Review

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Review

Zika Virus, an Emerging Arbovirus in India: A Glimpse of Global Genetic Lineages

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Abstract: Zika fever (ZIKAF) is an emerging mosquito-borne flavivirus illness of humans. The etiological agent, ZIKA virus (ZIKAV), though known to be distributed in the tropics causing sporadic cases, its rapid global expansion with pandemic potential raised global concern. Abrupt emergence in South American countries, Caribbean, and the Americas, the WHO declared ZIKA a public health emergency of international concern in 2016. ZIKAV usually causes mild infections, however, its recent unusual presentations of Guillen–Barré syndrome in adult and microcephaly in newborn babies of ZIKAV-infected mothers in Brazil alerted global public health authorities. Certain mutations on virus genome have been found correlating with clinical severity and its unusual transmission routes through sexual and blood transfusions emphasizes the necessity for understanding the virological determinants and its impact. Abrupt re-emergence in India (2018–2019) particularly in Gujarat (2016), Tamil Nadu (2017), Uttar Pradesh (2021), Maharashtra, Kerala (2021), and Karnataka (2023), indicate urgent measures for strengthening surveillance systems for designing effective prevention and control measures in the country. Given the global concern, here we reviewed the current knowledge of global ZIKAV genetic lineages vis-a-vis the Indian scenario and discusses the future priorities in ZIKAV research in India for effectively designing control strategies.

Keywords: Zika virus; Zika fever; microcephaly; Gulliean-Barr syndrome; African lineage; Asian lineage

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Vector-borne diseases are considered emerging public health problems globally. The term "Zika" has recently drawn widespread attention worldwide because of its abrupt global emergence and rapidly expanding potential in new areas. ZIKA virus (ZIKAV) is transmitted to humans by *Aedes aegypti* mosquitoes. In humans, the virus usually causes subclinical or mild febrile illness called ZIKA fever (ZIKAF). ZIKAF clinically presents with a mild febrile undifferentiated illness and is often considered to be self-limiting. However, recent reports have revealed severe clinical manifestations, such as microcephaly in newborn babies delivered to ZIKAV-infected mothers and Guillen–Barré syndrome (GBS), a serious neurological disorder that warns of a looming global public health emergency. No effective commercial vaccines or treatment strategies are available to prevent and control ZIKAV. Controlling the vector is the only way to effectively control this disease.

ZIKA virus (ZIKAV) was first recovered from a sentinel rhesus macaque in Zika forest in Entebbe, Uganda, Africa, in 1947 [1]. In 1948, the virus was subsequently isolated from *Aedes africanus* mosquitoes in Uganda. The first case of human infection due to ZIKAV was detected in 1952 in Tanzania, Africa. Later, the virus was found to be involved in causing outbreaks of human infection on Yap Island, Micronesia 2007 [2], French Polynesia in 2013–2014 [3] and Brazil (2015) [4]. Besides Africa, ZIKAV is widely distributed in several Asian countries, including, Vietnam, the Philippines, Thailand, Cambodia, Indonesia, Singapore, and Japan [5], and the Americas.

Although the virus is transmitted to humans primarily through mosquito bites, recent reports of infection through sexual contact [6] indicate its non-vectorial, multimodal route of transmission potential, which indicates that suitable intervention strategies need to be developed. Moreover, transmission through contaminated blood and blood products [7] further complicates the existing control measures. An effective commercial vaccine or antiviral drug for the successful prevention and

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control of the disease is lacking. Figure 1. Shows the time scale events of ZIKAV clinical manifestations that occurred globally.

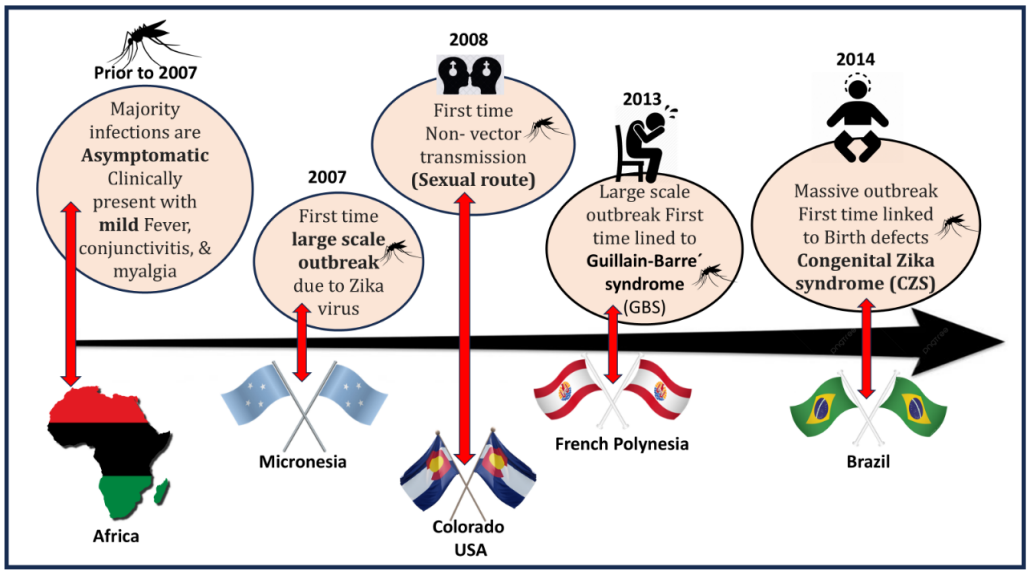

Figure 1. Illustration showing the time scale events of ZIKAV clinical manifestations that occurred globally.

Identifying a ZIKAV infection is difficult due to its overlapping clinical presentations with other commonly occurring mosquito-transmitted infections caused by dengue and Chikungunya infections. Additionally, the serological cross-reactions among closely related pathogens further complicate diagnostic decisions in endemic settings. Highly specific and sensitive diagnostic assays are needed for the timely and accurate detection of ZIKAV infections in endemic areas to control them. Along with the accurate detection of viruses, the development of an effective vaccine is another important part of research on the prevention and control of this disease. Knowledge of the circulating serotypes/genotypes of ZIKAV in particular geographic areas is necessary, as this information can complement diagnostic and effective vaccine development efforts. Additionally, owing to ongoing evolutionary events, the acquisition of mutational substitutions in circulating genotypes may reflect the clinical outcomes of the disease, such as the efficient transmissibility of mutant viruses and an increase in neurovirulence. Also, understanding the circulating genotypes is crucial for developing future control measures.

In India, although ZIKAV has been in circulation since 1947, information on the circulating genotypes in the country needs to be obtained and these genotypes need to be monitored in endemic areas and potential hotspots in the country.

Genomic Organization of ZIKAV

ZIKAV belongs to the Spondweni serogroup of arboviruses. As an enveloped RNA virus, its genome is about 11,000 bp in length and encodes three structural proteins (C, prM, and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (Figure 2). Based on the analysis of the C and E gene sequences of the virus, ZIKAV is classified into two major genetic lineages: Asian and African. The strains recovered from different outbreaks were grouped among the two major lineages. The strains recovered from Southeast Asia, the Pacific, and the American Islands were grouped under Asian lineages. Strains circulating in East Africa and West Africa were grouped under the African lineage. Interestingly, lineages recovered from epidemics have been grouped under Asian lineages, which indicates the emergence and epidemic potential of the genotypes, whereas strains originating from the African lineage are usually found endemically, particularly in East and West Africa. These endemic lineages of African origin are usually not involved in outbreaks

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and cause mild infections. A global picture of the distribution of genetic lineages of ZIKAV is shown in Figure 3.

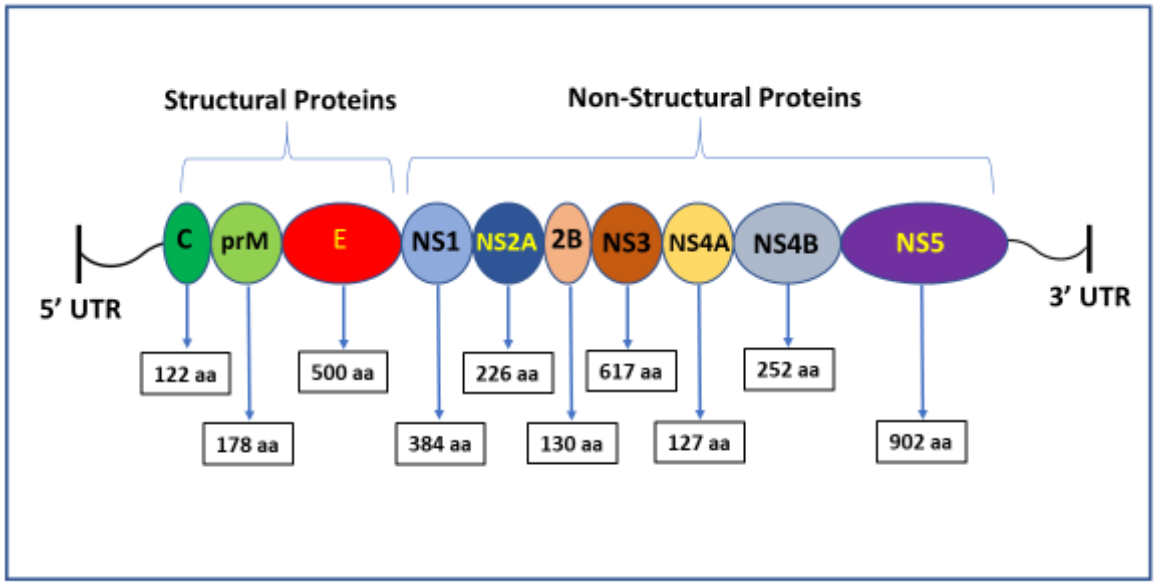

Figure 2. Genomic organization of the ZIKAV.

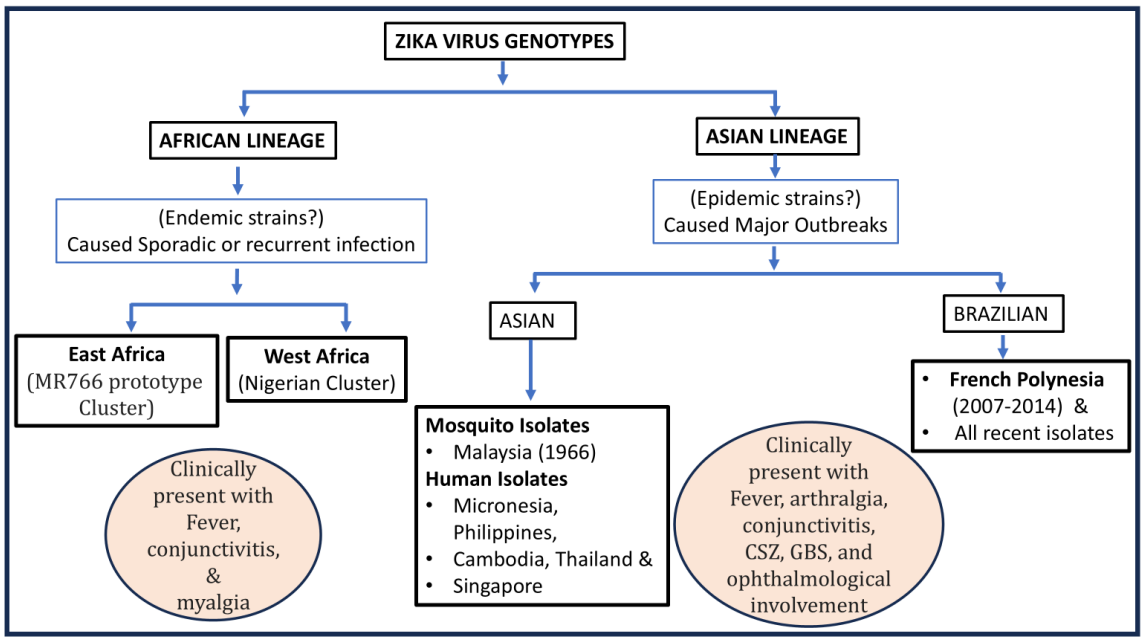

Figure 3. A schematic illustration of the circulating genotypes of ZIKAV around the world.

Zika viruses were discovered in 1947 and are found circulating extensively in Africa and Asia, with substantial evolutionary changes. Various studies comparing the genomes of global ZIKAV lineages have classified them into African and Asian lineages, which are found to have very few amino acid changes in their genomes. However, in-depth analysis has revealed more genetic variants among Asian lineages than African lineage viruses [8,9]. Additionally, African and Asian lineages of the ZIKAV behave differently in terms of pathogenesis and laboratory characteristics [10].

A global Picture of Genetic Lineages

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The ZIKA virus causes geographically localized mild-to-moderate infections among humans. Various studies have documented the genetic dynamics of ZIKAV over time in different parts of the world.

Although ZIKAV has been present in Africa and Asia (Malaysia) since 1947 and 1966, respectively, the abrupt emergence of ZIKAV in Brazil in 2015, attracted global attention for the development of urgent measures for its effective intervention. The massive outbreak of ZIKAV in Brazil (2015) was caused by the Asian lineage viruses, which were probably introduced from the Pacific Islands, particularly from French Polynesia. Thus, ZIKAV spread to other parts of the globe, particularly to the Americas, from French Polynesia. Studies suggest that after the detection of the Asian lineage of ZIKAV in Malaysia in 1966, it underwent continuous mutational changes throughout Southeast Asia and evolved into the most efficient ZIKAV with high outbreak potential, spread to FP, and caused massive outbreaks. From French Polynesia, the introduced ZIKAV, belonging to the Asian lineage, further spread to other parts of the world.

After the massive outbreak in Brazil (2015), a minor epidemic was recorded in Singapore in 2016 [11]. ZIKAV infection was subsequently detected in Malaysia, which has similar ecological conditions as Singapore and Brazil. Interestingly, despite the detection of ZIKAV among *A. aegypti* mosquitoes in Bentong Pahang, Malaysia, in 1966 [12], no outbreak of ZIKA has been recorded. The reason behind the mild form of the disease was found to be the existence of pre-immune antibodies in the local population and the circulation of local virus strains in the country[13]. This evidence indicates that compared to the Brazilian strains, the native strains of ZIKAV in Malaysia cause mild infections. Furthermore, understanding the virological dynamics of the circulating strains of ZIKAV and its interaction with local and exotic *Aedes* mosquitoes is important for developing future control efforts.

The ZIKA virus is an RNA virus similar to other mosquito-transmitted flaviviruses, such as the West Nile and JE viruses. It has undergone continuous evolutionary changes in endemic areas, and predicting its transmissibility and clinical dynamics based on the evolutionary events at the genetic level is difficult. The African lineages of ZIKAV are believed to cause mild infections; however, recent experimental evidence from appropriate cell lines, mosquitoes, and mouse models has shown that African lineage ZIKAV strains have greater transmissibility and pathogenicity than Asian lineage ZIKAV strains [14]. This new laboratory-based knowledge highlights that African ZIKAV lineages may give rise to potent outbreak strains. Adequate field-based data are needed in this direction to substantiate this trait to better understand its virological dynamics.

Mutation of ZIKAV and Its Impact on Phenotypes

Genomic analysis studies have revealed limited nucleotide divergence among the open reading frames (ORFs) of African (isolated from East Africa, Central Africa, and West Africa) and Asian (recovered in Southeast Asia, the Pacific Islands, and the Americas) ZIKAV strains [15].

Flaviviruses constantly undergo mutational substitutions, mainly due to the lack of proofreading activity, and exhibit alterations in virulence and tropism [16]. ZIKAV constantly undergoes mutational changes with a high rate of rapid mutation of about 10 mutations per year, i.e., RNA mutates at a rate equivalent to about 0.01% each year. However, when the African and Asian lineages were compared, the African lineages were generally considered endemic, and Asian lineages were found to have epidemic potential. Although ZIKAV was first detected in Africa (1947) and subsequently in Malaysia (1966), its geographical distribution is restricted. While comparing the original strain [MR766 (HQ234498)] and the subsequent variants (AY632535 and DQ859059) recovered from rhesus monkeys (Uganda, 1947), a low nucleotide variation of 0.4% of nucleotides encoding 0.6% amino acid sequences was observed [17]. This is probably related to the low infection efficiency required to circulate among humans, which mostly causes subclinical infections. However, at a later stage, many studies documented the acquisition of mutations, which may have facilitated the rapid global spread of the virus along with altered pathology [18]. Moreover, experiments have shown the altered phenotypic expression of isolated or even minimal mutations in the viral genome [19].

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Among ZIKA viruses, mutations vary between 12 and 25 substitutions per year. As vector-borne infections, these mutational substitution events might have taken place in human/vector mosquitoes and could have certain traits, such as increased transmissibility, increased virulence, and immune escape ability. Early isolates, i.e., the original isolates, were found to have nine and 30 mutations in a year. However, the recent outbreak isolates KU740184 (the GZ01/2016 strain isolated from a Chinese patient who returned from Venezuela in 2016) and KU 744693 (the VE-Ganxian isolate from China) presented 30 and 64 mutations per year, respectively [20]. The difference in mutational substitution between original and field-adapted recent outbreak isolates could be due to the availability of many human hosts and the opportunity to undergo rapid mutation; such mutations occurred mainly due to the continuous selective pressure exerted by hosts or vectors. Researchers have also reported differences in replication efficiency and the differential regulation of the host innate immune response between Asian and African lineages of ZIKAV [21]. Additionally, in the ZIKAV genome, mutational substitutions occur in almost all genes, such as structural and non-structural genes, and their respective traits enhance phenotypic properties, with the emergence of fit genotypes capable of causing outbreaks [22]. This evidence indicates the possible emergence of more virulent strains of ZIKAV. The list of different substitutions that take place in the gene regions of ZIKAV and their effect on the phenotypic expression of the virus is provided in (Table 1).

During the in-depth analysis of the genetic details of various ZIKAV strains, [23] found that 75 amino acids were different between pre-epidemic and epidemic strains of global ZIKAV isolates. Specifically, 15 substitutions have been found only in the epidemic lineages of ZIKAV and not in pre-epidemic strains. Evidence has indicated the possible occurrence of recombination events between ZIKAV and the Spondweni virus, particularly in the NS2B coding region between the 4237 and 4528 nucleotide positions. The analysis also showed that nine nucleotide bases form a large bulge at SL1 in the epidemic ZIKAV strains, which resembled the SLII of the pre-epidemic strain. Further studies are needed to determine the clinical relevance of these changes in the epidemic strains of ZIKAV.

Reports on the genome analysis of ZIKAV have revealed successful nucleotide substitutions in the structural and non-structural genes among the viruses analyzed. Among the structural coding proteins, amino acid substitutions in the coding region of C (five amino acid substitutions I113V, R101K, I110V, L27F, and N25S), PrM (V262A, K246R, V1581, H157Y, A148P, K143E, S139N, 1125 V, and V153M), and domain III of the E region (V603I and D679E) have been detected in the epidemic strains but not in the pre-epidemic strains. Extensive analysis including more samples may elucidate the behavior of ZIKAV. The accumulated laboratory evidence on amino acid substitutions indicates that the ongoing evolutionary events occurring among the wild ZIKAV lineages in nature may contribute to emerging potential and clinical dynamics.

On the other hand, among non-structural proteins, nucleotide substitutions have been detected (E842D, K859R, A984V, and V1026I) in the NS1 of Asian lineage epidemic strains but not in African pre-epidemic strains. Among the NS4B proteins, two amino acid substitutions, V2491 and L2451S, have been detected in the epidemic strains of ZIKAV. NS5 is the largest protein among the virus-encoded proteins, and eight amino acid substitutions, which include T2630V, A2783V, M1970L, K3046R, N2892S, P3158S, D3383N, and S3219D, have been detected in the epidemic Asian lineages but not in the epidemic African lineages.

An analysis of the genome sequences of the Brazilian and African lineages of ZIKAV revealed possible selection pressure events that are exerted at several amino acid positions in the Brazilian epidemic lineage compared to the African lineage of viruses, particularly in the non-structural protein NS4B. This phenomenon might interfere with the phosphorylation of Akt and mTOR, affecting the Akt-mTOR signaling pathway and causing developmental neuropathies in newborns [24].

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Table 1. Details of the mutations in various genes of the ZIKAV genome and their potential effect on the phenotype.

Reference cited	LOCATION	SUBSTITUTION	PHENOTYPIC CHANGE
Shan et al (2020)[22]	Envelop	EV-473M	-Increase neuro virulence; -Maternal to Fetal transmission -Viremia to increase urban transmission
Fontes-Garfias, Camila R 2017 et. al. [25]	Envelop	Asn 154	- mosquito-cell infectivity & -virus assembly
Liu et al (2021) [26]	Capsid	C-T106A	-Virus fitness advantage -accelerated the spread in both mosquitoes & rodents -enhancing transmissibility between vectors & hosts
Phumee et al (2023) [5]	Pre-Membrane	prM-V1A	-Linked with high mortality rate
Yuan et al (2017) [27]	Pre-Membrane	prM-S17N	Increased microcephaly in fetus?
Liu *et al* (2021) [26]	Pre-Membrane	prM-V123A,	Virus fitness advantage
Yuan et al (2017) [27]	Pre-Membrane	PrM-S139N	-Accelerated virus infectivity for mouse & human neural progenitor cells. -Enhanced apoptosis.
Xia et al (2018) [28] Liu et al (2017) [29]	NS1	NS1-A188V	-Enhance virus infectivity in *Aedes aegypti* -Suppress Type -T interferon
Liu *et al* (2021) [26]	NS1	NS1-A982V	Virus fitness advantage
Zhang et al (2023) [30]	NS2A	NS2A-A1204T	Associated with Neurovirulence
Regla Nava et al (2022) [31]	NS2B	NS2B-139V	-Enhance virus virulence -Escape from pre-immune Dengue antibody
	NS5	NS5-M872	However, to be determined
Peng et al (2022) [32]	NS5	NS5-M114V	No role on virus replication and transmission potential.
Liu *et al* (2021) [26]	NS5	NS5-M3392V	Virus fitness advantage

Indian Status

In India, evidence of ZIKAV circulation was recorded in the 1950s through a serological survey in which anti-ZIKAV neutralizing antibodies were detected by [33]. However, their public health importance in causing human infections in India is not known or documented.

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After the explosive outbreaks of ZIKAV in Brazil in 2013, the eruption of the ZIKAV outbreak occurred in Rajasthan, India, in 2018, affecting 159 individuals, including 63 pregnant women. Between the demonstration of its first activity during the 1950s and subsequent detection during the 2018 episode in Rajasthan, no virus activity was documented in the country. This could be due to the absence of the virus in circulation or the absence of cases along with the presence of arbovirus in the country, particularly dengue/JE/CHIKV, etc., which have common clinical manifestations that overlap with ZIKAV. Following the episode in Rajasthan, the first laboratory evidence of ZIKAV infection was detected in a pregnant woman from Ahmadabad, Gujarat, India, in 2017 [34]. Genomic characterization showed the close relationship of the Gujarat Isolate with the Malaysian ZIKAV isolate (MYS/P6-740/1966 [KX694533]) recovered in 1966, which indicated the dispersal and silent establishment of the Asian isolate in the Indian subcontinent. Further studies are needed to determine the extent of its distribution and its virological dynamics in vectors and hosts in India. In 2017, a few ZIKAF cases were recorded in a local population, and laboratory evidence was generated from a single case from Krishnagiri district, Tamil Nadu, India. In 2018, outbreaks were subsequently reported in Jaipur, Rajasthan (158 cases) and Madhya Pradesh (127 cases), India. Interestingly, the ZIKAV isolate recovered in Jaipur was found to cluster with the Asian lineage viruses.

After the Rajasthan episode, where three isolates of ZIKAV belonging to the Asian lineage were detected from human cases, about 130 human cases were reported from Madhya Pradesh, India, and a single case from Gujarat (WHO, 2019), which indicated that ZIKAV could spread to other Indian states. In response, countrywide surveillance was conducted against ZIKAV among *Aedes* vectors [(79,492 samples (6,492 pools) covering 49 districts in high-risk zones in 14 states from 2016–2019 [35]. The RT-PCR results revealed the activity of ZIKAV in three pools collected from Jaipur. The detected strains were also clustered among the Asian lineage of ZIKAV, which confirmed the wide circulation of the virus in the country and its ability to cause outbreaks.

In 2021, more than 100 human cases were reported and confirmed to be ZIKAV infections in Uttar Pradesh, India. The cases were confirmed with RT-PCR by detecting the virus-specific genome during the outbreak (https://scroll.in/article/1010555/inside-uttar-pradeshs-zika-outbreak-can-indias-most-populous-state-contain-the-virus-spread) [36]. Suspected cases of ZIKAV infection were reported among OPD patients and healthcare workers at private clinics in the Trivandrum area of Kerala, India in 2021. The complete genome sequence data of two strains (MCL-21-H-8900 and MCL-21-H-8901) recovered from the outbreak were found to be closely related to those of Rajasthan (accession number: MK238037.1), with nucleotide similarities of 99.33% and 99.4%, respectively [37]. However, none of the affected patients from the Trivandrum episode had a history of travel or family members with a history of ZIKAV exposure in the ZIKAV-affected area. Although the source of ZIKAV that infected these patients is not known, the findings strongly suggest the ongoing circulation and silent establishment of ZIKAV in these localities. During the outbreak of ZIKAV in Kerala, Uttar Pradesh, and Maharashtra in 2021, the silent circulation of ZIKAV activity was demonstrated in other states, such as Amritsar, Punjab; New Delhi; Aligarh, Uttar Pradesh; Jodhpur, Rajasthan; Ranji, Jharkhand; Hyderabad, Telangana; and Trivandrum, Kerala.

A recent report on the emergence of a new genotype, i.e., the African genotype of ZIKAV that replaced the circulating genotype in Brazil, alerted public health authorities about possible re-emergence in the country. This finding indicates that the existing genotype of ZIKAV can also be replaced by an emerging new genotype and can lead to outbreaks. The phenomenon of shifting or replacing arboviral strains from one genotype to another has been documented in the cases of dengue, JE, etc. Long-term studies are needed to confirm such events in the case of ZIKAV in India. Countries such as India need proactive efforts at the hotspots to track the movement of ZIKAV via improved surveillance tools for timely control.

The available data indicate that ZIKAV is in circulation and is expected to incur new loci, undergo evolution, establish hotspots, and emerge with outbreak potential in the country. Adequate preparedness and strengthening of the surveillance system are essential for mitigating future outbreaks in India.

Origin and Global Dispersal of ZIKAV

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ZIKA virus (ZIKAV) originated in East Africa, and its activity was first detected accidentally in monkeys in Uganda in 1947 [1] during yellow fever (YF) surveillance. It later dispersed to West Africa, as determined by its detection in a human in Nigeria. Simultaneously, it was dispersed to Cote d'Ivoire in the 1940s and to Senegal (1985) in the West African region. From Africa, it was introduced and established in Southeast Asia and was in circulation for about 50 years. In Southeast Asia, the virus underwent several evolutionary events for about 50 years and adapted to spread to other parts of the globe. From Southeast Asia, particularly from Cambodia, it spread to French Polynesia, where it caused massive outbreaks from 2013 to 2014. This was confirmed by the close relationship between Cambodia ZIKAV-2010 and the strain that caused the outbreak in French Polynesia (Figure 4).

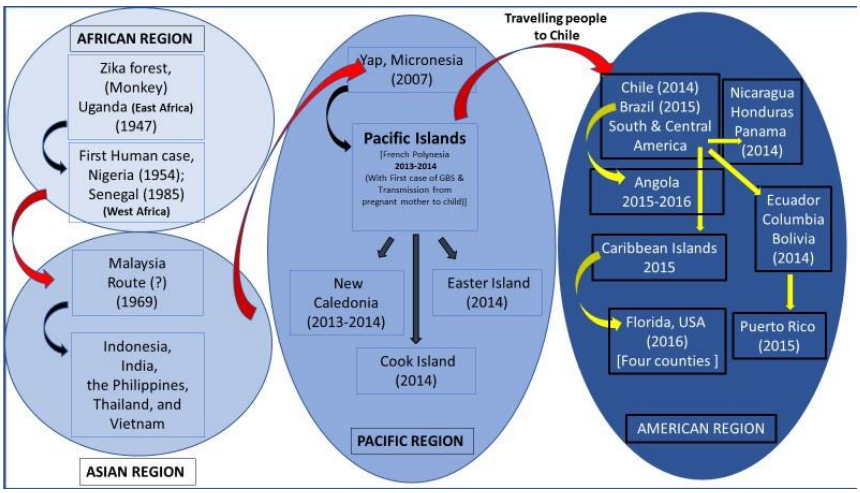

Figure 4. Proposed routes of region-wise global movement of ZIKAV in the African, Asian Pacific, and American regions.

In Southeast Asia, ZIKAV was first detected in Malaysia from field-caught mosquitoes in 1969. The results of a phylogenetic analysis of African and Southeast Asian ZIKAV showed that a geographically distinct lineage of ZIKAV was introduced into SEA regions compared to the African genotype [17]. In 2007, a massive outbreak of ZIKAV was reported from Yap Island, Micronesia; this outbreak affected many people [38]. The virus that caused the outbreak at Yap Island was closely related to the Asian lineage, which has been circulating in SEA. This finding highlights the evolutionary, emerging, and dispersal potential of ZIKAV(Figure 5).

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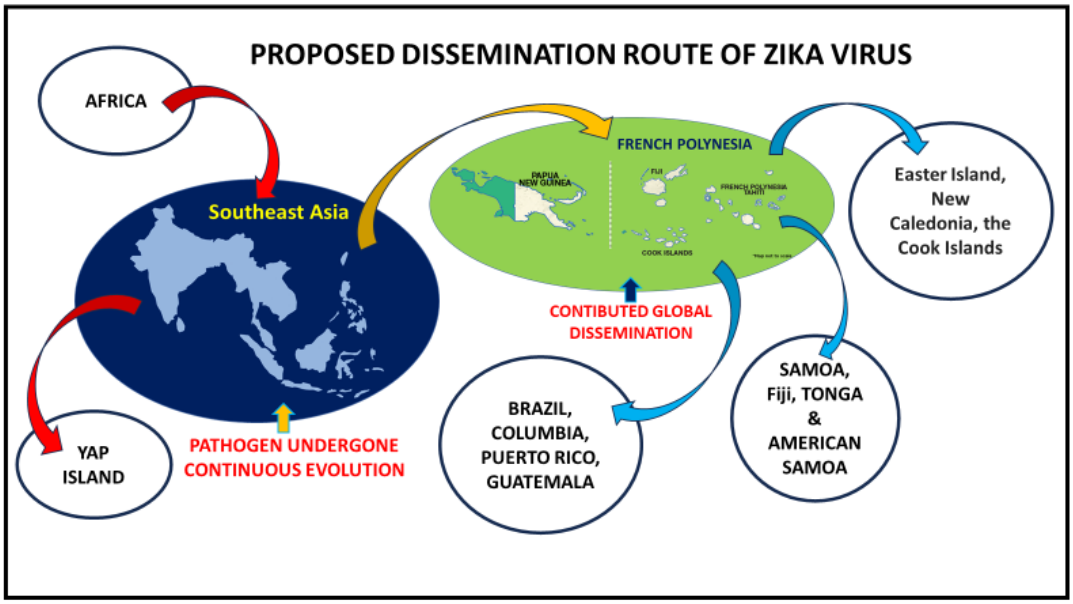

Figure 5. Illustration showing the global dissemination of ZIKAV after undergoing major evolutionary events at Asia and rapid global spread from French Polynesia.

The virus probably dispersed to the Pacific Islands, where it caused outbreaks, including those in French Polynesia [39], New Caledonia (2013–2014), the Cook Islands (2014), and the Easter Islands (2014). The unusual phenomenon of GBS and mother-to-fetal transmission of ZIKAV has been detected in French Polynesia. From the Pacific Islands, particularly from French Polynesia, the virus further spread to the Americas through a single introduction event via the Easter Islands [40], as determined by assessing the cases in Brazil in 2015 [4]. The virus subsequently caused outbreaks in December 2014; South Ecuador, Colombia, Bolivia, Venezuela, etc. in September 2014; and Central American countries such as Nicaragua and Honduras, including most of the Caribbean Islands, in April 2015. An outbreak of ZIKAV was reported from Angola in 2015–2016, probably due to the introduction of an Asian lineage of ZIKAV closely related to the one circulating in Brazil in 2015 [41].

During the entire pandemic in the Americas, around 100 million people were infected by the ZIKAV [42], and a strong correlation between ZIKAV infection during pregnancy and fetal congenital malformations was detected [43]. Additionally, following viral infection, severe neurological (encephalitis) and autoimmune (Guillain–Barré syndrome) complications were also recorded [44].

After being introduced to the Americas, ZIKAV cases were reported in Florida in 2016. The virus later dispersed to the four counties of Florida with most cases observed in Miami-Dade County. The main source of ZIKAV introduction to Florida was predicted to be the Caribbean Islands.

Multiple virus introduction events resulting from the ZIKAV outbreak were also noted in Puerto Rico in 2015–2016. The results of the Bayesian reconstruction analysis of Puerto Rico strains revealed that the outbreak isolates clustered into two clades: Puerto Rico Clade 1 (PRC1) and Puerto Rico Clade 2 (PRC2). PRC1 included viruses originating from South America and the Caribbean Islands, including French Guyana, Brazil, Suriname, the US Virgin Islands, and the Dominican Republic. In contrast, PRC2 included viruses originating from Central America, including Honduras and Nicaragua [45].

ZIKAV (NIV1720741), which was recovered from a patient in Gujarat, India, was speculated to have evolved along with the cluster of strains isolated in Central and South America between 2015 and 2016 [34]. However, further investigation on the full-length sequences of the virus strains is needed for confirmation. Recently, based on the NS5 and E gene analysis, [35] proposed a probable separate Indian lineage of ZIKAV that was recovered from Thiruvananthapuram, Kerala, India, which caused an outbreak. However, a complete genome-based analysis is needed to support this claim.

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During a recent episode in Pune, Maharashtra, out of 66 confirmed cases of ZIKAF, no severe clinical manifestations of microcephaly were detected among the 26 infected pregnant women. (https://www.business-standard.com/india-news/dengue-cases-rising-in-west-bengal-govt-says-situation-under-control-124080800235_1.html).

The complete genome sequences of the 197 Asian and African lineages of ZIKAV are available in the global database. However, the limited number of partial genomic sequences of ZIKAV of Indian origin available in the database (Table 2) indicates future attempts to map the circulating strains in India.

Table 2. Details of the Indian isolates of ZIKAV available in public databases.

Accession no:	Place	Source	Year	Reference
MCL-21-H-8900 & MCL-21-H-8901	Thiruvananthapuram, Kerala, India	Human	2021	Yadav et, al. (2022) [37]
MK238037.1	Rajasthan, India	Human	2018	Yadav et. al. (2019) [46]
OP678998	Thiruvananthapuram, Kerala, India	Human	2021	Pradeep Kumar et. al. (2023) [35]
OP678999	Thiruvananthapuram, Kerala	*Aedes albopictus*	2021	Pradeep Kumar et. al. (2023) [35]
OM666892.1	Maharashtra		2021	
NIV1720741/1845ZKV	Gujarat	Human	2016	Sapkal et al (2017) [34]

The above evidence demonstrates the dissemination potential of the ZIKAV in the Indian subcontinent and its spread to newer areas. Strengthening virological surveillance is essential to track their movements and clinical outcomes, particularly among pregnant women, for possible severe manifestations.

Conclusion

ZIKAV is an emerging mosquito-borne virus infection in India. The global isolates of ZIKAV are broadly classified into African and Asian lineages. A detailed analysis of the genomes of virus isolates recovered from different geographical regions showed the continuous process of mutation of structural and non-structural genes in the field isolates of ZIKAV. The detection of ZIKAV in Uttar Pradesh (2019), Gujarat (2019), Tamil Nadu, Kerala (2022), and Karnataka (2023) in India indicates its silent geographical expansion and its emerging potential in the country. The strengthening of surveillance systems should be prioritized to identify hotspots for implementing proactive control strategies.

Global experiences have shown that ZIKAV outbreak can occur after the viruses acquire appropriate mutational changes, which can contribute to its virulence, manifestation, and transmissibility characteristics in India. Efforts should also be focused on developing field-adapted quick ZIKAV detection assays for the timely detection of viral antigens in wild vectors to implement vector control efforts. The application of promising unbiased NGS-based methodologies for quick detection of ZIKAV should be investigated extensively from an Indian perspective. ZIKAV surveillance needs to be strengthened using advanced tools to determine the magnitude of the problem in the country for adequate public health preparedness.

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Ethics statement: As this is a review article, the present work was not approved by any form of Ethical committees from the home Institute.

Reference

1. Dick, G.W.; Kitchen, S.F.; Haddow, A.J. (1952) Zika virus. I. Isolations and serological specificity. *Trans. Roy. Soc. Trop. Med. Hyg*. **1952**,46, 509–520.
2. Lanciotti, R.S.; Kosoy, O.L.; Laven, J.J.; Velez, J.O.; Lambert, A.J.; Johnson, A.J.; Stanfield, S.M.; Duffy, M.R. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg. Infect. Dis.* **2008,** *14(8)*,1232-9. doi: 10.3201/eid1408.080287
3. Musso, D.; Bossin, H.; Mallet, H.P.; Besnard, M.; Broult, J.; Baudouin, L.; Levi, J.E.; Sabino, E.C.; Ghawche, F.; Lanteri, M.C.; et. al. Zika virus in French Polynesia 2013-14: anatomy of a completed outbreak. *Lancet Infect. Dis*. **2018**, *18(5),* e172-e182. doi: 10.1016/S1473-3099(17)30446-2
4. Zanluca, C.; Melo, V.C.A.; Mosimann, A.L.P.; Santos, G.I.V.D.; Santos, C.N.D.S.; Luz, K. (2015) First report of autochthonous transmission of Zika virus in Brazil. *Mem. Inst. Oswaldo. Cruz*. **2015**, 110, 569–572. 10.1590/0074-02760150192
5. Phumee, A.; Chitcharoen, S.; Sutthanont, N.; Intayot, P.; Wacharapluesadee, S.; Siriyasatien, P. Genetic diversity and phylogenetic analyses of Asian lineage Zika virus whole genome sequences derived from *Culex quinquefasciatus* mosquitoes and urine of patients during the 2020 epidemic in Thailand. *Sci. Rep.* **2023**, *13*, 18470. https://doi.org/10.1038/s41598-023-45814-9
6. Musso, D.; Roche, C.; Robin, E.; Nhan, T.; Teissier, A.; Cao-Lormeau, V.M. Potential sexual transmission of Zika virus. *Emerg. Infect. Dis*. **2015**, 21(2), 359-61. doi: 10.3201/eid2102.141363
7. Musso, D.; Nilles, E.J.; Cao-Lormeau, V.M. Rapid spread of emerging Zika virus in the Pacific area. *Clin. Microbiol. Infect*. **2014**, *20(10),* O595-6. doi: 10.1111/1469-0691.12707
8. Shrivastava, S.; Puri, V.; Dilley, K.A.; Ngouajio, E.; Shifflett, J.; Lauren, M.; Oldfield.; Fedorova, N.B.; Hu, L.; Williams, T.; et. al. Whole genome sequencing, variant analysis, phylogenetics, and deep sequencing of Zika virus strains. *Sci*. **2018,** *8*, 15843. https://doi.org/10.1038/s41598-018-34147-7
9. Barzilai, L.; Schrago, C.G. The range of sampling times affects Zika virus evolutionary rates and divergence times. *Arch. Virol.* **2019,** *164(12)*, 3027-3034. doi: 10.1007/s00705-019-04430-7
10. Simonin, Y.; van Riel, D.; Van de Perre, P.; Rockx, B.; Salinas, S. Differential virulence between Asian and African lineages of Zika virus. *PLoS Negl. Trop. Dis.* **2017**, *11(9),* e0005821. doi: 10.1371/journal.pntd.0005821
11. Fisher, D.; Cutter, J. The inevitable colonisation of Singapore by Zika virus. *BMC Med*. **2016,** *14*, 188. https://doi.org/10.1186/s12916-016-0737-9
12. Marchette, N.J.; Garcia, R. Rudnick A. Isolation of Zika virus from *Aedes aegypti* mosquitoes in Malaysia. *Am. J Trop. Med, & Hyg*. **1969**,*18*, 411–415.
13. Woon, Y.L.; Lim, M.F.; Abd Rashid, T.R.T.; Thayan, R.; Chidambaram, S.K.; Abdul Rahim, S.S.S.; Mudin, R.N.;Sivasampu, S. Zika virus infection in Malaysia: an epidemiological, clinical and virological analysis. *BMC Infect. Dis.* **2019,** *19*, 152. https://doi.org/10.1186/s12879-019-3786-9
14. Fabien, A.; Sofie,J.; Maïlis, D.; Sebastian,L.; Leen,D.; Albin, F.; Natapong, J.; Elliott, F.; Miot, S. D.; Caroline, M.; et. al. Recent African strains of Zika virus display higher transmissibility and fetal pathogenicity than Asianstrains. *Nat.Commun*. **2021**,*12*,916. https://doi.org/10.1038/s41467-021-21199-z
15. Haddow, A.D.; Nasar, F.; Guzman, H.; Ponlawat, A.; Jarman, R.G.; Tesh, R.B.; Weaver, S.C. Genetic Characterization of Spondweni and Zika Viruses and Susceptibility of Geographically Distinct Strains of *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* (Diptera: Culicidae) to Spondweni Virus. *PLoS Negl. Trop. Dis.* **2016**, *26*, 10(10):e0005083. doi: 10.1371/journal.pntd.0005083
16. Neal, J.W. Flaviviruses are neurotropic, but how do they invade the CNS? *J Infect.* **2014**, *69*, 203–15. doi: 10.1016/j.jinf.2014.05.010
17. Haddow, A.D.; Schuh, A.J.; Yasuda, C.Y.; Kasper, M.R.; Heang, V.; Huy, R.; Hilda, G.; Robert, B.T.; Scott,C.W. Genetic Characterization of Zika Virus Strains: Geographic Expansion of the Asian Lineage. *PLoS Negl. Trop. Dis.* **2012**, *6(2)*, e1477. https://doi.org/10.1371/journal.pntd.0001477

18. Yokoyama, S.; Starmer, W.T. Possible roles of new mutations shared by Asian and American zika viruses. *Mol. Biol. Evol.* **2017**, *34*, 525–34. 10.1093/molbev/msw270
19. Smith, D.R.; Sprague, T.R.; Hollidge, B.S.; Valdez, S.M.; Padilla, S.L.; Bellanca, S.A.; Golden, J.W.; Coyne, S.R.; Kulesh, D.A.; et. al. African and Asian Zika virus isolates display phenotypic differences both in vitro and in vivo. *Am. J Trop. Med. Hyg*. **2018**, 98,432–44.
20. Logan, I.S. ZIKA--How fast does this virus mutate? *Dongwuxue Yanjiu.* **2016**, *37(2)*, 110-5. doi: 10.13918/j.issn.2095-8137.2016.2.110.
21. Österlund, P.; Jiang, M.; Westenius, V.; Kuivanenm, S.; Jarvi, R.; Kokkola, L.;Lundberg, R.; Kelen, K.; KorvaT.A.; et al. Asian and African lineage Zika viruses show differential replication and innate immune responses in human dendritic cells and macrophages. *Sci. Rep.* **2019,** *9*, 15710. https://doi.org/10.1038/s41598-019-52307-1
22. Shan, C.; Xia, H.; Haller, S.L.; Azar, S.R.; Liu, Y.; Liu, J.; Muruato, A.E.; Chen, R.; Rossi, S.L.; Wakamiya, M.; et. al. A Zika virus envelope mutation preceding the 2015 epidemic enhances virulence and fitness for transmission. *Proc. Natl. Acad. Sci.* **2020**, *18;117(33),* 20190-20197. doi: 10.1073/pnas.2005722117
23. Zhu, Z.; Chan, J.F.; Tee, K.M.; Choi, G.K.; Lau, S.K.; Woo, P.C.; Tse, H.; Yuen, K.Y. Comparative genomic analysis of pre-epidemic and epidemic Zika virus strains for virological factors potentially associated with the rapidly expanding epidemic. *Emerg. Microbes. Infect*. **2016**, *16*, 5(3):e22. doi: 10.1038/emi.2016.48
24. Jun,S.R.; Wassenaar,T.M.; Wanchai,V.; Patumcharoenpol, P.; Nookaew, I.; Ussery,D.W.; Suggested mechanisms for Zika virus causing microcephaly: what do the genomes tell us? *BMC Bioinfo***. 2017**, *18(14)*, 471. doi: 10.1186/s12859-017-1894-3
25. Fontes-Garfias, C.R.; Shan, C.; Luo, H.; Muruato, A.E.; Medeiros, D.B.; Mays, E.; Xie, X.; Zou, J.; Roundy, C.M.; Wakamiya, M.; et. al. Functional Analysis of Glycosylation of Zika Virus Envelope Protein. *Cell Rep*. **2017**, 21, 1180–1190.
26. Liu, J.; Liu, Y.; Shan, C.; Nunes, B.T.D.; Yun, R.; Haller, S.L.; Rafael, G.H.; Azar, S.R.; Andersen, C.R.; Plante, K.; et. al. (2021). Role of mutational reversions and fitness restoration in Zika virus spread to the Americas. *Nat. Commun.* **2021,** 12, 595. doi: 10.1038/s41467-020-20747-3
27. Yuan, L.; Huang, X.Y.; Liu, Z.Y.; Zhang, F.; Zhu, X.L.; Yu, J.Y.; et al. A single mutation in the prM protein of Zika virus contributes to fetal microcephaly. *Sci*. **2017**, *358*, 933–6.
28. Xia, H.; Luo, H.; Shan, C.; Muruato, A.E.; Nunes, B.T.D.;Daniele, B.A.; Zou, J.; Xie, X.; Girldo, M.I. et al. An evolutionary NS1 mutation enhances Zika virus evasion of host interferon induction. *Nat. Commun*. **2018,** *9(1)*, 414. https://doi.org/10.1038/s41467-017-02816-2
29. Liu, Y.; Liu, J.; Du, S.; Shan, C.; Nie, K.; Zhang, R.; Li, XF.; Zhang, R.; Wang, T.; Qin, CF.; et. al. Evolutionary enhancement of Zika virus infectivity in *Aedes aegypti* mosquitoes. *Nature*. **2017**, *545(7655)*, 482–486. https://doi.org/10.1038/nature22365
30. Zhang, Y.F.; Guo, J.J.; Yang, F.; Zhou, H.Y.; Zhang, N.N.; Xiong, X.C.; Feng, Y.; Deng, Y.Q.; Qin, C.F. Characterization and phylogenetic analysis of a neurovirulent Zika virus isolated from Cambodia in 2019. *J Med. Virol*. **2023**, *95(1)*, e28290. doi: 10.1002/jmv.28290
31. Regla-Nava, J.A.; Wang, Y.T.; Fontes-Garfias, C.R.; Liu, Y.; Syed, T.; Susantono, M.; Gonzalez, A.; Viramontes, K.M.; Verma, S.K.; Kim, K.; et. al. A Zika virus mutation enhances transmission potential and confers escape from protective dengue virus immunity. *Cell Rep*. **2022**, *39(2)*, 110655. doi: 10.1016/j.celrep.2022.110655
32. Peng, N.Y.G,; Amarilla, A.A.; Hugo, L.E.; Modhiran. N.; Sng, J.D.J.; Slonchak, A.; Watterson, D.; Setoh, Y.X,; Khromykh, A.A. The distinguishing NS5-M114V mutation in American Zika virus isolates has negligible impacts on virus replication and transmission potential. *PLoS Negl. Trop. Dis*. **2022,** *16(5),* e0010426. doi: 10.1371/journal.pntd.0010426
33. Smithburn,K.C.; Kerr, J.A.; Gatne, P.B. Neutralizing antibodies against certain viruses in the sera of residents of India. *J Immunol.* **1954,** *72(4)*, 248-57.
34. Sapkal,G.N.; Yadav,P.D.; Vegad,M.M.; Viswanathan,R.; Gupta,N.; Mourya,D.T.; (2017). First laboratory confirmation on the existence of Zika virus disease in India. *J Infect.* **2017**, 76(3), 314-317. doi:10.1016/j.jinf.2017.09.020
35. Pradeep Kumar, N.; Ajithlal, P.M.; Prasanta Saini.; Abidha Suresh.; Philip Samuel, P.; Balasubramaniam, M.; Jessu, M.; Sonia, T.; Amju, K.P.; Aiswarya, R.S.; et. al. Recent Outbreak of Zika in Kerala State, India; is Zika Virus Re-Emerging as a Distinct Genetic Lineage in India? Preprint • April 2023 DOI: 10.2139/ssrn.4396363
36. Scroll.in . Inside Uttar Pradesh's Zika outbreak: can India's most populous state contain the virus spread? 2021. Accessed November 30, 2021. https://scroll.in/article/1010555/inside-uttar-pradeshs-zika-outbreak-can-indias-most-populous-state-contain-the-virus-spread.
37. Yadav, PD.; Niyas, V.K.M.; Arjun, R.; Sahay, R.R.; Shete, A.M.; Sapkal, G.N.D.; Pawar, S.; Patil, D.Y.; Gupta, N.; Abraham, P. Detection of Zika virus disease in Thiruvananthapuram, Kerala, India 2021 during the second wave of COVID-19 pandemic. *J Med. Virol.* **2022**,*94(6)*,2346-2349. doi: 10.1002/jmv.27638

 doi:10.20944/preprints202412.0254.v1

38. Duffy, M.R.; Chen, T.H.; Hancock, W.T.; Powers, A.M.; Kool, J.L.; Lanciotti, R.S.; Pretrick, M.; Marfel, M.; Holzbauer, S.; Dubray, C.; et. al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl. J Med*. **2009**, *360*, 2536–2543. 10.1056/NEJMoa0805715
39. Cao-Lormeau, V.M.; Roche, C.; Teissier, A.; Robin, E.; Berry, A. L.; Mallet, H.P.; Sall, A.A.; Musso, D. (2014). Zika virus, French polynesia, South pacific, 2013. *Emerg. Infect.Dis.* **2014**, 20, 1085. doi: 10.3201/eid2006.140138
40. Faria, N.R.; Azevedo, R.D.S.D.S.; Kraemer, M.U.G.; Souza, R.; Cunha, M.S.; Hill, S.C.; Theze, J.; Bonsall, M.B.; Bowden, T.A.; Rissanen, L.; et. al. Zika virus in the Americas: Early epidemiological and genetic findings. *Science*. **2016**, *352(6283)*, 345-349. doi: 10.1126/science.aaf5036
41. Hill, S.C.; Vasconcelos, J.; Neto, Z.; Jandondo, D.; Zé-Zé, L.; Aguiar, R.S.; Xavier, J.; Thézé, J.; Mirandela, M.; Micolo Cândido, A.L.; et. al. Emergence of the Asian lineage of Zika virus in Angola: an outbreak investigation. *Lancet Infect. Dis.* **2019**, *19(10),* 1138-1147. doi: 10.1016/S1473-3099(19)30293-2
42. Perkins, T.A.; Siraj, A.S.; Ruktanonchai, C.W.; Kraemer, M.U.G.; Tatem, A.J. Model-based projections of Zika virus infections in childbearing women in the Americas. *Nat. Microbiol*. **2016**, 1:16126.
43. Mlakar, J.; Korva, M.; Tul, N.; Popović, M.; Poljšak-Prijatelj, M.; Mraz, J.; Kolenc, M.; Resman Rus, K.; Vesnaver Vipotnik, T.; Fabjan Vodušek, V. Zika virus associated with microcephaly. *N. Engl. J Med.* **2016**, *374*, 951–958.
44. Parra, B.; Lizarazo, J.; Jiménez-Arango, J.A.; Zea-Vera, A.F.; González-Manrique, G.; Vargas, J.; Angarita, J.A.; Zuñiga, G.; Lopez-Gonzalez, R.; Beltran, C.L. Guillain-Barré syndrome associated with Zika virus infection in Colombia. *N. Engl. J Med.* **2016**,*375*, 1513–1523.
45. Santiago, G.; Kalinich, C.C.; Cruz-López, F.; Gonzalez, G.L.; Flores, B.; Hentoff, A.; Charriez, K.N.; Fauver, J.R.; Adam, L.E.; et. al. Tracing the Origin, Spread, and Molecular Evolution of Zika Virus in Puerto Rico, 2016–2017. *Emer. Infec. Dis.* **2021**, *27(11)*, 2971-2973. doi:10.3201/eid2711.211575
46. Yadav, P.D.; Malhotra, B.; Sapkal,G.; Nyayanit, D.A.; Deshpande,G.; Gupta, N.; Padinjaremattathil, U.T.; Sharma, H.; Sahay,R.R.; Sharma,P.; et al. Zika virus outbreak in Rajasthan, India in 2018 was caused by a virus endemic to Asia. *Infect. Genet. Evol.* **2019**,*69*, 199-202.