a polyprotein which includes three structural proteins viz., capsid, pre-membrane, and envelope and seven non-structural (NS) proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5). Hitherto studies elucidate phylogenetic relationship among Zika virus strains targeting selected genes (e.g. envelope, NS3, NS5, etc.) from a few strains/isolates. Still, we are falling short of a broad spectrum picture about Zika virus evolution.

The first Zika virus isolation was done in 1947 (Dick et al. 1952). Since the Zika disease outbreak in Brazil during early 2015 (Zanluca et al. 2015; WHO 2016; Rodriguez-Morales 2015; Nereida 2015), the disease is spreading rapidly across South and Central America, and Mexico (Summers et al. 2015). After Brazil, Colombia is the most-affected country, with well over 25,000 suspected human cases reported and 1331 Zika virus cases confirmed in man since October 2015 (Petersen, Wilson, et al. 2016; Roa 2016). These outbreaks are more challenging as they are unpredictable and rapidly emerging/spreading among the people affected. Considering the above situation, the WHO on 1 February 2016, declared it as a 'Public Health Emergency of International Concern' (WHO 2016; Gulland 2016b; Higgs 2016).

As of 30th March 2016, locally transmitted (autochthonous) cases of Zika virus infection have been reported from 61 countries or territories worldwide (Zanluca et al. 2015; Calvet, Filippis, et al. 2016). In literature, Zika disease is described as a mild, self-limiting febrile illness lasting four to nine days without severe complications, no fatalities, and a low hospitalization rate (CDC 2016). There is a link between Zika virus spread and an increase in cases of fetal abnormalities like microcephaly, hydranencephaly, ventriculomegaly, cerebral calcifications, abnormally formed or absence of brain structures, cataracts of both eyes, calcifications of eye, and hydrops fetalis during pregnancy and yet an unproven association with Guillain–Barré syndrome (GBS) in adult people (Oehler et al. 2014; de Paula Freitas et al. 2016; Karwowski et al. 2016). Since October 2015, more than 4000 babies in Brazil had been born with abnormally small heads and brains – a rare condition known as microcephaly (Martines et al. 2016; Schuler-Faccini et al. 2016; Mlakar et al. 2016; Rodriguez-Morales 2016; Heymann et al. 2016). The Zika virus transmission occurs through the *Aedes* mosquito, perinatal transmission (Besnard et al. 2014), sexual intercourse, and blood transfusion (Musso, Nhan, et al. 2014; Franchini & Velati 2015). From infected pregnant mothers, the Zika virus can be transmitted via transplacental route and as a consequence might affect the developing brain of the fetus (Roa 2016; Rodriguez-Morales 2016; Torjesen 2016). The methods available for diagnosis of Zika virus include real-time (RT) polymerase chain reaction (PCR) (Faye et al. 2008; Gourinat et al. 2015) and IgM-based ELISAs, with the latter one suffering from limitation of cross-reaction of antibodies with other *Flaviviruses* (Buathong et al. 2015). Currently, there is no effective treatment for this disease. Though vaccination seems to be a promising option for controlling the disease, no effective vaccine against Zika disease is available yet (Dyer 2016a). Prevention and control are the only available weapon in the arsenal to keep this disease under check. Several approaches for controlling mosquito population like mechanical control, use of chemicals, and genetic control methods are available (Araújo et al. 2015). The WHO has issued an alert to the public regarding Zika disease on 1 February 2016 (Citrome 2016). The guidelines issued recently need to be followed to prevent further spread of disease. Strict biosecurity measures need to be adopted worldwide in order to prevent further spread of the disease to different parts of the world.

2. Etiology

The family *Flaviviridae* comprises of four genera viz., 1. *Flavivirus* (53 Species), *Hepacivirus* (one Species), *Pegivirus* (two Species), and *Pestivirus* (four Species) (http://www.ictvonline.org/virusTaxonomy.asp). Based

on virus transmission, the genus *Flavivirus* is further divided into three major groups viz., (1) tick-borne viruses (mammalian tick-borne virus group, seabird tick-borne virus group), (2) mosquito-borne viruses (Aroa virus group, Dengue virus group, Japanese encephalitis virus group, Kokobera virus group, Ntaya virus group, Spondweni virus group, and Yellow Fever virus group), and (3) viruses with no known arthropod vector (Entebbe bat virus group, Modoc virus group, and Rio Bravo virus group). The Zika virus belongs to the genus *Flavivirus* and is a representative of the Spondweni virus group of mosquito-borne virus group.

The viruses in the genus *flavivirus* possess a single-stranded, positive-sense RNA genome of approximately 11 kbp with a 5′-type I cap structure that is methylated at the guanine N-7 and the ribose 2′-OH positions of the first transcribed nucleotide adenine (m7GpppAm) (Ray et al. 2006). The RNA cap structure is crucial for RNA stability and translation (Picard-Jean et al. 2013). The particle size of Zika virus (strain 766) was estimated to be between 30 and 45 nm (Dick 1952). The earlier reports speculated that phylogenetically Zika virus relates more closely to Spondweni virus; the two viruses are the only members of their clade within the mosquito-borne cluster of flaviviruses (Kuno et al. 1998; Cook & Holmes 2006; Lanciotti et al. 2008; Hayes 2009).

Recently, the Zika virus structure was decipehreed using cryo-electron microscopy with 3.8 Å resolution. Higher similarity exists with other *Flavivirus* members excepting for nearly 10 amino acids which enfold the Asn154 glycosylation site and constitutes the icosahedral shell. Furthermore, the probable virus attachment site to the host cell was predicted, where any change can alter further virus transmission and disease progression. This provides critical insights into the development of antiviral treatments and vaccines in days to come (Sirohi et al. 2016).

3. Zika virus genomic configuration

As on 31st March 2016, 59 complete coding sequences and/or genome information is available on Zika virus in the public database, representing human ($n = 39$), monkey ($n = 7$), and *Aedes* mosquito ($n = 13$) isolates (Table 1). In 2007, for the first time, two full-length sequences of Zika virus strain 'MR 766' from monkey (NC_012532 and AY632535) were deposited to the database by Kuno and Chang from the National Center for Zoonotic, Vector-Borne, and Enteric Diseases, USA. The former sequence is being considered as the reference sequence. With the advancement in next generation sequencing platforms, the database was continuously enriched with the addition of new strains/isolates, with the highest number of submission of Zika viral sequences ($n = 44$) in the year 2016 itself. As per the genomic information available, the ORF within the Zika virus genome ranges between 10,254 and 10,272 nucleotides, encoding a polyprotein of 3417 (e.g. isolates ARB15076 from mosquito and MR 766 from monkey) to 3423 amino acid residues as depicted in Table 1. The insertion of few amino acids is seen at position 441–442 in the genomic region encoding E protein. This observation needs further analysis regarding their role in host−pathogen interaction or other functions. Of the note, all human Zika virus strains/isolates possess a polyprotein of 3423 amino acids, whereas, Zika virus with smaller polyprotein could only be isolated from mosquitos and monkey (Table 1). Deletion of certain amino acids in polyprotein may be attributed to the passage history of a particular strain; however, it needs strong experimental support (Haddow et al. 2012). The un-translated regions (UTRs) in *Flaviviruses* contain conserved sequences (designated as CS1, CS2, and CS3) that are believed to play important roles in genome stabilization and genomic cycle such as recognition by cellular or viral factors, translation, RNA packaging, replication, etc. (Lindenbach & Rice 2003; Kuno & Chang 2007). It is noteworthy that variation in CS1 and CS3 is also reported in Zika virus strain MR 766 (Kuno & Chang 2007). Likewise, polymorphism (additions as well as deletions) in length of Zika viral polyproteins is also reported in a few strain/isolates (Haddow et al. 2012; Berthet et al. 2014). It would be of interest to know what causes the polymorphism in length of Zika viral polyproteins and to explore if this phenomenon has any influence/role on viral replication and pathogenicity. The polyprotein is co- and post-translationally cleaved by host and viral proteases to generate three structural proteins, designated the capsid (C), membrane precursor/membrane (prM/M) and envelope (E) proteins, and seven NS proteins in the gene order: 5'−C−prM(M)−E−NS1−NS2A−NS2B−NS3−NS4A−2K−NS4B−NS5-3' (Rice et al. 1985; Blitvich & Firth 2015; Baronti et al. 2014). These three structural proteins (C, prM, and E) form capsids and seven NS proteins are involved in the assembly and viral replication. The Zika virus envelope plays a role in various aspects of the life cycle including binding and membrane fusion (Lindenbach & Rice 2003). The protein C (core protein; 11 kDa) is important for the formation of the nucleocapsid; protein E (50 kDa − the largest structural protein) is an envelope glycoprotein functioning as a fusion protein and it is the primary target for neutralizing antibodies. The protein M (8 kDa) is translated as precursor protein viz; 'prM' (26 kDa), which is cleaved by a cellular enzyme (furin) into mature protein M − and a 'pr' segment at the time of virion release from the cell. The prM complex protects protein E from degradation during virion assembly. The 'pr' and the M proteins can provide host protection from infection. The seven NS proteins play a vital role in replication of the flaviviruses and invoking cell-mediated immune response (Ryan et al. 1998;

Table 1. Details of Zika virus strains/isolates (full length or near to full length sequences) available in the database.

S. No.	Host	Strain/isolate	Country	Year[#]	Accession No.	CDS (nucleotide)	Polyprotein (amino acid)
			Mosquito				
1	*A. aegypti*	P6-740	Malaysia	1966	HQ234499	10,269	3423
2	*A. africanus*	ArB1362	CAR	1968	KF383115	10,272	3423
3	*A. luteocephalus*	ArD7117	Senegal	1968	KF383116	10,272	3423
4	*A. africanus*	Isolate ARB13565	CAR	1976	KF268948	10,269	3422
5	*A. africanus*	Isolate ArD_41519	Senegal	1984	HQ234501	10,269	3423
6	*A. taylori*	41662-DAK-tc	Senegal	1984	KU955592	10,272	3423
7	*A. africanus*	41525-DAK-tc	Senegal	1984	KU955591	10,272	3423
8	*A. taylori*	41671-DAK-tc	Senegal	1984	KU955595	10,272	3423
9	*A. luteocephalus*	ArD128000	Senegal	1997	KF383117	10,272	3423
10	*A. dalzieli*	ArD157995	Senegal	2001	KF383118	10,272	3423
11	*A. dalzieli*	ArD158084	Senegal	2001	KF383119	10,272	3423
12	*A. opok*	Isolate ARB15076	CAR	2013	KF268949	10,254	3417
13	*A. africanus*	Isolate ARB7701	CAR	2014	KF268950	10,269	3422
			Human				
1	*H. sapiens*	IbH_30656	Nigeria	1968	HQ234500	10,251	3417
2	*H. sapiens*	EC Yap	Micronesia	2007	EU545988	10,272	3423
3	*H. sapiens*	Isolate FSS13025	Cambodia	2010	JN860885	10,269	3423
4	*H. sapiens*	FSS13025-tc	Cambodia	2010	KU955593	10,272	3423
5	*H. sapiens*	Isolate CPC-0740	Philippines	2012	KU681082	10,269	3423
6	*H. sapiens*	H/PF/2013	French Polynesia	2013	KJ776791	10,272	3423
7	*H. sapiens*	Haiti/1225/2014[*]	Haiti	2014	KU509998	10,272	3423
8	*H. sapiens*	Isolate SV0127/14	Thailand	2014	KU681081	10,269	3423
9	*H. sapiens*	ZikaSPH2015[*]	Brazil	2015	KU321639	10,272	3423
10	*H. sapiens*	PRVABC59[*]	Puerto Rico	2015	KU501215	10,272	3423
11	*H. sapiens*	Isolate Z1106033	Suriname	2015	KU312312	10,272	3423
12	*H. sapiens*	BeH818995	Brazil	2015	KU365777	10,272	3423
13	*H. sapiens*	BeH819015	Brazil	2015	KU365778	10,272	3423
14	*H. sapiens*	BeH819966[¶]	Brazil	2015	KU365779	10,272	3423
15	*H. sapiens*	BeH815744	Brazil	2015	KU365780	10,272	3423
16	*H. sapiens*	8375	Guatemala	2015	KU501217	10,272	3423
17	*H. sapiens*	MRS_OPY_Martinique_PaRi_2015	Martinique	2015	KU647676	10,272	3423
18	*H. sapiens*	Natal RGN	Brazil	2015	KU527068	10,272	3423
19	*H. sapiens*	ZKV2015	Brazil	2015	KU497555	10,272	3423
20	*H. sapiens*	SSABR1	Brazil	2015	KU707826	10,272	3423
21	*H. sapiens*	FLR	Colombia	2015	KU820897	10,272	3423
22	*H. sapiens*	BeH828305	Brazil	2015	KU729218	10,272	3423
23	*H. sapiens*	BeH823339	Brazil	2015	KU729217	10,272	3423
24	*H. sapiens*	103344	Guatemala	2016	KU501216	10,272	3423
25	*H. sapiens*	ZJ03	China	2016	KU820899	10,272	3423
26	*H. sapiens*	VE_Ganxian	China	2016	KU744693	10,272	3423
27	*H. sapiens*	Dominican Republic/2016/PD1	Italy	2016	KU853012	10,272	3423
28	*H. sapiens*	Dominican Republic/2016/PD2	Italy	2016	KU853013	10,272	3423
39	*H. sapiens*	MEX/InDRE/Lm/2016	Mexico	2016	KU922923	10,272	3423
30	*H. sapiens*	MEX/InDRE/Sm/2016	Mexico	2016	KU922960	10,272	3423
31	*H. sapiens*	Rio-U1	Brazil	2016	KU926309	10,272	3423
32	*H. sapiens*	Rio-S1	Brazil	2016	KU926310	10,272	3423
33	*H. sapiens*	FB-GWUH-2016	USA	2016	KU870645	10,272	3423
34	*H. sapiens*	Z16006	China	2016	KU955589	10,272	3423
35	*H. sapiens*	Z16019	China	2016	KU955590	10,272	3423
36	*H. sapiens*	GDZ16001	China	2016	KU761564	10,272	3423
37	*H. sapiens*	GD01[¶]	China	2016	KU740184	10,272	3423
38	*H. sapiens*	GZ01	China	2016	KU820898	10,272	3423
39	*H. sapiens*	SZ01	China	2016	KU866423	10,272	3423
			Monkey				
1	*M. mulatta*	MR 766	Uganda	1947	HQ234498	10,269	3423
2	*M. mulatta*	MR-766-tc	Uganda	1947	KU955594	10,260	3419
3	*M. mulatta*	MR 766-tc	Uganda	1947	KU720415	10,272	3423
4	*M. mulatta*	MR 766	Uganda	2006	DQ859059	10,254	3417
5	*M. mulatta*	MR 766[*¶]	Uganda	2010	AY632535	10,260	3419
6	*M. mulatta*	MR766-NIID[*]	Uganda	2014	LC002520	10,272	3423
7	*M. mulatta*	MR 766[*§]	Uganda	2016	NC_012532	10,260	3419

[#]Represents year of collection/isolation/submission of the sequence.
[*]Complete genome.
[§]Reference sequence.
[¶]Putative ancestral sequences (please refer Figure 3). CAR; Central African Republic, tc; Cell cultured strains/isolates.

Zhang et al. 2003; Bollati et al. 2010; Shiryaev & Strongin 2010; Smit et al. 2011; Blitvich & Firth 2015). The structure of Zika virus and its genome is depicted in Figure 1. The NS5, being the largest, serves as a multi-tasking protein, as its C-terminus performs RNA-dependent RNA polymerase (RdRP) activity while the N-terminus possesses methyltransferase activity and is involved in RNA capping (Lindenbach & Rice 2003).

Zika virus genome encodes for only 10 proteins. Hence, it may be assumed that one or more proteins

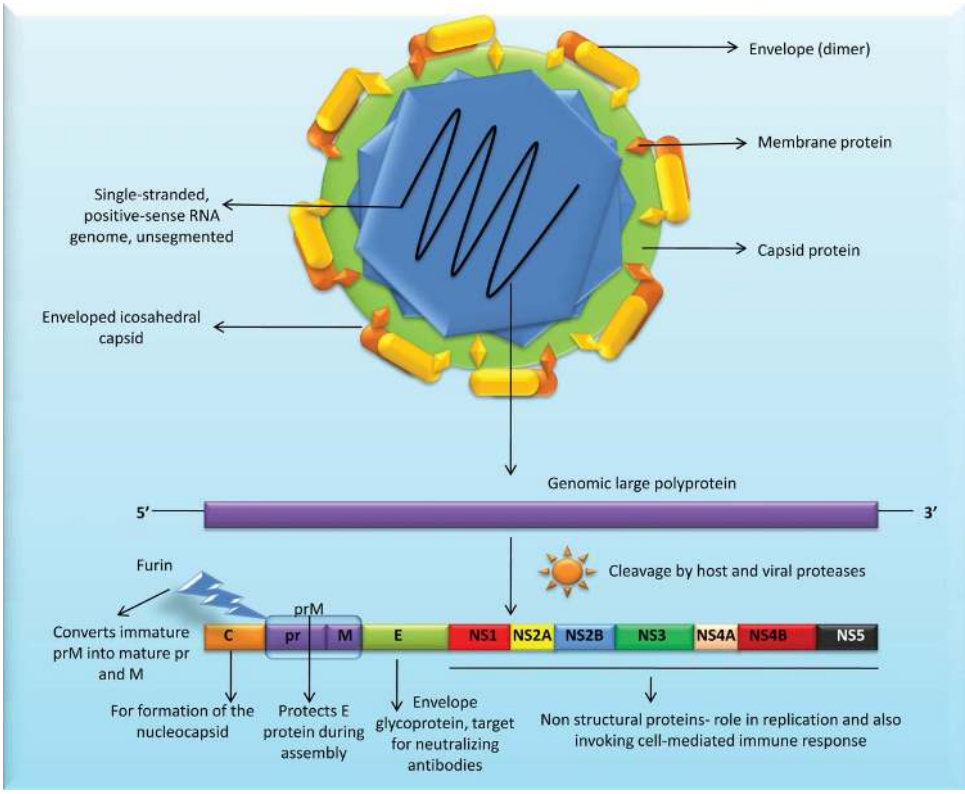

Figure 1. Structure of Zika virus and its genome. Zika possess ss+ve sense RNA which has seven non-structural genes and three structural genes (C, M, E). M exists as prM (immature form) which gets cleaved to pr and M with the aid of furin enzyme.

may possess post-translational modifications (PTM) to exert different functions. PTM is a crucial phenomenon that leads to altered protein function in various subcellular locations. It becomes further important when genome size is less and only a few proteins are translated as in the case of viruses. A number of putative N and O-linked glycosylation sites have been predicted in a few proteins viz., membrane, envelope, and NS1 of several Zika virus strains (Kuno & Chang 2007; Lanciotti et al. 2008; Haddow et al. 2012; Faye et al. 2014). N-linked glycosylation is known to be important in infectivity and assembly of flaviviruses (Mondotte et al. 2007; Lee et al. 2010). The glycosylation sites in Zika virus genome exhibit polymorphism and may have adaptive values during evolution. It is imperative to remark here that loss of glycosylation sites occurs in Zika viruses (Haddow et al. 2012; Hanna et al. 2005; Lee et al. 2010). Recently, the correlation between glycosylation sites polymorphism and the vector was suggested to cause the evolution of Zika viruses (Faye et al. 2014). Though the absence of glycosylation may be important for the sylvatic cycle of the virus (Mondotte et al. 2007), the functional importance of glycosylation is still a matter of research.

Moreover, we predicted putative phosphorylation sites in the polyprotein from different Zika virus strains isolated from human (Haiti/1225/2014, $n = 156$, and ZikaSPH2015, $n = 155$), monkey (MR 766, $n = 148$), and mosquito (ARB7701, $n = 146$) following the method described earlier (Chattopadhyay et al. 2010). Likewise, sites for potential SUMOylation could also be predicted with a high threshold in human (Haiti/1225/2014, $n = 4$, and ZikaSPH2015, $n = 4$), monkey (MR 766, $n = 6$), and mosquito (ARB7701, $n = 5$) Zika virus polyproteins. Till date, only glycosylation sites are reprted in Zika viral proteins, whereas predictions emerged out of the analysis we conducted suggest the presence of other PTM modifications also. Biological functions such as the protein−protein interaction of proteins are known to be influenced by phosphorylation, whereas SUMOylation provides functional flexibility to proteins and is involved in different biological phenomena such as protein localization and functions. Hence, future Zika virus research strategies should also include exploration of PTM in polyproteins and their biological significance.

4. Zika virus evolution

To study the Zika virus evolution, complete genome sequences encoding polyprotein were retrieved from NCBI database ($n = 59$) and subjected to the phylogenetic analysis with the help of MEGA (Molecular Evolutionary Genetic Analysis version 6.05) tool (Tamura et al. 2013). Nucleotide sequences were aligned using the default pairwise and multiple alignment parameters of ClustalW in MEGA and applied for selection of the best substitution model for further analysis using FindModel tool (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). Gaps were

positioned to minimize nucleotide mismatches and treated as missing data in all analyses. The Maximum likelihood statistical method was applied to construct a phylogenetic tree with the GTR + G + I model. The robustness of tree was tested using the bootstrap method based on 1000 replicates. Branch corresponding to partitions reproduced in <50% bootstrap replicates was collapsed. The genealogy of Zika virus was studied to identify the ancestral sequence(s) among the existing viral populations by TCS network (Clement et al. 2000) implemented in PopART i.e. Population Analysis with Reticulate Trees software version 1.7 (http://popart.otago.ac.nz).

Hitherto studies report evolutionary analysis of pandemic Zika virus with elucidation of genetic relationship among different Zika virus strains/isolates (Lanciotti et al. 2008; Haddow et al. 2012; Faye et al. 2014; Berthet et al. 2014; Grard et al. 2014; Buathong et al. 2015; Gatherer & Kohl 2016) based either on a few genes e.g. envelope, NS3, NS5, etc. (Lanciotti et al. 2008; Faye et al. 2014; Grard et al. 2014) or some strains/isolates (Haddow et al. 2012; Gatherer & Kohl 2016; Buathong et al. 2015). Use of envelope and other non-structural genes (NS5) for phylogenetic analysis in most of the studies could be attributed to the presence of high phylogenetic signal contents in these genes (Faye et al. 2014). Broadly, these studies have outlined that all Zika virus strains/isolates cluster into two major clades, one representing the African and the other the Asian lineage. The African lineage is further grouped into two sub-clades, the MR766 prototype clade from East Africa (Ugandan cluster) and another from West Africa (Nigerian cluster) (Haddow et al. 2012; Faye et al. 2014; Berthet et al. 2014; Grard et al. 2014; Gatherer & Kohl 2016; Buathong et al. 2015). The African and American strains share more than 87.5% and 95% homologies with Asian strains/isolates, respectively (data not shown). The phylogenetic analysis carried out herein, targeting ORF of polyprotein gene, was in agreement with previous observations based on selected genes. Moreover, it also supported the notion that American as well as Pacific Island strains/isolates are relatively closer to those from Asian lineage (Zanluca et al. 2015; Buathong et al. 2015; Alera et al. 2015; Enfissi et al. 2016). The African strains clustered together forming three sub-clades representing strains from West Africa, Central African Republic (CAR), and East Africa, respectively (Figure 2). This observation was also evident in TCS network analysis, where Asian and African lineages also exhibit clear distance (Figure 3). There were few variations observed in Asian and American lineage, as strains/isolates from Americas and Carrebian Islands exhibited diversity and formed four different clusters (Figure 2). Asian strains formed independent clusters, except those isolated from China, suggesting relatively more diversity than African Zika viral strains. Between the two Zika viral lineages, the Asian lineage is thought to have evolved with higher epidemic potential (Kelser 2016) in line with with the diversity seen in Asian lineage strains. Furthermore, strains from Pacific Islands (two representations in our study) clustered distinctly in the phylogram. The strain that caused the outbreak in Micronasia (2007) clustered with those from Phillipines and Malaysia, while that from French Polynesia formed an independent cluster. It was concomitant with previous phylogenetic observations (Enfissi et al. 2016). Of the note, two isolates from Italy (Accession no. KU853012-13) were found to have closer similarity with Brazilian isolates. Further examination revealed that these two actually belong to Dominican Republic and were carried to Italy with a traveler (Barzon et al. 2016). Strains from Senegal in African lineage were present in two sub-clades from West and East Africa as observed earlier (Faye et al. 2014) which hypothesize the presence of more than one distinct lineage in Senegal (Faye et al. 2014). Likewise, strains from China as well as Brazil were also part of different sub-clades, suggesting circulation of more than one type of Zika viral population in those regions (Figure 2). Especially Brazilian isolates exhibit highest diversity among them when compared to strains/isolates from any other country. This diverse Zika viral population may pose serious challenge to Zika viral vaccination research in the continent. Phylogenetic studies by other researchers on Zika virus also emphasized that it emerged in Uganda (most probably the strain 'MR 766' accession no. AY632535, as evidenced in our analysis also; Figure 3). This emergence is followed by movement of the virus towards East African countries such as Senegal, Nigeria, and CAR. The Asian lineage also emerged from African strain (from Uganda), moved towards Pacific Islands causing epidemic in Micronesia (Faye et al. 2014; Gatherer & Kohl 2016; Buathong et al. 2015; Alera et al. 2015) and eventually reached the Americas (Gatherer & Kohl 2016; Zanluca et al. 2015). Additionally, TCS network suggested two more putative ancestral sequences of human origin circulating in China (GD01; KU740184) and Brazil (BeH819966; KU365779). Our observation advocates the school of thought that the Zika virus was widely distributed throughout the distinctly situated continents such as Africa, Asia, and America since long (Faye et al. 2014). Therefore, time and place of isolation might not be linked to their spread.

Recently, recombination events – although a rare phenomenon in Flaviviruses (Loriere and Holmes 2011) – are also found with significant statistical support in different Zika viral strains (Faye et al. 2014). The study advocated that recombinations are also being experienced by Zika viruses in the field. However, the occurrence of this phenomenon in Zika viruses should be addressed with additional strain/isolates available in the database and eventually must be evaluated for

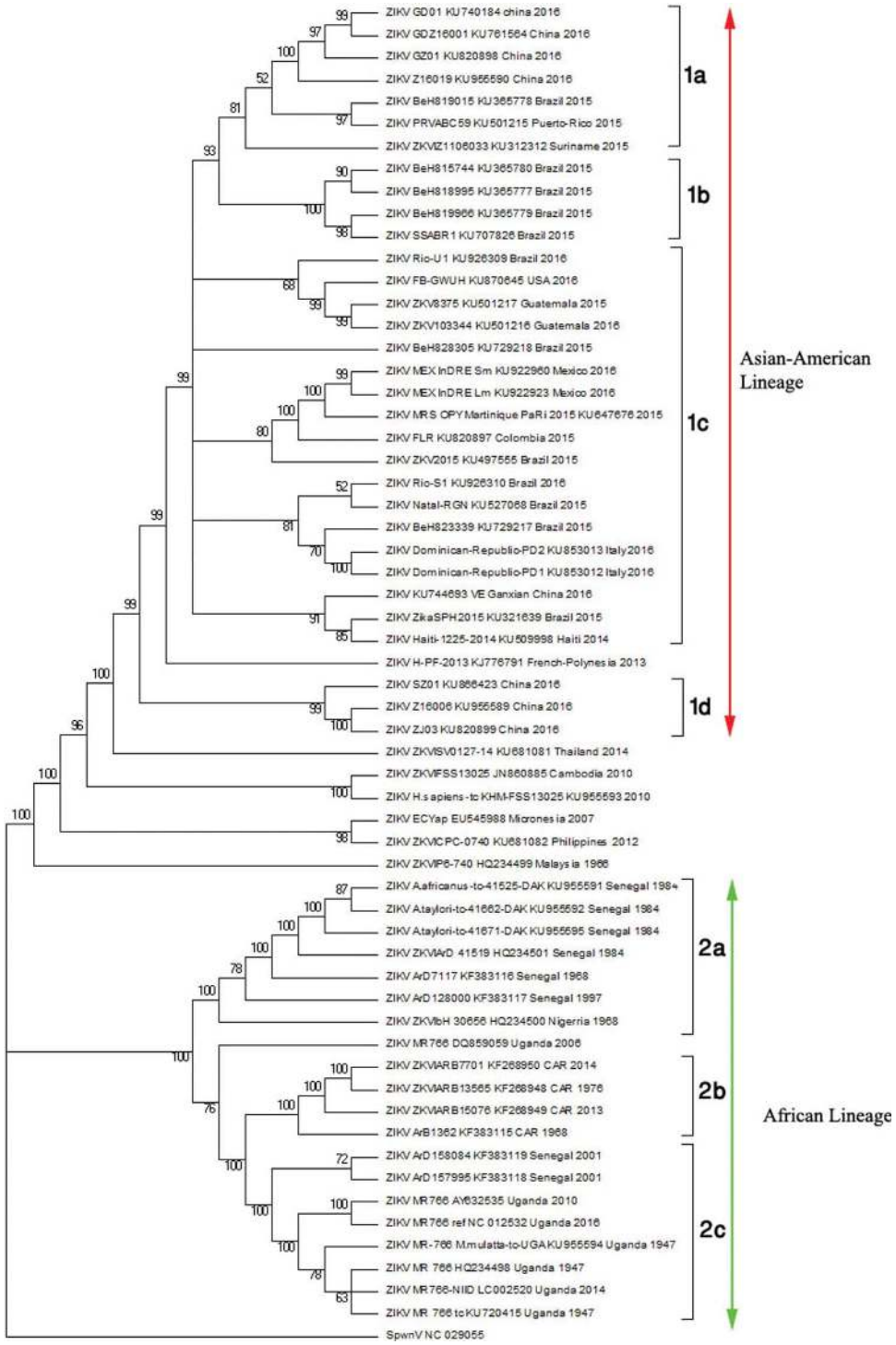

Figure 2. Phylogenetic analysis of genes encoding polyprotein in Zika virus. Maximum likelihood tree was generated from nucleotide sequences of 59 Zika virus strains (complete open reading frame) retrieved from GenBank utilizing the GTR + G + I model. The tree was rooted with Spondweni virus reference sequence (GenBank accession number NC_029055). Numbers at the nodes represent percentage bootstrap support values based on 1000 replicates. Strains/isolates are represented according to their name, accession number, country of origin, and year of collection/isolation/submission. Strains from Americas (1a−1c) together with those from Asian and Oceania constituted the Asian-American lineage and grouped into 1a, 1b, 1c, and 1d. The African strains/isolates clustered to form the African lineage and grouped into east (2a), central (2b) and west (2c) African sub-clades.

their impact on pathogen distribution, epidemiological, and zoonotic potential. Additionally, the study also identified that a few Zika viral genes e.g. envelope and NS5 are under strong negative selection pressure. Infection and transmission mode of RNA viruses such as Zika viruse permit the negatively selected sites and synonymous substitutions (Hanada et al. 2004). Hence, episodes of negative selections together with no sign of positive selection are indicative of eradication of unwanted polymorphism in genes with functional

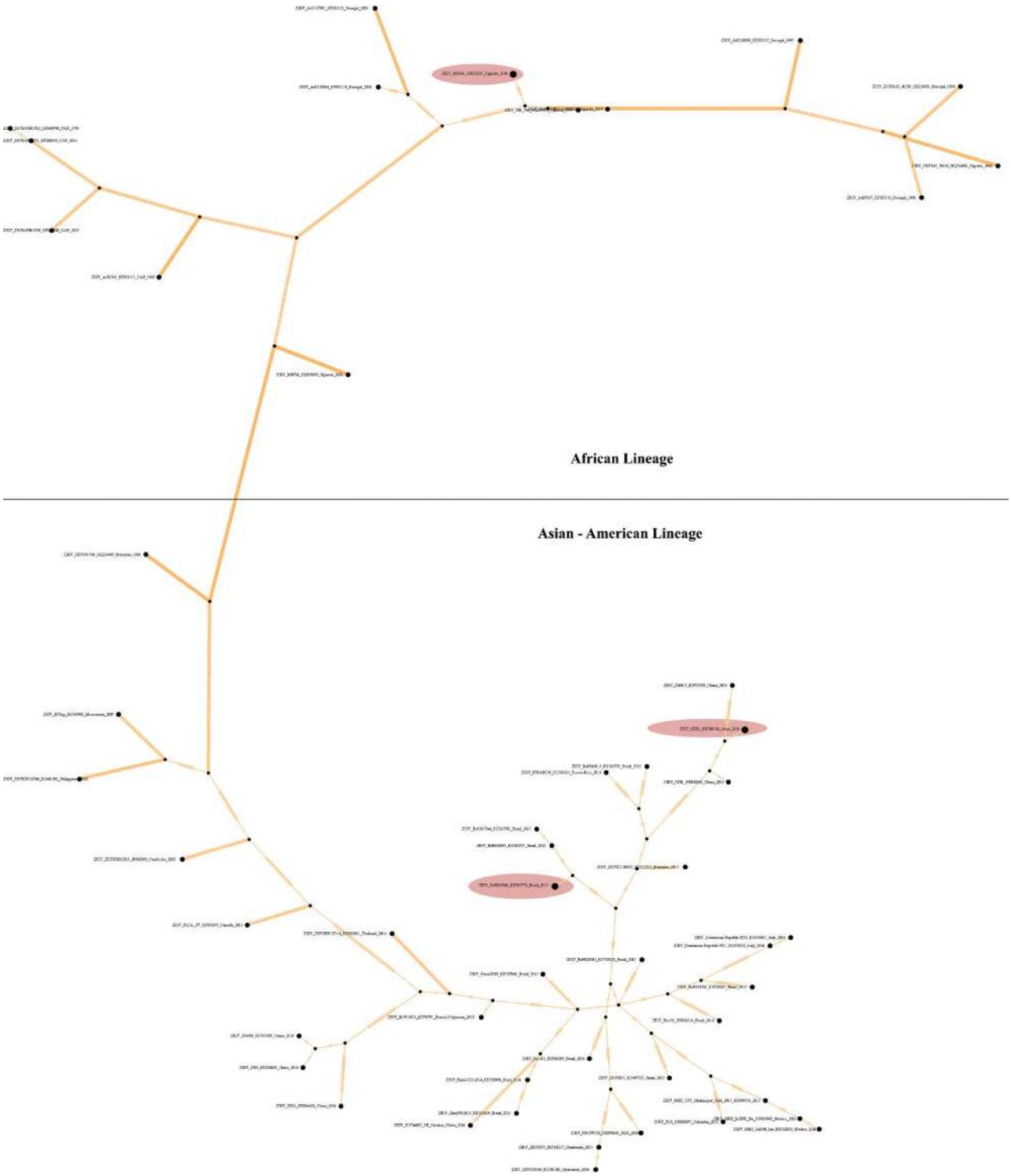

Figure 3. The genealogy of Zika viral strains/isolates. The TCS network showing 59 haplotypes connected parsimoniously. Individual discs indicate haplotypes with the size of the disc proportional to the number of accessions in the haplotype. Strains viz; 'MR 766' from monkey (AY632535), BeH819966 (KU365779) and GD01 (KU740184) both of human origin represented in dark eclipses were identified as basal (ancestral) haplotypes in Zika viral population. Hatch marks represent nucleotide changes during evolution.

significance. One can assume that deletions that remove glycosylation sites in the envelope gene which, in turn, increase the infectivity of Zika virus and the outbreak of Zika virus is due to negative selection. However, all these speculations need experimental validation. One speculation is that Zika virus undergoes recombination and due to loss of its N154 glycosylation site in the envelope protein got adapted to *Aedes dalzieli,* a mosquito vector in Africa (Faye et al. 2014).

5. Epidemiology, transmission, and spread

In recent times, a lesser known mosquito-borne virus, Zika virus, is emerging as a serious public health

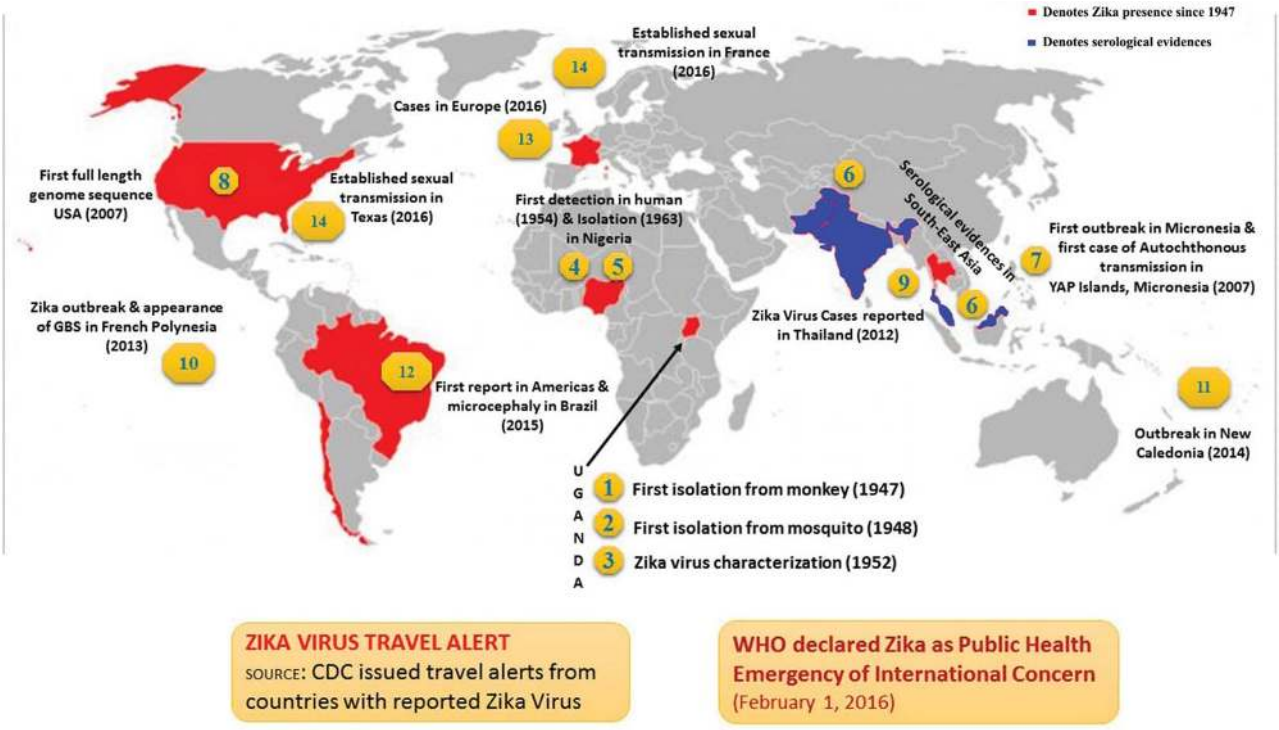

Figure 4. Landmarks in Zika virus epidemiology. The red area depicts Zika virus presence and/or serological reports (Since 1947—2016) and in blue denoted serological evidences of Zika in Southeast Asia.

concern (Chen & Hamer 2016; Fauci & Morens 2016; Higgs 2016; WHO 2016; CDC 2016). It produces a comparatively mild disease in adult humans. As shown in Figure 4, Zika virus was first isolated from a rhesus monkey in 1947, at Zika forest in Uganda (Dick et al. 1952; Goma 1965; Derraik & Slaney 2015). Subsequently, the second isolation of Zika virus was made from a batch of wild-caught *Aedes africanus* in January 1948, in the same forest (Weinbren & Williams 1958; Haddow et al. 1964). A study conducted in semi-captive orangutans in Malaysia suggested that these animals were exposed to Zika virus at some point in time (Kilbourn et al. 2003). In 1954, during the investigation of an outbreak of jaundice suspected of being Yellow Fever in Eastern Nigeria, three cases were confirmed as positive for Zika virus infection; virus could be isolated from a serum sample of a 10-year-old girl with a history of fever and headache only, whereas rise in antibodies was detected against this virus in two other cases (MacNamara 1954). During the research on new strains of Zika virus isolated from *A. africanus* collected in Zika forest, one of the researchers became ill with maculopapular rash on the face, neck, trunk, and upper arms, slight malaise with pain in the back and febrile response and Zika virus could be isolated from a serum sample while he was febrile (Simpson 1964). Subsequently, virus isolation has been made from different countries including Nigeria (Moore et al. 1975; Fagbami 1979), Ivory Coast in 1999 (Akoua-Koffi et al. 2001), Cambodia (Heang et al. 2012), Thailand (Buathong et al. 2015), the Philippines (Alera et al. 2015), Indonesia (Perkasa et al. 2016), and Brazil (Calvet, Filippis, et al. 2016; Calvet, Aguiar, et al. 2016).

Serological surveys in Africa and Southeast Asia indicated a most likely silent Zika virus circulation with detection of specific antibodies in various animal species (large mammals such as orangutans, zebra, elephants, water buffaloes) and rodents (Geser et al. 1970; Jan et al. 1978; Lanciotti et al. 2008). However, so far the natural reservoir for Zika virus is unknown. Furthermore, serosurveys and viral isolation from mosquitoes and man had confirmed that before 2007, the geographical distribution of Zika virus was restricted to tropical Africa and Southeast Asia (Olson et al. 1983; Martinez-Pulgarin et al. 2016). Of note, in 2007, the first documented transmission and outbreak of Zika virus in man (with 185 confirmed cases), outside of its traditional endemic areas of Africa and Asia, was reported on Yap Island of the Federated States of Micronesia (Duffy et al. 2009).

Subsequently, between 2013 and 2015, several disease outbreaks were notified on islands and archipelagos from the Pacific region including a large scale outbreak in French Polynesia (Hancock et al. 2014; Musso, Nilles, et al. 2014; European CDC 2014; Attar 2016) and New Caledonia (Cao-Lormeau et al. 2014; Besnard et al. 2014; Teurlai et al. 2015; Tognarelli et al. 2015). In May 2015, the Pan American Health Organization (PAHO) issued an alert after the first confirmed Zika virus case in Brazil. The emergence of Zika virus in South America led to a rapid spread throughout South and Central America, reaching Mexico in November

2015 (Zanluca et al. 2015; WHO 2016). The first case of Zika virus infection in the USA was recorded recently in a traveler returning from Latin America to Texas (McCarthy 2016a). It has appeared sporadically in travelers from the United States of America and Europe but has not established person-to-person spread in those areas. As on 30 March 2016, 61 countries and territories were reporting local transmission of Zika virus (Zanluca 2015; Sabogal-Roman et al. 2015; Calvet, Filippis, et al. 2016; Petersen, , Wilson, et al. 2016; Roa 2016; Fauci & Morens 2016; Gulland 2016a).

In October 2015, Brazil public health authorities detected an unusual increase in human microcephaly cases in public and private healthcare facilities in Pernambuco state, Northeast Brazil. By 30th March 2016, 6776 suspected cases of microcephaly and/or central nervous system (CNS) deformations potentially linked to Zika virus have been reported in Brazil which are mainly concentrating in Northeastern parts. Furthermore, an increase in GBS has also been observed in Brazil, Colombia, El Salvador, Martinique, and Suriname (Campos et al. 2015). Lately, from the United States of America, locally acquired cases, around 14 in number without vector-borne transmission, likely to have been contracted by sexual contact, were reported (Musso, Roche, Robin, et al. 2015; McCarthy 2016b; McCarthy 2016c), and indirect evidence of local transmission were further documented in six additional countries (Lucey & Gostin 2016). There is not a single known case of someone contracting Zika virus from mosquitoes in the United States. Very recently in Thailand, a study showed that seven samples out of 21 tested showed immunoreactivity to Dengue, Chikungunya, Zika, and Japanese encephalitis by Western blot. Of note, two samples reacted only to Zika virus antigen confirming its presence in Thailand, which marked the first report of this virus in this part of the world (Wikan et al. 2016). Japan has also reported Zika virus in urine from a traveler visiting Thailand (Shinohara et al. 2016) and two earlier cases have been reported in traveler's returning from French Polynesia and Japan (Kutsuna et al. 2014). It was reported in an Australian returning from Indonesia. Hence, tourism and travel have also resulted in transmission of the disease (Kwong et al. 2013).

The geographical distribution of Zika virus has been steadily increasing since it was first detected in the Americas in 2015 (Gatherer & Kohl 2016; Hennessey et al. 2016). It is likely that Zika virus will continue to spread and will likely reach all countries and territories of the Americas where *Aedes* mosquitoes are found. In less than a year, Zika virus epidemic has evolved in a WHO-declared global health emergency (Jacob 2016; Kindhauser 2016). The research community urgently needs to understand why Zika virus has shifted from being a virus that caused mild illness to one associated with more than 4000 suspected microcephaly cases in Brazil (Petersen, , Wilson, et al. 2016), as well as possibly GBS (Lucey and Gostin 2016). The WHO has outlined its $56 million strategic plan to respond to the ongoing outbreaks of Zika virus disease in the Americas (McCarthy 2016d). The plan provides a framework for WHO and its partner organizations' Zika response through to June 2016. Recently, there are four cases of Zika virus reported from the United Kingdom which may be due to travel to affected countries (O'Dowd 2016).

The Zika virus-specific antibodies could be detected from serum samples of six out of 99 residents of Uganda and one out of 15 wild monkeys tested (Dick 1952). Subsequently, the antibodies against Zika virus could be detected in man in Nigeria (MacNamara 1952), Uganda, and Tanganyika (United Republic of Tanzania) (Smithburn 1952). During 1952−1953, in India, serum samples have been collected from residents of 38 localities in 6 states and tested for neutralizing antibodies against 15 different arthropod-borne viruses and a significant number of samples showed neutralizing activity against Zika virus (Smithburn, Kerr, Gatne 1954). Subsequently, the presence of antibodies against Zika virus was reported in several countries − Malaysia (Smithburn 1954), Borneo (Smithburn 1954), Vietnam (Pond 1963), Thailand (Smithburn 1954), Egypt (Smithburn et al. 1954), Philippines (Hammon et al. 1958), Somalia (Henderson et al. 1968), Kenya (Geser et al. 1970), Gabon (Jan et al. 1978; Saluzzo et al. 1982), Nigeria (Monath et al. 1973; Fagbami 1977; Fagbami 1979; Adekolu-John & Fagbami 1983), Sierra-Leone (Robin & Mouchet 1975), Indonesia (Olson et al. 1981), Central African Republic (Saluzzo et al. 1981), Pakistan (Darwish et al. 1983), Senegal (Renaudet et al. 1978; Monlun et al. 1993), Nigeria (Baba et al. 1999), Côte d'Ivoire (Akoua-Koffi et al. 2001),Yap Island, Federated States of Micronesia (Lanciotti et al. 2008; Duffy et al. 2009), Cameroon (Fokam et al. 2010), USA (Foy et al. 2011), Cambodia (Heang et al. 2012), Zambia (Babaniyi et al. 2015), and French Polynesia (Aubry et al. 2015).

In non-human primates, antibodies against Zika virus have been reported both in wild and experimental animals in Borneo (Smithburn 1954; Wolfe et al. 2001) and Uganda (Smithburn 1952; Dick 1952; Dick et al. 1952; Weinbren & Williams 1958; Haddow et al. 1964; Henderson et al. 1968; Henderson et al. 1970; Kirya & Okia 1977; McCrae & Kirya 1982; Rodhain et al. 1989; Duffy et al. 2009). Since serological cross-reactivity may occur between Zika virus and other Flaviviruses (e.g. Dengue, Yellow Fever, St. Louis encephalitis, Japanese encephalitis, West Nile, etc.), molecular assay in addition to serology such as RT-PCR should be used for distinguishing these viruses.

Until the large-scale outbreak of Zika virus in 2007 in the Yap Island (comprises four closely grouped islands and several outer islands) of the Federated States of

Micronesia in the North Pacific, 14 cases of human Zika virus were previously documented (Duffy et al. 2009) within Africa and Asia. The physicians on Yap Island noted an outbreak of illness characterized by rash, conjunctivitis, subjective fever, arthralgia, and arthritis. Ten out of 71 samples (14%) were confirmed as Zika virus positive by RT-PCR by CDC, Arbovirus Diagnostic, and Reference Laboratory in Fort Collins, Colorado, USA. Further investigation was carried out in the group of four main islands (total population of 7391 persons). From a total of 185 suspected cases, 49 (26%) were confirmed as positive and 59 (32%) classified as probable (based on a set of criteria mainly by detection of Zika virus RNA and serology) with an overall morbidity rate of 14.6 per 1000 Yap Island residents. No deaths or neurological complications were reported in this outbreak (Lanciotti et al. 2008; Duffy et al. 2009). Analysis of Zika virus RNA revealed that the outbreak on Yap Island originated in Southeast Asia (Lanciotti et al. 2008; Duffy et al. 2009; Haddow et al. 2012; Buathong et al. 2015).

In October 2013, Zika virus outbreak started in French Polynesia – a French overseas territory located in the South Pacific. A total of 8510 suspected cases were reported by the sentinel network from the beginning of the epidemic to 14 February 2014, leading the estimation at 29,000 (10% of the population), the number of patients having consulted for a Zika virus infection. The clinical symptoms observed in this outbreak were mild Dengue-like illness consisting of low fever (<38 °C), asthenia, wrist and fingers arthralgia, headache, rash, conjunctivitis, swollen ankles, and aphthous ulcers (Cao-Lormeau et al. 2014). Out of 746 patient samples, Zika virus RNA was confirmed in 396 (53%) by RT-PCR, in which 72 cases of severe presentations with acute neurological symptoms were notified. Among these, 40 GBS were also diagnosed within three months (Besnard et al. 2014; European CDC 2014; Cao-Lormeau et al. 2014; Ioos et al. 2014). The phylogenetic tree shows that the Zika virus that recently emerged in French Polynesia is similar to Cambodia 2010 and Yap State 2007 strains, which suggests an expansion of Zika virus of Asian lineage (Cao-Lormeau et al. 2014). The results of retrospective investigations were submitted to WHO. These reports indicate a possible association between Zika virus infection and congenital defects, severe neurological and autoimmune complications, in particular, an increase in the incidence of GBS. However, the Island was also experiencing an outbreak of Dengue in association with Zika virus infection and GBS (Ioos et al. 2014; Kindhauser et al. 2016). Further, in 2013–2014, the outbreak spread to other Pacific Islands: New Caledonia, Cook Islands, Easter Island, Vanuatu, and Solomon Islands (Roth et al. 2014; Cao-Lormeau et al. 2014; Musso, Nhan, et al. 2014).

The first autochthonous cases of Zika virus in Brazil (in the city of Natal, state of Rio Grande do Norte) was confirmed in May 2015. In the year 2015, a total of Zika virus cases in Brazil amounted to the tune of 440,000–1,300,000, along with 4783 cases of microencephaly, where 76 were associated with mortality in the northeast province of Brazil (Heukelbach et al. 2016). Symptoms observed in these cases were arthralgia, oedema of extremities, mild fever, maculopapular rashes frequently pruritic, headaches, retro-orbital pain, no purulent conjunctivitis, vertigo, myalgia, and digestive disorders. Eight out of 21 samples were positive for Zika virus RNA and shared a high nucleic acid identity with sequences from the Zika virus of Asian lineage (Zanluca et al. 2015). Since then, cases of Zika virus are being continuously reported from several states. The major concern is a several fold increase in infants with microcephaly after the introduction of the Zika virus into Brazil. Before 2015, the annual reported cases of microcephaly were consistently below 200 whereas the number of suspected cases reported between November 2015 and 13 February 2016 was 5280. In the initial screening of 1345 samples, Zika virus was detected in 41 samples (Victora et al. 2016) (http://apps.who.int/iris/bitstream/10665/204454/1/zikasitrep_19Feb2016_eng.pdf?ua = 1). Between 1 January 2007 and 17 February 2016, a total of 48 countries and territories reported local (autochthonous) transmission of Zika virus (http://apps.who.int/iris/bitstream/10665/204454/1/ zikasitrep_19Feb 2016_eng.pdf?ua = 1).

The Zika virus transmission can be classified into vector-borne and non-vector-borne. In the vector-borne mode, several species of the genus *Aedes* are involved. The non-vector-borne mode includes sexual transmission, blood transfusion, perinatal or intra-uterine transmission, postnatal transmission, and animal bites (Patiño-Barbosa et al. 2015).

Zika virus is maintained in the community through its – (1) sylvatic cycle, where the virus circulates between non-human primates and *Aedes* mosquitoes, (2) urban cycle, where the virus circulates between man and domestic mosquitoes. Till the year 2007, the most known facts about zika outbreaks demonstrate sylvatic origin/cycle (Weaver et al. 2016). The primary mode of transmission of vector-borne Zika virus is through the bites of female mosquitoes in the *Aedes* genus. The main species in the Zika virus mosquito transmission is *Aedes aegypti* and there are at least 20 other species that have been tested positive to Zika virus including *Aedes albopictus, A. africanus, Aedes luteocephalus, Aedes vitattus, Aedes furcifer, Aedes hensilii, Aedes apicoargenteus, Aedes. polynesiensis, A. dalzieli, Aedes hirsutus, Aedes metalicus, Aedes taylori, Aedes unilineatus, A. apicoargenteus, Anopheles coustani, Culex perfuscus, and Mansonia uniformis* (MacNamara 1952; Dick et al. 1952; Dick 1953; MacNamara 1954; Weinbren & Williams 1958; Haddow et al. 1964; Simpson 1964; Fagbami 1979; Haddow et al. 2012; Petersen, Wilson,

et al. 2016; Zanluca & dos Santos 2016; Li et al. 2016). Male mosquitoes live only for 10 days while female mosquitoes live for one to two months suggesting that female mosquitoes have a role in transmission and harbouring of the virus (Chang et al. 2016). Since the *Aedes* mosquitoes are found in many parts of the world, it is likely that outbreaks of Zika virus might spread to new naïve areas (Petersen, Wilson, et al. 2016). Transmission potential of Zika virus was found to be similar to Dengue and Chikungunya since mosquito is mainly involved in all three diseases (Nishiura et al. 2016). Recently, in Senegal mosquito species like *A. unilineatus, A. vittatus,* and *A. luteocephalus* were studied for their vector competence and it was found that all species were susceptible to infection by oral route (Diagne et al. 2015). A study in Yap Island revealed that *A. hensilli* was the most important vector for transmission of Zika virus and Chikungunya (Ledermann et al. 2014). Similarly, a study from Singapore reports that *A. Stegomyia* is the potential vector for Zika virus transmission (Li et al. 2012).

In August 2008, two American scientists who worked in the village of Bandafassi in southeastern Senegal while performing a mosquito-sampling project in surrounding villages, after returning to the USA, showed Zika-like clinical illness within a week. Furthermore, the wife of one of the above researchers also showed clinical signs but she never traveled to Africa or Asia and had not left the United States of America since 2007. The Zika virus infection was confirmed by a serological assay. Circumstantial evidence suggests direct person-to-person, possibly sexual, transmission of the virus (Foy et al. 2011). In December 2013, during a Zika virus outbreak in French Polynesia, a patient in Tahiti sought treatment for hematospermia, and replicative Zika virus could be detected from semen samples (Musso, Roche, Robin, et al. 2015). Sexual transmission of Zika virus is not assumed to be a major factor in its spread. Over 80% of Zika virus infection is silent in persons without overt clinical signs including blood donors. Blood-borne transmission of Zika virus via blood transfusion is a major concern (Marano et al. 2015; Petersen, Wilson, et al. 2016). From November 2013 to February 2014, 42 (3%) of 1505 blood donors, although asymptomatic at the time of blood donation, were found positive for Zika virus by PCR (Musso, Nhan, et al. 2014).

During Zika virus outbreak in French Polynesia, two mothers and their newborns had detectable Zika virus RNA in serum collected within four days post-delivery by RT-PCR. The infants' infection most probably occurred by transplacental transmission or during delivery (Besnard et al. 2014). Zika virus RNA could be detected from some of the microcephaly samples (Mlakar et al. 2016; Rubin et al. 2016) and from amniotic fluid of two pregnant women with microcephaly fetuses in Brazil (Calvet, Aguiar, et al. 2016). In the latter study, the virus could be isolated. The other plausible mode of transmission is monkey bite (Leung et al. 2015). Serological studies reveal that Zika antibodies can be detected in goats, sheep, bats, and rodents. Serosurvey studies have detected antibodies to Zika virus in bats, goats, and rodents of the species *Bandicota bengalensis, Tatera indica,* and *Meriones hurrianae*, but such serological data may be misleading since there is cross reaction between flaviviruses, however it cannot be excluded (Musso & Gubler, 2016). Hence, there is no well-documented reservoir animal for Zika and further studies will reveal such animals if found so.

6. The disease

6.1. Clinical symptoms

Zika virus has an incubation period of 3−12 days (Burke et al. 2016; Goeijenbier et al. 2016). Clinical signs include acute fever, maculopapular skin rashes, non-purulent conjunctivitis, arthralgia, headache, myalgia, asthenia, edema of hand and feet and less evident signs like anorexia, abdominal pain, vomiting, diarrhoea, vertigo, burning sensation of sole and palm and at times there may be pain in the retro orbital region, and pruritits (Korhonen et al. 2016; Shinohara et al. 2016; Zammarchi, Stella, et al. 2015; Zammarchi, Tappe, et al. 2015**;** Basarab et al. 2016; Goeijenbier et al. 2016). Symptomps are usually self-limiting and may last for four to seven days (Barrera-Cruz et al. 2016). Haematuria and haematospermia (there is no report of haematospermia caused by other arboviruses) have been documented in some cases (Foy et al. 2011; Musso, Roche, Robin, et al. 2015). These clinical signs are overlapping with the clinical findings of Dengue and Chikungunya, but Zika virus-induced symptoms are milder than those of others (Carneiro & Travassos 2016; Tappe et al. 2016). Abrupt onset of fever and appearance of rashes, often pruritic which disappear quickly with time, are characteristic features. Occasionally, Zika virus may cause nerve-associated abnormalities tentatively referred to as GBS (Oehler et al. 2014; Gatherer & Kohl 2016; Pinto et al. 2015). Infected pregnant woman traveling to an area where Zika virus outbreak has recently occurred should be routinely subjected to ultrasound to assess the abnormal cranial development of the fetus (Schuler-Faccini et al. 2016; Dyer 2015; Dyer, 2016b; McCarthy 2016e). If the infants are getting an infection from their mother *in utero*, the virus has been reported to be a reason for the foetal** abnormalities like microcephaly, intracranial calcification, hydrops fetalis, hydranencephaly, and eye abnormalities mainly affecting macular and optic nerve development (McCarthy 2016f; Sarno et al., 2016; Vest 2016). A study reported 29% of the 42 pregnant women infected with Zika virus had fetal abnormalities revealed ultrasonically and 12% of the

examined womens foetus had microcephaly, cerebral atrophy, and brain calcifications (Driggers et al. 2016). There was no adverse effect seen on retinal development in such infants (Ventura, Maia, Bravo-Filho, et al. 2016; Schuler-Faccini et al. 2016; Ventura, Maia, Ventura, et al. 2016). In the case of no cranial abnormalities in infants born from infected mothers, they need to be checked during the first year of life routinely by periodic cranial ultrasound, routine eye checkup, and hearing tests to diagnose subclinical cases (Staples et al. 2016). Microcephaly has been reported mainly from Latin American countries though Africa and Asia are endemic and the hypothesis suggested was that women exposed to zika virus at early age gain immunity against zika hence may resist fetal anomalies (Frank et al. 2016). Recently, hypertensive iridocyclitis in Zika virus infection has been reported (Fontes 2016). An ocular abnormality has been noticed which include chorioretinal atrophy and also gross pigment mottling as well as hypoplasia of the optic nerve (Ventura, Maia, Ventura, et al. 2016). Co-infection with Zika virus, Chikungunya, and Dengue virus has been reported in Colombia (Faccini-Martínez et al. 2016; Villamil-Gómez et al. 2016). An overview of Zika viral disease is presented in Figure 5.

6.2. Pathology and pathogenesis

Zika virus isolates from French Polynesia exhibited their affinity towards human immature dendritic cells, dermal fibroblasts, and epidermal keratinocytes. Zika virus may replicate in the midgut when Aedes mosquitoes feed blood meal as like other flaviviruses and later in the salivary glands. Vertical transmission of Zika virus by transovarian route also cannot be excluded (Chan et al. 2016). Aedes mosquito injects Zika virus while feeding on man and the virus enters the cells through receptors like AXL, DC-SIGN, Tyro3 and Tim-1 (lesser extent) that are found on the surface of both skin and nerve cells (Hamel et al. 2015). Inside the cells, they use host machinery and finally cause apoptosis and autophagy of the cells thereby entering other cells. Zika virus stimulates transcription of TLR-3, MDA5, RIG-I, and also interferon genes like OAS2, ISG15, and MX1. Viral replication in the cells causes the release of type I interferon (Hamel et al. 2015). Replication of Zika virus is increased by inducing autophagy in the host. Thus, agents inhibiting autophagy can decrease virus load in the cells (Carneiro & Travassos 2016). After replication locally, virus gets distributed to muscles, heart, CNS, and also to foetus by crossing the

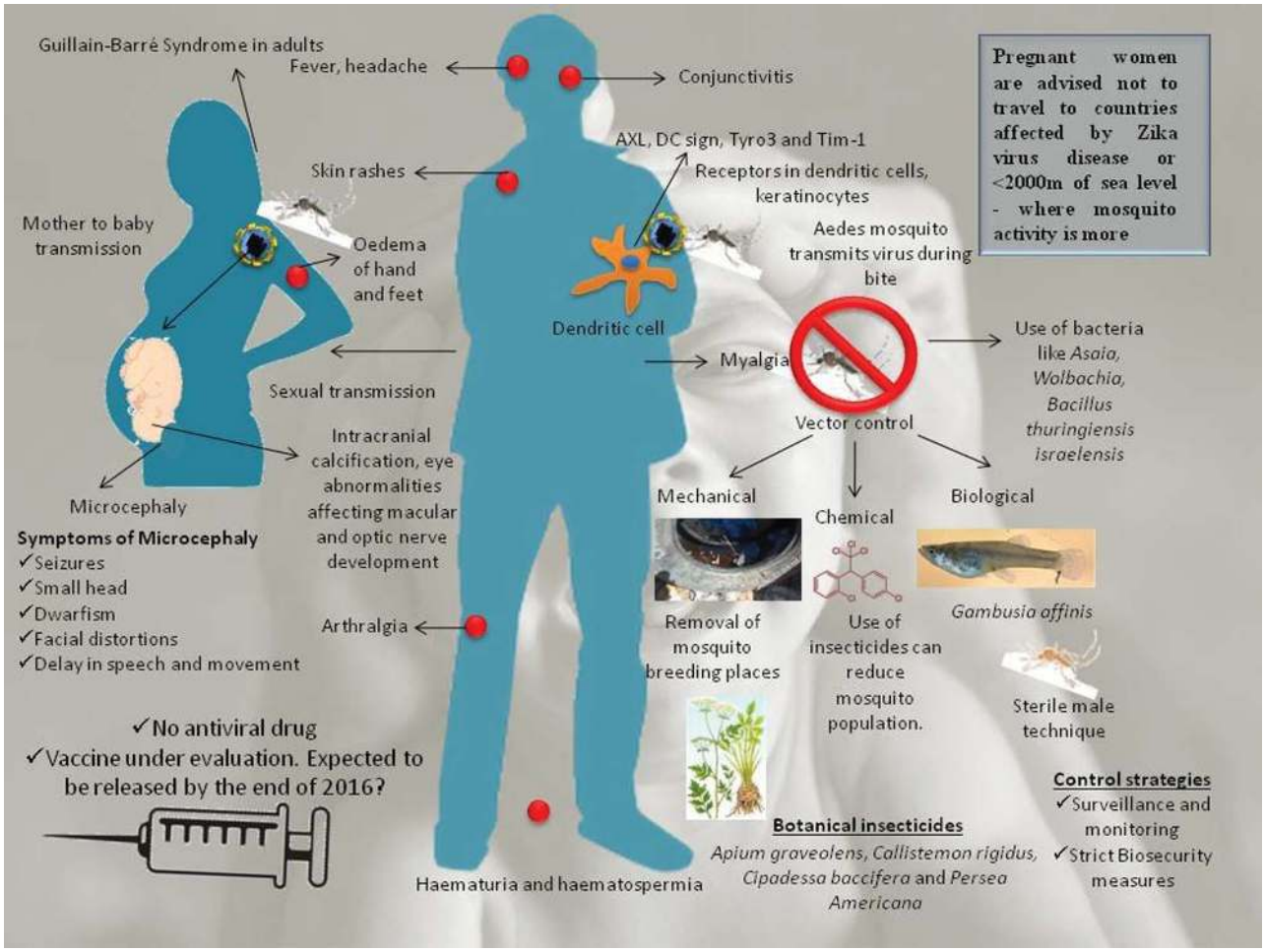

Figure 5. An overview of Zika viral disease and its prevention and control.

placental barrier through blood vessels and lymphatics (Chan et al. 2016). The damage in the eye of infants is presumed to be due to Zika virus infection and needs further detailed pathophysiological investigations (McCarthy 2016f). Zika virus might have evolved certain means to defeat the defence of the trophoblast thereby attacking neuronal tissues causing neuronal abnormalities in the fetus (Sadovsky et al. 2016).

In earlier reports, Zika virus had been shown to have affinity towards brain cells which was demonstrated with the aid of intraperitoneal injection of mice that showed that Zika virus can cross the blood−brain barrier (Dick 1952). Neurons and glial cells were infected by virus producing intracytoplasmic inclusions called viral factories having their origin from the endoplasmic reticulum and also from mitochondria and nucleus (Bell et al. 1971). Recent studies indicate that Zika virus can infect neuroblast cells *in-vivo* (Tang et al. 2016). Autophagy, as noticed in the dermal fibroblast cells is not noticed in the neurons. Microcephaly has been proposed to be due to abnormalities in the centrosomes yet several studies need to be conducted before confirmation (Tetro 2016). There was enlargement of astrocytes and there was also the destruction of hippocampus especially regarding pyriform cells (Bell et al. 1971). Inflammatory responses of the placenta due to Zika virus can also cause damage to the fetus (Mor 2016). Transient leukopenia and thrombocytopenia have been reported in few cases. Serum biochemistry shows that there may or may not be an increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities (Tappe et al. 2014).

6.3. Immunobiology and immunity

Antibody-dependent enhancement (ADE) of infection is common among Dengue virus serotypes. The recent outbreak of Zika virus has caused more panic than earlier outbreaks and one reason that can be related is that most of the recent outbreaks occurred in hyper-endemic regions of Dengue virus. ADE may be a reason where antibody raised against Dengue virus might have caused enhancement of Zika infection (Lazear & Diamond 2016). Immunity against re-infection develops for an unidentified time period (Tappe et al. 2015). The increase in chemokines is shown at a higher level than cytokines in Zika viral disease and cytokines level declined during the recovery phase. During acute disease, there is a rise in IL-2, IFN-γ, IL- 4, IL-13, IL-17, and IL-9 (Tappe et al. 2015). Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES) or CCL5 was elevated in the acute phase of disease while IP-10 was elevated in the later recovery phase (Tappe et al. 2015). Further studies are needed to elucidate the immune response of host against Zika virus disease. Recently, a study revealed that Zika virus and man have a peptide in common, hence this may be the reason for microcephaly and GBS (Lucchese & Kanduc, 2016).

7. Public health importance

Zika virus disease has been a major threat throughout the world, but it has shown its hideous face in Brazil. It is not only Zika virus but also Dengue and Chikungunya that have caused damage in Brazil. A recent report shows that there is co-infection of Zika, Dengue, and Chickungunya in Pernambuco, Brazil (Pessôa et al. 2016). This has kindled interest among researchers why these diseases affect mainly this part of the world. The study shows that it was mainly due to climate change causing an alteration in the vector population (Gautret & Simon 2016). Crowding at a particular place due to meetings like World cup, Olympics, etc. could also aid in transmission of diseases. Good knowledge about the diseases prevailing in the native population should be identified and they should be segregated so as to prevent transmission to the tourists. Arboviral diseases like Dengue, Chikungunya, and recently, Zika have created panic amongst tourists who planned to visit countries like Brazil where disease threat is more predominant (Gautret & Simon 2016). FIFA world cup was held in Brazil in the year 2014 and several studies were conducted to assess the arboviral diseases influenced by it and it was found that four to nine people out of 100,000 tourists who visited Brazil succumbed to Dengue (van Panhuis et al. 2014). Some researchers are also of the opinion that Zika virus has also entered during FIFA world cup 2014 into Brazil, but there is no evidence to support this speculation (Zanluca et al. 2015). Another major sports event took place later in August 2014 named as 'Va'a World Sprint Championship canoe race' where there were participants from French Polynesia, New Caledonia, Cook Islands, and Easter Islands, hence this was also speculated for the reason of disease introduction into Brazil. Hypothesis and possibilities were made that people from Chile has transmitted this disease to Brazil during this event (Salvador & Fujita 2015). It was speculated that Zika virus might have entered northeast of Brazil through Natal, Recife, Salvador, and Fortaleza where the 2014 World cup football took place. Phylogenetic analysis by researchers revealed that Zika virus might have been introduced in the year 2013 around May to December. By the end of 2015, Zika virus has spread the entire length of Brazil (Weaver et al. 2016). Summer Olympics 2016 is around the corner and Zika virus has pressed the alarm button on all health and sports officials in Brazil to look after the health issues of athletes traveling from different parts of the world (Citrome 2016).

8. Diagnosis, surveillance and monitoring

Zika virus infection can be diagnosed based upon clinical symptoms, the prevalence of vector in the region, and by serological and molecular detection assays. The isolation of Zika virus from mosquito samples was carried out using newborn Swiss albino mice through different routes like intraperitoneal, intracerebral, and subcutaneous injections (Marchette et al. 1969; Way et al. 1976). Passages from brain material revealed Zika virus which was further confirmed using hemagglutination inhibition test (Haddow et al. 1964). Zika virus can be cultivated in cell lines like Vero cells, rhesus monkey kidney cells like LLC- 192 MK2, and cell lines of mosquito origin like *A. albopictus* (C6/36) and *A. psuedoscutellaris* (MOS61 or AP-61) (Waggoner & Pinsky 2016). Vero and Vero E6 cells were used for isolation of Zika from saliva (Barzon et al. 2016). Recently, a murine model for studying Zika virus has also been reported which could aid in understanding the disease pattern (Rossi et al. 2016). Haemagglutination inhibition test, serum neutralization test, and complement fixation tests have been employed for the diagnosis of Zika virus disease (Fagbami 1979; Monath et al. 1980). The serological diagnosis involves detection of IgG and IgM antibodies. For detecting antibodies against Zika virus in serum of patients, IgM antibodies can be monitored by ELISA. It is positive if Zika virus neutralizing antibody titre is higher than fourfold from Dengue neutralizing antibodies, else the test is considered to be inconclusive (Staples et al. 2016). Cross reaction between other members of the flavirus hinders the use of ELISA techniques and hence plaque reduction neutralization test (PRNT) can be done to identify Zika virus induced antibodies (Petersen, Jamieson, et al. 2016). PRNT helps in distinguishing cross-neutralizing antibodies generated by various Flaviviruses (Staples et al. 2016). Seroconversion is confirmed by analyzing IgM and IgG titres in paired serum samples from the acute and convalescent phase of infection (Pyke et al. 2014). Earlier haemagglutination inhibition and mouse protection tests were also used to detect Zika virus antibodies in serum (Fagbami 1979; Kirya & Okia 1977).

Microcephaly is detected by measuring occipitofrontal circumference as suggested by standard charts (WHO 2006). Ultrasound scanning can be employed for detection of microcephaly in pregnant women (SMFM Publications Committee 2016; Oliveira et al. 2016). Several foetal abnormalities can be identified around five months of gestation, but microcephaly can only be identified around fix toseven months of gestation or after birth (Lazear et al. 2016). Further confirmation can be done using molecular and serological tests (Staples et al. 2016).

Blood picture reveals neutropenia and thrombocytopenia (Rafiei et al. 2016). Molecular diagnosis involves detecting RNA of Zika virus by RT-PCR. In the initial phase of the disease (first seven days) viral nucleic acids can be detected in the serum (Haug et al. 2016). Sample involves serum of patients or umbilical cord sample of infants, urine, nasopharyngeal swab, saliva, amniotic fluid, CSF, and frozen and fixed placenta (Staples et al. 2016; Shinohara et al. 2016; Tognarelli et al. 2015; Leung et al. 2015; Musso, Roche, Nhan, et al. 2015; Gourinat et al. 2015; Balm et al. 2012; de M Campos et al. 2016). Shedding of virus in different body fluids like urine (persists for 15−20 days) has to be elucidated to establish a better diagnostic assay combined with exact sample of choice (Moulin et al. 2016). A report suggests that viruria can be seen more than 15 days after the onset of symptomps (Rozé et al. 2016). Recently, in order to establish the relationship between Zika virus and microcephaly, scientists started to investigate antibodies against Zika virus in amniotic fluid and also in the blood of neonates (Jacob 2016). RT-PCR followed by DNA sequencing is considered confirmatory (Zanluca et al. 2015). Further sequencing of NS5, NS3, and envelope gene confirms the relation of various strains of Zika virus (Tognarelli et al. 2015; Fonseca et al. 2014; Grard et al. 2014). Real-time PCR (rRT-PCR) can be employed to detect the virus in the early stage of infection (Faye et al. 2013; Faye et al. 2008). Furthermore, it can be confirmed by employing immunohistochemical staining of antigen fixed on the umbilical cord and placenta of infected infants (Staples et al. 2016).

The rapid spread of the Zika virus and its epidemic potential along with the presence of other arboviruses create difficulties in the differential diagnosis (Pinto et al. 2015). Till now, no commercial diagnostic tests exist for diagnosing this viral infection. Advances in diagnostic techniques need to be applied for Zika virus detection viz., multiplex PCR, LAMP, recombinant diagnostics, gene sequencing and phylogenetic analysis, biosensors, biochips, microarrays, and nanotechnology-based diagnostics (Jain 2007; Kawadkar et al. 2011; Dhama, Karthik, et al. 2014; Ratcliff et al. 2007; Bergquist 2011; Van den Hurk and Evoy 2015). Apart from these newer tools for extensive epidemiological and surveillance studies, alternatives need to be explored for tracking and monitoring Zika virus infections and spread, such as geographical information system and establishing networking programs to counter this important pathogen. More recently, a mobile App named as ZIKA-Tracker (zikatracker.net) has been developed in four languages to report the cases as they occur so that they can be effectively controlled and provided on time (Kelvin et al., 2016). Such early reporting helps in understanding the pattern of spread of the disease from one area to another. It is essential to use such techniques to report Zika virus cases at earliest possibility keeping in mind the rapid spread of the virus (Nishiura et al. 2016). All developments and timely progress would help in rapid detection of Zika virus and design effective disease prevention and control programmes.

9. Prevention and control strategies

Prevention and control of Zika virus are mainly aimed at prevention of vector population (mosquitoes) as they play an important role in the transmission of this virus (Ahmad et al. 2016). Control measures are similar to the measures adopted for Dengue and Chikungunya viruses that are also transmitted by vectors (Pinto et al. 2015). Mosquito of the genus *Aedes* is the vector transmitting Zika virus and also another important vector-borne disease namely Dengue (Araújo et al. 2015). Hence, control of vector population is of prime importance to control the transmission of these important dreadful diseases among humans. Day-time avoidance of the mosquito bites from infected patients can reduce a human-to-mosquito transmission thereby preventing transmission to other humans. These vectors can be controlled either by mechanical, chemical, and biological measures (Araújo et al. 2015).

Mechanical control methods involve removal of any objects that can aid in unwanted storage of water in the premises that serve as a breeding point for female mosquitoes. Removal of those objects like plastic bags, unused tyres, unused containers, bottles, and also closing water tanks with lids can prevent breeding areas of the mosquitoes (Araújo et al. 2015). Chemical control of insects and vectors is an age-old technique and can be used with caution as it can cause toxicity to animals (Dick et al. 2012). Recently, chemicals like pyrethroids, organochloride, and organophosphorus which acts on the nervous system of the vectors are being used to prevent mosquitoes. The major disadvantage in the use of these chemicals is the problem of resistance development and also these compounds are toxic to higher mammals thereby questioning its use for the control of vectors (CDC 2012). Preparations like N,N-diethyl-meta-toluamide (DEET) and picaridin insect repellent can be used as they are safe for pregnant women (Kline & Schutze 2016). Herbal therapy has not only been revolutionizing as an antimicrobial regimen but also has been implemented recently in the control of vectors. These botanical insecticides can be produced from leaves, seeds, and even fruits of different plant varieties (Ghosh et al. 2012). Some of the plants that can be used for extraction of botanical insecticides include *Apium graveolens*, *Callistemon rigidus, Cipadessa baccifera,* and *Persea Americana* (Kumar et al. 2014; Pierre et al. 2014). Extracts can be isolated from these plants and can be employed to find the cidal action against larvae and other stages of the mosquitoes so that it can be employed as a safe insecticide (Dias et al. 2014). These extracts can also be used as a repellent in the household day-to-day to effectively control mosquito bites so that prevention of deadly diseases like Zika virus becomes easy (Ramkumar & Karthi 2015). *Cymbopogon citratus* and *Eucalyptus citriodora* possess a principle named Citronella which has been used in mosquito repellents these days. Another study also reported *Apium graveolens* seed oil has a good repelling power against *Aedes* mosquito (Kumar et al. 2014). Biological control of vectors might also involve the use of bacteria namely *Bacillus thuringiensis israelensis* producing deadly endotoxins when mosquitoes ingest those bacteria (Boyce et al. 2013). Fungi like *Beauveria bassiana* has also been used to control *Aedes* mosquitoes (Darbro et al. 2012). Another approach is the use of larvivorous fishes (e.g. *Gambusia affinis*) in the water-logging areas and flower pots which can eliminate larvae of mosquitoes (Kant et al. 2013).

Lately, the use of microbes to control vector population gained interest and certain genus of bacteria named *Asaia* has been explored for its ability to colonize the gut and reproductive tract of mosquitoes and also to get transmitted vertically to its progeny (Favia et al. 2008). Another bacterium named *Wolbachia* has the potential to feminize male vectors thereby preventing the reproduction of vectors hence controlling their population (Brelsfoard & Dobson 2009). Trails are on the way using this bacterium and sterile males have been released into the environment to monitor the effect on reproduction (Frentiu et al. 2014). Trails are conducted in Australia, Vietnam, Indonesia, and also in Brazil using these sterile males created by the use of *Wolbachia* (Blagrove et al. 2012). *Wolbachia*-infected mosquitoes need to be realesed over a wide geographical area as the *A. aegypti* has a poor flight range and also, it can evolve mechanisms to resist *Wolbachia* (Weaver et al. 2016). Other techniques like irradiation have been carried out to generate sterile males to control the mosquito population (Alphey 2014).

Genetic modification of mosquitoes is another approach that can be used to control its population by interrupting their reproduction. Two different approaches can be adopted to achieve this as— (1) suppression or complete eradication of the vectors with the aid of genes that can kill the vector or make them sterile and (2) substitution method where a constructed gene will prevent the transmission of disease (Reis-Castro 2012; Carvalho et al. 2014). The approach of using sterile male is not new since it is an age-old approach which has successfully aided in local control of certain vectors like *Cochliomyia hominivorax* (New World screwworm fly) in the United States of America, Central America, and Mexico, the *Ceratitis capitata* (Mediterranean fruit fly) and *Pectinophora gossypiella* (pink bollworm) in the United States of America, *Cydia pomonella* (codling moth) in Canada and *Glossina fuscipes* (tsetse fly) in Zanzibar (Alphey et al. 2010; Burt 2014). Use of gamma irradiation that can damage the DNA leading to mutation has been the theme of sterile male techniques (Alphey 2014). Use of sterile male mosquitoes of *A. aegypti* in the Grand Cayman (first experiment of its kind) resulted in around 80%

reduction in the population of wild mosquito (Harris et al. 2012). Oxitec released in a trial in Piracicaba, Brazil, an engineered mosquitoe with 'self-limiting' gene that halts the reproduction of other mosquitoes by not allowing their offsprings to develope into adult mosquitoes, revealed 82% larval reduction during last year (Al-Qahtani et al. 2016).

Apart from vector control, proper surveillance and monitoring has to be carried out at the highest level in countries where there are reports and also countries adjoining them. Strict biosecurity measures need to be ensured so that disease transmission can be kept under check (Musso & Nhan 2015). All international airports, harbors, and places of international tourist attraction is needed to be under the scanner for the screening of this important disease. Especially pregnant woman should be advised not to travel to countries under threat of Zika viral disease and is advised to avoid travel to areas below 2000 m of sea level since a study reported that mosquito-borne Zika viral infection is possible above 2000 m (Petersen et al. 2016c; Cetron 2016). Several guidelines have been issued periodically by CDC and other health departments regarding do's and dont's during an outbreak (Oster et al. 2016; Ahmad et al. 2016; Fleming-Dutra 2016) and need to be strictly followed. Since sexual transmission has been reported recently in humans, guidelines have been issued to both men and women that have to be adopted to prevent further spread. These guidelines include: (1) men returning from an area of Zika disease should avoid or have safe sex with partner (Oster et al. 2016), and (2) a recent study also reports that Zika virus has been detected in semen though it could not be detected in blood hence men who traveled to countries under disease outbreak or who had been bitten by *Aedes* mosquitoes should be screened since cases are mostly (80%) asymptomatic (Musso, Roche, Robin, et al. 2015). Summer Olympics 2016 is around the corner hence thorough surveillance is needed as Brazil is worst hit by this virus so as to prevent further damage to the human population (Bogoch et al. 2016). An overview of prevention and control of Zika virus is presented in Figure 5.

10. Vaccines

Vaccines are not yet available to counter the Zika virus infection (Borchardt 2016). The development of Zika virus vaccine requires a long time-line process. Only after the recent emergence and high public health concerns associated with this virus, efforts are being made towards developing a vaccine which could be in production by this year end (Dyer 2016a; O'Dowd 2016). As a front-runner in the race for a Zika virus vaccine development, this perspective Zika virus vaccine could easily be produced to very high levels in a short time period, and could meet the demand to some extent of any sudden emergency appearing in future. Around 23 groups from different countries are working on the development of vaccine against Zika (Gulland 2016c). Approaches employed for the development of vaccine against three Flaviviruses (Yellow fever virus, Japanses encephalitis virus, and tick-borne encephalitis virus) can be used for zika vaccine development. An ideal Zika virus vaccine should have important qualities like administration of a single dose vaccination protecting from Zika viral disease, a safe vaccine which can be used in pregnancy, and rendering long lasting immunity (Palacios et al. 2016). Apart from conventional vaccines (live attenuated or inactivated), newer and advanced technologies of vaccine development need to be explored to their full potential for developing effective vaccine against Zika virus such as DNA vaccines, subunit vaccines, recombinant vaccines, vectored vaccines, transgenic plant vaccines, reverse vaccinology, virus−like particles, gene/mutant−deleted vaccines, nanovaccines, and adjuvanted vaccines which are showing promising results for countering various infectious pathogens (Petrovsky and Aguilar 2004; Dhama, et al. 2008; Dhama, Wani, et al. 2013; Delany et al. 2014; Finco and Rappuoli 2014; Kim et al. 2014; Reed et al. 2013; Singh et al. 2015; Zhou et al. 2014; Pany et al. 2015; Ulmer et al. 2015). A futuristic perspective needs to have a better understanding of the immune mechanisms, immunobiology, pathogenesis and molecular characterization of Zika virus, necessary modifications of vaccine components with immunogenic antigens, and incorporating modern adjuvants such as TLRs, improving the vaccine delivery systems and appropriate immunomodulatory approaches.

11. Treatment

Currently, there is no antiviral drug that can save the human population against potential pandemic threats of Zika virus. Only supportive treatment is in use for Zika virus disease like rest, use of fluids, and analgesics to reduce pain and antipyretics to reduce fever (Petersen et al. 2016c). Very recently, there is a report from China on the use of Xiyanping injection combined with supportive therapy with promising results (Deng et al. 2016). Xiyanping is a semi-synthetic component extracted from *Andrographis paniculata*, a Chinese herb (Xiao et al. 2013). The chemical component present in Xiyanping is 9-dehydro-17-hydro-andrographolide and sodium 9-dehydro-17-hydro-andrographolide-19-yl sulfate and possesses anti-inflammatory and antiviral activity (Chong et al. 2013). Antipyretics-like acetaminophen or dipyrone can be used to reduce fever, while aspirin should be avoided to reduce hemorrhages until confirmation of Dengue (Rasmussen et al. 2011; Petersen, Staples, et al. 2016). Although aspirin and other non-steroidal anti-inflammatory

drugs are not typically used in pregnancy, these medications should specifically be avoided until Dengue is ruled out (Petersen, Staples, et al. 2016). Pregnant women with confirmed Zika viral disease should regularly have a check up of the fetus to assess its health (Petersen, Staples, et al. 2016). Review of the literature reveals that upcoming and emerging therapeutic regimens have not been explored yet for possibilities to treat Zika viral disease, as have been studied recently with other infectious pathogens and are showing promising therapeutic values for treating various diseases including viral diseases. Like for countering recent pandemic threats of flu viruses (bird flu and swine flu), Tamiflu was developed and similarly for Ebola virus emergency ZMapp (humanized mouse antibodies) was developed. There is also a dire need to find out the suitable antiviral drug for prevention and control of Zika virus. Hence, seeing the public health significance of this virus and global concerns, research needs immediate attention to exploit valuable therapeutic options of novel and emerging/upcoming regimens such as cytokines, RNA polymerase inhibitors, microRNA (mi-RNAs), small interfering RNA (si-RNA), avian egg yolk antibodies (IgY), phages, toll-like receptors (TLRs), probiotics, herbs/plant extracts, nutritional immunomodulation, immunotherapeutic, and nanotechnology-based treatments along with attempting for drug discovery and developments (Blecher et al. 2011; Kawadkar et al. 2011; Dhama, Chakraborty, et al. 2013; Dhama,,Chakraborty, et al. 2014; Dhama, Saminathan, et al. 2015; Mahima et al. 2012; Malik et al. 2013; Junquera et al. 2014; Tiwari et al. 2014). For this purpose, much research and clinical trials are required to find a suitable and/or novel therapeutic to treat and control the disease

12. Conclusion and future prospectives

A few years back, Zika virus was thought to be innocuous but since its emergence into pandemic status, now the researchers are searching globally for a solution to stop its spread. The main mode of transmission of Zika virus is mosquito species (*Aedes*), which is also serving as a vector for other related Flaviviruses such as Dengue and Chikungunya. Although Zika virus is currently a concern in Western Hemisphere, it may not take the time to reach the remaining parts of the world, due to its vector and rapid movement of the human population globally. Though there are also other modes of virus transmission such as intrauterine, sexual intercourse, and blood transfusion, priority should be given to the mosquito control. For example, the use of *Wolbachia pipientis* – a common bacterium that infects mosquitoes as well as genetically modified mosquitoes – may reduce the spread of these viruses. However, much research needs to assess their effectiveness and safety. Since the pathogenic potential of Zika virus is comparatively new, not much information is available regarding its genetic characterization which is most important in understanding – why the virus became virulent?, What kind of mutation is involved regarding its virulence?, What are molecular differences between mosquito, mice, and human adopted by viruses? The other areas of interest are host–pathogen interaction studies which will provide the answers to how the viruses are crossing the placental barrier? Why it causes microcephaly and/or neurological disorders? Why is the Brazilian population more susceptible to Zika virus infection especially regarding fetal abnormalities? As the currently available serological assays including neutralization assay are cross-reactive with other related Flaviviruses such as Yellow Fever, Dengue, and Chikungunya, a subsequent confirmatory test is necessary. Therefore, identifying the unique portion of Zika virus genome and developing recombinant-based diagnostic assays are in immediate need. Abortion has been banned in many countries of the world, hence strict precautionary measures needs to be undertaken by the pregnant women residing in the countries with Zika virus disease so as to prevent damage to the foetus. Currently, there is no vaccine available and scientists should focus on isolation and development of vaccine on war foot basis. Currently, detailed epidemiological data are scarcly available and these should be extended, including developing countries, to be able to determine the true prevalence of the virus and disease manifestations within their borders. Can domestic animals get infected with Zika virus? – the veterinary community should think about it and start the research immediately. Finally, researchers and policy-makers should be generous enough to share their data between researchers/countries.

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Authors declare that there is no conflict of interest that could possibly arise.

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