REVIEW ARTICLE

Congenital Chikungunya Virus Infections

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Abstract

Structure: Chikungunya virus (CHIKV) is an arthropod-borne ribonucleic acid (RNA) virus, classified in the genus alphavirus in the family Togaviridae.

Clinical presentation: Perinatal/neonatal infections are rare, but some infants can develop fever, thrombocytopenia, lymphopenia, pigmentary changes, and a maculopapular rash. The neurocognitive outcome of some infants with vertically transmitted mother-to-child perinatal infections and CHIKV neonatal encephalopathy can be poor.

Diagnosis: The diagnosis of CHIKV infections can be confirmed by the detection of chikungunya viral RNA via real-time reverse-transcription polymerase chain reaction (RT-PCR) and/or specific immunoglobulin (Ig)M and IgG serology.

Treatment: Currently, no specific antiviral treatment(s) are available for CHIKV, and management is limited to supportive care by maintaining adequate intravascular volume by intravenous fluids and oral rehydration. Infants exposed *in utero* or during the perinatal period need to be monitored for adverse neurocognitive outcomes.

Keywords: *Aedes aegypti*, *Aedes albopictus*, Brownie nose, Chikungunya sign, Chikungunya virus encephalitis, Infant, Neonate, Newborn, Thrombocytopenia, Vertical transmission.

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Key Points

- Chikungunya virus is widely transmitted in tropical and subtropical areas by Aedes (Ae.) mosquito vectors: *Aedes aegypti* and *Aedes Albopictus*.
- Pregnant mothers with recent CHIKV infections can transmit the virus to the fetus *in utero* or to the newborn infant during the perinatal period. These infants are diagnosed as infected if they test positive for viral RNA or specific IgM antibodies before postnatal day 10 in blood or day 15 in the cerebrospinal fluid (CSF). The virus is carried throughout the body in infected monocytes.
- Chikungunya virus encephalitis can show as white matter (WM) hyperintensities on T1-weighted magnetic resonance imaging (MRI) in the choroid plexus, leptomeninges, and ependyma. The long-term neurocognitive outcome of these children is poor.
- There is no specific treatment. Supportive management includes close monitoring of vital signs and maintenance of adequate intravascular volume.
- A live-attenuated, measles-vectored vaccine expressing CHIKV structural proteins (MV-CHIK), a chikungunya (CHIK) virus-like particle (VLP) vaccine, and a messenger RNA (mRNA)-based vaccine (VLA-181388) are under trials.

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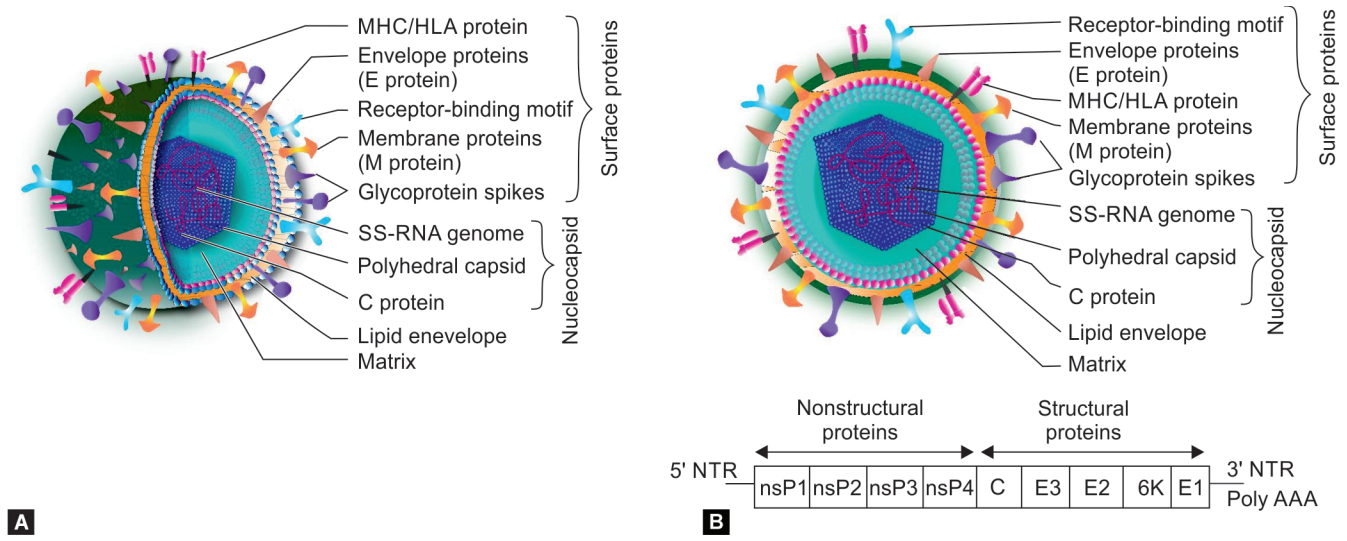
Introduction

Chikungunya virus (CHIKV) is an arthropod-borne (arbovirus) classified in the genus alphavirus, the arthritogenic Semliki forest virus serocomplex, and the family Togaviridae.[1–3] In adults, it has been associated with acute febrile polyarthralgia, inflammatory arthritis, and dermatologic and systemic presentations.[4,5] It was first isolated by Ross in 1952 in the Newala district of Tanzania[6] and then described in more detail in 1955 by Robinson and Lumsden after an earlier outbreak on the Makonde Plateau, along the border between Tanganyika and Mozambique.[7,8] The name Chikungunya is derived from the Kimakonde language spoken in southeast Tanzania and northern Mozambique, meaning "that which bends up" referring to the debilitating arthralgia caused by this disease.[9,10] It has now increasingly been recognized as a global health concern.[11]

Viral Structure

The chikungunya virus originated in Tanzania and is closely related to the O'nyong'nyong virus, which originated in Uganda.[12–14] It



Figs 1A and B: Schematic diagrams showing (A) surface and side dissection and (B) cross-section of the chikungunya virus

is an enveloped, positive-sense, single-chain linear ribonucleic acid (RNA) virus with a diameter of 60–70 nm (Fig. 1).[15] The RNA genome of around 11.8 kb is divided into two open reading frames (ORFs) surrounded by 5′ and 3′ nontranslated regions.[15,16] The 5′ ORF encodes four nonstructural proteins (nsPs): nsP1, nsP2, nsP3, and nsP4. The 3′ ORF also encodes four structural proteins: capsid (C), envelope 3 (E3), envelope 2 (E2), and envelope 1 (E1).[17,18] The nsPs are important mediators in viral pathogenesis and neuroinvasiveness due to their role in viral replication inside the host cytoplasm. The structural proteins facilitate the recognition of host cells, binding, and entry.[19,20] The E2 subunit of the E protein binds the Mxra8 receptor on fibroblasts and skeletal muscle cells and promotes viral entry into host cells by clathrin-mediated endocytosis.[20–24]

In RNA viruses, the replacement of only a few amino acids can bring about major changes in biological properties.[25,26] The RNA-dependent RNA polymerase (RdRp) of CHIKV is a low-fidelity enzyme, and hence can promote the formation of new viral variants during successive cycles of RNA replication.[27–29] These features promote adaptation to varying hosts and determine their pathogenicity.[30–32] The CHIKV has been traditionally classified into three lineages based on the sequences of the envelope E1 gene.[33,34] Since 2006, a new classification with four geographic lineages has been recognized: (1) the West African, (2) the Asian genotypes with varying E protein expression, (3) the East/Central/South African (ECSA) with mutations in the E1 protein, and (4) the Indian ocean lineage that has diverged from the ECSA.[35–38] The ECSA variants have also been isolated in Rio de Janeiro, Brazil, and in Reunion Island.[39] These variants can cause severe cutaneous lesions and multisystem disease in neonates.[19,33] Some mutants may show enhanced transmission via *Aedes albopictus* mosquitoes[26] and are associated with neuroinvasive disease by upregulating Toll-like receptor-3 in neuronal cells.[40,41] The ECSA strains with an arginine-to-valine mutation on position 226 seem to be the most pathogenic, CHIKV-Western Hemisphere (CHIKV-WH) as moderately so, and the unmodified ECSA and West African strains the least pathogenic lineages.[42,43] Table 1 summarizes the information on the major viral components.

Epidemiology

Geographical Areas at Higher Risk of CHIKV Infections

Chikungunya virus infections can be both endemic and epidemic.[10,44] The virus is transmitted via the *Aedes* mosquito vectors with a typical incubation period of 3–7 days, although the infectious period may range from 1 to 12 days.[45,46] It is endemic in West Africa, but outbreaks have also been recorded in other parts of Africa, Asia, Europe, islands in the Indian and Pacific Oceans, and in the Americas.[47,48] Over one-third of the inhabitants of La Reunion Island, a French territory in the Indian Ocean, were affected in the 2005–2006 outbreak.[49] Most outbreaks in the tropics occur during rains.[11] The CHIKV may rarely be transmitted by blood products.[50,51] The chikungunya (CHIK) viremia can precede the onset of symptoms and disappears after 6–7 days of illness.[52,53]

Mother-to-Fetus Transmission

Pregnant women infected with CHIKV before 16 weeks' gestation can develop deep trophoblastic invasion and consequently, fetal sequelae and deaths. In these women, the viral genome is detectable in high titers in the amniotic fluid, placenta, and/or brain of the fetuses.[54] The mothers are usually asymptomatic other than the occasional miscarriage.[55,56] During the second trimester, the placenta is a strong barrier to CHIKV and maternal-to-fetal transmission of the virus is generally infrequent.[47] In the third trimester, transmission is infrequent; even most of the stillborn fetuses born to mothers with CHIKV fever do not test positive for the virus.[57]

We do not have consistent and detailed epidemiological data implying a strong association between first-trimester infections and increased risk of miscarriage or congenital malformations. Chikungunya virus infections may occur with higher frequency in mothers from the lower socioeconomic strata of society.[58]

Maternal viremia is seen frequently in the peripartum period, particularly in the preceding 2 to the subsequent 2 days after delivery.[56] Vertical transmission rates during this period may range between 27.7% and 49%;[24,55,59] the risk is higher when peripartum

Table 1: Major structural components of CHIKV

Structure	*Available information*
Lipid envelope	The lipoprotein envelope is derived from the nuclear membrane of an infected host cell and covers the nucleocapsid.[191]
Glycoproteins	Glycoproteins, E1 and E2, form membrane spikes in an icosahedral shell on the virion surface. Glycoprotein E1 is a class II fusion protein that mediates low pH-triggered membrane fusion during infection. E2 is a type I transmembrane glycoprotein and binds cell surface receptors.[192,193] It is derived from furin cleavage of p62 precursors.[66,194]
Receptor-binding motifs	Receptor-binding motifs are involved in virion attachment to host cell surface receptors during the process of infection and endocytosis. Receptor binding is facilitated by the E2 glycoprotein of CHIKV,[195,196] which contains recognized receptor binding sites.[194,197] E2 domain B contains a class III PDZ-binding motif,[198,199] which mediates protein–protein interactions.[200,201] A phosphatidylserine residue in the viral envelope also binds cell–surface receptors on the cell surface.[202]
Envelope protein	Envelope proteins, E1 and E2, form membrane spikes on the viral surface.[203] These spikes facilitate attachment to cell surfaces and viral entry into the cells.
Membrane protein	E1 protein contains three β-barrel domains. Domain I is between domains II and III, and the fusion loop is at the distal end of domain II.[194] Heterodimers of the E1 and E2 proteins assemble into spikes on the virion surface and facilitate the infection of target cells.[194] The E1 protein contains a hydrophobic fusion peptide and is necessary for viral and cellular membrane fusion.[21]
Major histocompatibility complex (MHC) or human leukocyte antigens (HLA) proteins	Conserved B- and T-cell epitopes of CHIKV structural proteins may play an important role in evoking immune responses against CHIKV. B-cell epitopes "PPFGAGRPGQFGDI" is highly immunogenic, while among T-cell epitopes, MHC class I peptides "TAECKDKNL" and MHC class II peptides "VRYKCNCGG" are important. All T-cell epitopes are conserved between CHIKV genomic sequences belonging to 17 different countries.[204]
Spike protein	Glycoproteins, E1 and p62, bind to form heterodimers that subsequently trimerize into a viral spike in the endoplasmic reticulum. The CHIKV spikes show intraspike contacts between three constituent E2 molecules. The glycoprotein E1 wraps around E2 and contributes to interspike interactions with E3 being located at the periphery of the E2 molecules. The spikes undergo a structural rearrangement during maturation, with the cleavage of p62 into E2 and E3, thereby exposing the fusion loop on E1 and arranging the glycoprotein spikes into a mature conformation. The association of mature spikes with the nucleocapsid makes these less compact and nucleocapsid disassembly upon release into the host cell cytoplasm corresponding to the release of the genome into a host cell after virus entry.[199]
Surface tubules	Either not expressed or relevance unclear fetal/infantile disease.
Palisade layer	Either not expressed or relevance unclear fetal/infantile disease.
Viral tegument	Either not expressed or relevance unclear fetal/infantile disease.
Lateral bodies	Either not expressed or relevance unclear fetal/infantile disease.
Capsid	The capsid is composed of 240 copies of specific proteins and encloses the viral genomic RNA in nucleocapsid cores. These cores interact with the E1–E2 glycoproteins produced in the endoplasmic reticulum and the Golgi. The mature virions bud from the plasma membrane.[205]
Capsomeres	Structural subunits of the capsid and can be seen in electron micrographs.[206]
Core membrane	Either not expressed or relevance unclear fetal/infantile disease.
Protein core	The polyprotein is expressed from the ORF1 of CHIKV. It is processed into four nsPs (nsP1, 2, 3, and 4), which undergo proteolysis and assemble into the viral replication complex.[201] Mature nsPs function collaboratively to replicate the viral genomic RNA and to transcribe the subgenomic RNA, which encodes the structural genes for virus particle assembly.[207]
Core fibrils	Either not expressed or relevance unclear fetal/infantile disease.
Matrix	Either not expressed or relevance unclear fetal/infantile disease.
Enzymes	The cerebral palsy (CP) sindbis virus (SINV) is divided into three regions: region I (residues range: 1–80), region II (residues range: 81–113), and region III (residues range: 114–264). The regions I and II are part of the N-terminal domain of CP and are involved in the encapsidation of the genomic RNA.[205] The region III is part of the C-terminal domain, which is responsible for the serine protease activity of CP. The CP has a cis-proteolytic activity that cleaves itself from the nascent structural polyprotein precursor.[206] The nsP1 displays the unique N7-guanine-methyltransferase and guanylyltransferase activities required for viral RNA 5′ cap-0 synthesis. The nsP2 is the largest nsP that has the N-terminal RNA helicase/nucleoside triphosphatase/RNA triphosphatase domain and the C-terminal cysteine protease domain. The nsP4 is the RNA-dependent RdRp.[201]
RNA elements	A transient double-stranded replicative RNA intermediate composed of viral plus- and minus-strand RNAs is synthesized by a replicase complex formed by the non-structural proteins nsP1–4.[208] The newly synthesized minus strand serves in turn as a template, allowing the RNA-dependent RdRp to synthesize additional plus-strand genomic RNA.[17] Following the complete processing of the ns-polyprotein, the replicase then promotes the synthesis of the viral genomes and production of the subgenomic RNAs that encode the viral capsid and envelope proteins.[209]

(Contd...)

Table 1: *(Contd...)*

Structure	*Available information*
Nucleus	Either not expressed or relevance unclear fetal/infantile disease.
Nucleosome	Either not expressed or relevance unclear fetal/infantile disease.
DNA	No DNA genome exists.
RNA	The CHIKV virion contains a positive-sense RNA genome ~11.8 kb in length, which is translated into a large polyprotein during the infectious life cycle. The genome contains two ORFs flanked by 5′- and 3′-untranslated regions (UTRs) and separated by a noncoding intergenic region. The 5′-UTR is 76 nt in length and contains a 5′ type-0 N 7-methylguanosine cap for initiation of cap-dependent translation. The 3′-UTR varies in length between ~500 and ~900 nt and includes a 3′ polyadenylate tail.[17]
Genome-associated polyprotein	RNA genome is translated into a replicase complex consisting of four nsPs that are expressed as a polyprotein precursor. These nsPs are initially produced as a nonstructural polyprotein precursor that is processed by the viral protease.[210]
DNA polymerase	Either not expressed or relevance unclear fetal/infantile disease.
RdRp	The C-terminal domain of nsP4 acts as an RNA-dependent RdRp and catalyzes the formation of negative-sense, genomic, and subgenomic viral RNAs. Viral replication begins with the synthesis of minus-strand RNA from the positive-strand RNA genome, which then acts as a template for the formation of plus-strand RNA genomes. Production of new viral particles is catalyzed by the RNA-dependent RdRp.[211]
Reverse transcriptase	Either not expressed or relevance unclear fetal/infantile disease.
Head	Either not expressed or relevance unclear fetal/infantile disease.
Base plate	Either not expressed or relevance unclear fetal/infantile disease.
Integrase	Either not expressed or relevance unclear fetal/infantile disease.
Tail	Either not expressed or relevance unclear fetal/infantile disease.
Tail fiber	Either not expressed or relevance unclear fetal/infantile disease.
Neck	Either not expressed or relevance unclear fetal/infantile disease.

PDZ, post-synaptic density-95, disks-large and zonula occludens-1

maternal viremia coincides with breaches in the placental barrier and, consequently, results in high placental viral loads.[47,52,55] Cesarean sections are not protective and are, therefore, not recommended.[54–56,59] However, even though the epidemiological data are scant, there are many reports of infants who got infected during the peripartum period as developing neurocognitive delays and arrested head growth after birth.[47,54,60]

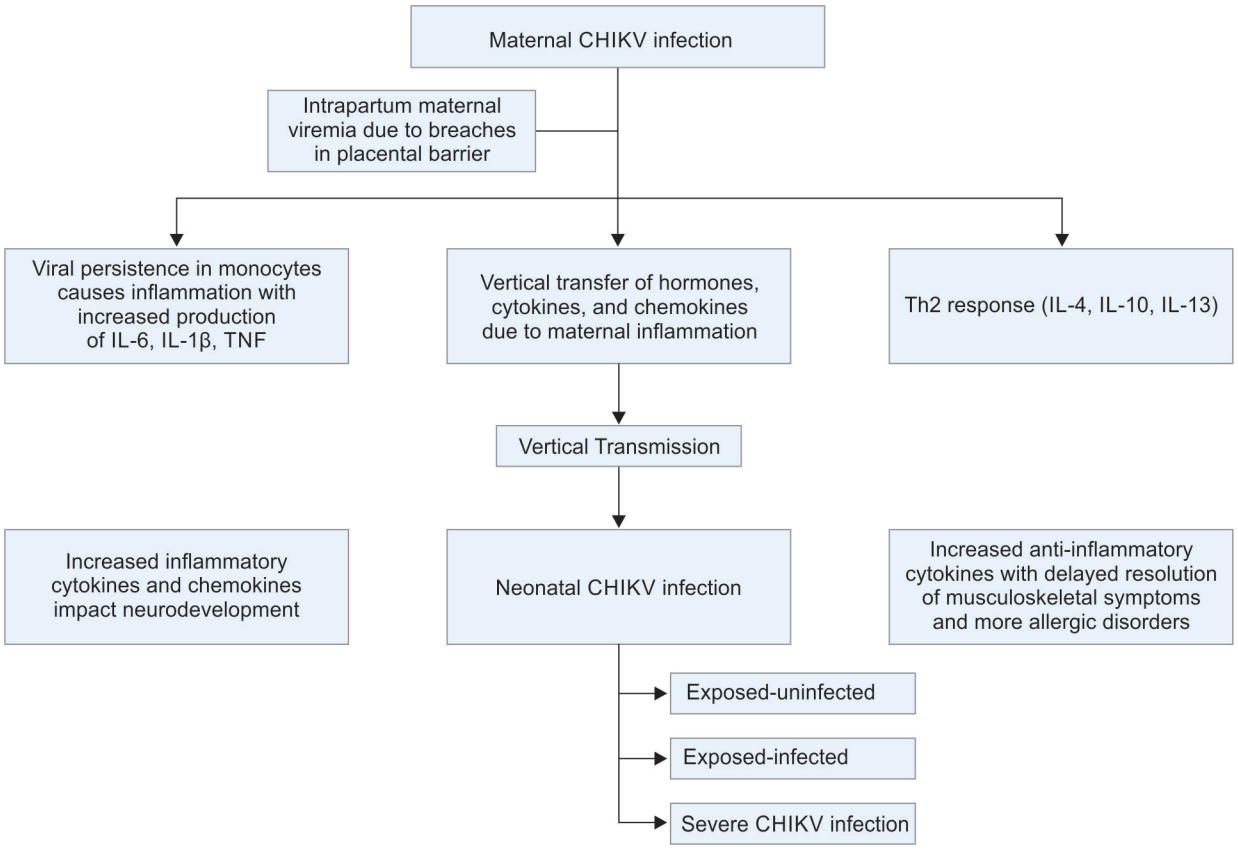
Pathogenesis

The CHIKV infection is followed by viremia within a few days of infection. Animal models of CHIKV infections suggest that the virus first infects the synovium, tenosynovium, and muscle, and then may persist in joints for several days to weeks.[61] This promotes the recruitment of leukocytes, particularly monocytes, and increased expression of inflammatory cytokines, chemokines, and other inflammatory mediators.[62–64] Disease severity correlates with the persistence of CHIKV in monocytes and the systemic inflammatory response with increased production of interleukin (IL)-6, IL-1β, tumor necrosis factor (TNF), and CC ligand 2 (CCL2, monocyte chemoattractant protein-1) (Flowchart 1).[24,47,65,66]

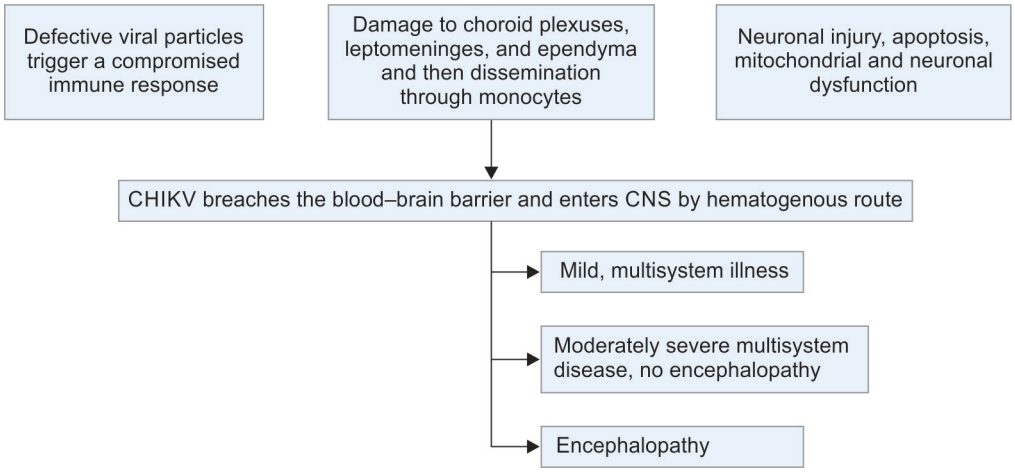
The CHIKV infections during pregnancy can manifest with maternal sepsis, preterm delivery, premature rupture of membranes, decreased fetal movements, intrauterine death, oligohydramnios, and preterm labor pains.[67,68] Cluster of differentiation 163 (CD163), an activation marker,[69] is detected in the CHIKV-infected placenta as an indication of the presence of Hofbauer cells.[70] The placenta is hyperplastic with enlarged $CD163^{+}$ cells due to immunological activation. Mitochondrial swelling, a characteristic of apoptosis,[71] and dilated endoplasmic reticulum cisterns are seen in the cytotrophoblasts of CHIKV-infected placenta, thereby affecting cell homeostasis and signaling.[72] There is a thickening of the endothelial basement membrane, which can alter the absorption of gases and nutrients in the placenta.[73] Maternal viral infections during gestation can cause epigenetic changes and alter the inflammatory microenvironment leading to developmental changes; there is a need for long-term follow-up.[74,75]

The CHIKV-induced maternal inflammation can influence fetal development through the vertical transfer of cytokines, chemokines, and hormones.[76] Neonates, with still evolving specific adaptive immunity, depend on innate immune responses with exaggerated inflammation and considerable morbidity.[77] The CHIKV-exposed infants have high levels of inflammatory cytokines such as TNF, which can impact neurodevelopment, and inhibit the proliferation and differentiation of neuronal cells (Flowchart 2).[78,79] However, the effects of various cytokines are not consistent. The CHIKV-exposed infants may have increased circulating chemokines such as chemokine (C-X-C motif) ligand 8 (CXCL-8), chemokine (C-C motif) ligand 3 (CCL3) macrophage inflammatory protein, (MIP)-1α and CCL4 (MIP-1β), which recruit neutrophils and monocytes.[80–82] Elevated plasma/cerebrospinal fluid (CSF) levels of CXCL9, CXCL10, and the eotaxins (CCL11, CCL24, and CCL26) can promote neuronal damage and possibly be associated with Zikavirus (ZIKV) microcephaly.[47,83–86] CXCL10 and eotaxins can also promote neurological damage in these patients.[47,84] Low levels of T-helper cell 2 (Th2)-related cytokines such as IL-4, IL-10, and IL-13 can delay the resolution of musculoskeletal symptoms.[87,88] However, CCL3/MIP-1α may be neuroprotective.[82] Interferon (IFN)-γ and IL-12p70 may also be

Flowchart 1: Pathogenesis of perinatal chikungunya infection



Flowchart 2: Pathogenesis of neurological manifestations



neuroprotective in some situations.[79,89,90] However, neonates have limited expression of Toll-like receptors that induce IFN production.[91] Type I IFN (IFNα, –β, and –ω) are an important component of anti-CHIKV immunity and can suppress viral replication in the early stages of the disease.[92]

Asymptomatic CHIKV-infected infants may present with serum ferritin > 600 ng/dL, which is a by-product of IL-8 activation in viral infections.[93] It has been noted earlier as a predictor of the severity of dengue fever.[94] Compared to plasma, CSF samples frequently contain different or defective viral mutants with insertions/deletions and stop codons in nonstructural genes. Defective viral particles can trigger an atypical immune response that may cause placental or blood–brain barrier damage, followed by vertical or brain transmission.[95]

Murine models of CHIKV infection show increased expression of genes presumably involved in neuronal injury, mitochondrial and neuronal dysfunction, and apoptosis.[96] Even though only a minority of infected patients develop neuroinvasive disease, immune imbalance may play an important role in neurodegeneration. Because in addition to the CHIKV infection

itself, some immuno mediators such as TNF and IL-6 are known to cause neuronal death.[96]

The CHIKV encephalopathy is associated with neurotropism as evidenced by white matter (WM) and corpus callosum atrophy, microglial activation and demyelination, neuronal loss due to WM damage and leading to microcephaly, cerebral palsy, or neurocognitive dysfunction, which is similar to neonatal encephalitis caused by enterovirus or parechovirus infections.[97,98] These encephalopathic changes involve damage to the highly vascular choroid plexuses, leptomeninges, and the ependyma,[92,99] and are followed by the dissemination of the virus in monocytes.[100] This is seen as WM hyperintensities on T1-weighted magnetic resonance imaging (MRI), consistent with microglial activation leading to demyelination.[47] On MRI, the most distinctive lesion of CHIKV neonatal encephalopathy is reversible diffusion restriction of WM associated with transient ischemia with cytotoxic edema.[101] In an animal model, viral infection is mainly detected at the meningeal and ependymal levels rather than in the brain parenchyma.[92] The CHIKV affects stem cell production by ependymal cells, neuron migration,[102] and myelin sheath production.[13,103,104] Demyelination is the hallmark of CHIKV neonatal encephalopathy, which is caused by autoreactive $CD8^+$ T lymphocytes to clear infected cells.[105,106] $CD8^+$ T cells are frequently seen in the CSF of cynomolgus macaques, the only nonhuman primate model challenged by CHIKV.[107]

Clinical Presentations

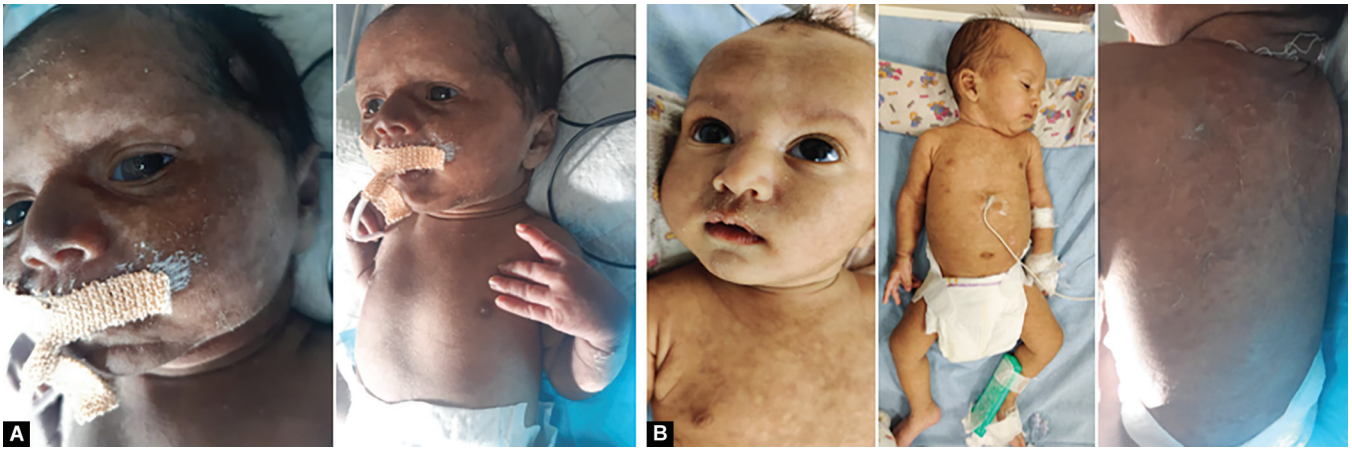
For infants presenting during the neonatal period, the median age of presentation is 9.5 (range: 3–15) days, whereas for babies presenting after the neonatal period, the median age is between 1 and 3 months.[47,108] In the Réunion outbreak, neonates presented earlier, within 3–7 days after delivery with fever, poor feeding, rash, and peripheral edema with 89% having thrombocytopenia.[55] Some infants present with meningoencephalitis, cerebral edema, and intracranial hemorrhage or myocardial disease.[54]

Vertically transmitted CHIKV infections in neonates typically present during the first week of life but not at the time of birth and present with fever, polyarthralgias, limb edema, irritability, poor feeding, and rash. Twenty-five percent show skin manifestations such as maculopapular rashes (Fig. 2), freckle-like pigmentation in the centrofacial area, and vesiculobullous lesions. Acute inflammatory lesions typically last 5–7 days and are often followed by hyperpigmentation due to a postinflammatory response or CHIKV-induced intraepidermal melanin retention.[109] The pigmentary changes are seen most frequently in the axilla, perioral, and genital areas. Some infants show tenderness and edema of the hands and feet. Arthritis is seen very rarely.[108] There may be mucosal changes such as nasal blotchy erythema and multiple aphthous-like ulcerations. Some infants may show purpuric or hemorrhagic vasculitic lesions, toxic epidermal necrolysis-like rash, or nail changes like black lunulae, longitudinal melanonychia, and transverse pigmented bands.[109–111]

Severely afflicted infants may have sepsis-like syndrome with multiorgan dysfunction, meningoencephalitis, recurrent apnea, shock, and/or disseminated intravascular coagulation. Unlike dengue, hemorrhagic manifestations and shock are infrequent in CHIKV infections.[112]

One systemic review[47] showed that the pooled combined disease impact on the fetus and newborn was 17%. The overall risk of symptomatic disease in neonates born to mothers with active infection was 15.5%. The risk was higher, nearing 50%, among intrapartum maternal infections. The pooled risk of long-term neurodevelopmental delays in infants with symptomatic neonatal infections neared 50%. The mean interval between the onset of maternal illness and the onset of neonatal illness was 5 days (range: 3–9). The most frequent clinical signs in neonates were fever (79%), pain (100%), rash (82%), and peripheral edema (58%). Thrombocytopenia (76%), lymphopenia (47%), altered coagulation (65%), and elevation of aspartate aminotransferase (77%) were detected with some neonates developing complications such as seizures, hemorrhage, and hemodynamic disorders. Reverse transcription-polymerase chain reaction (RT-PCR) in CSF was positive in 22 of 24 cases. The brain MRI showed WM lesions, intraparenchymal hemorrhages, or both. Echocardiography showed myocardial hypertrophy, ventricular dysfunction, pericarditis, and coronary artery dilatation. One neonate died of necrotizing enterocolitis.[47]

In neonates, the incidence of symptomatic infections varies by region, although most have no or relatively mild symptoms. In contrast to adults with CHIKV infections, infants have a fever lasting only for 24–48 hours, and this is followed by the appearance of the maculopapular rash. Some may develop vesicles and bullae by the fourth day along with acrocyanosis without any hemodynamic alteration.



Figs 2A and B: Clinical manifestations in two neonates with congenital chikungunya. (A) Images from one infant show prominent pigmentary changes comprising the "CHIK sign" of congenital chikungunya infection on the central part of the face. (B) The pigmentary changes in a second infant extended to the chest

One report described 12% of those infected vertically as symptomatic.[113] Joint involvement can be seen in a few cases.[114] One case of neonatal CHIKV infection has been reported from India with a fixed flexion deformity of the right thumb on follow-up at 6 months, suggestive of tenosynovitis manifesting as a sequela of arthritis.[115] In another report, the authors have described painful arthralgia in 78–100% neonates, associated with distal joint edema and persistent prostration.[55] In highly endemic zones, neonates can acquire CHIKV after birth, coincidental with other family members.[116] There is a reported case of congenital CHIKV infection who had hyperpigmented macules and extensive dystrophic calcifications at birth, suggestive of *in utero* skin affliction.[117] These skin lesions resolved without any sequelae with supportive therapy (Table 2).[118]

The severe neonatal disease is frequently associated with thrombocytopenia, where low platelet counts were seen in the more severe neonatal diseases.[119] Another report has described a newborn who was infected postnatally, confirmed by positive immunoglobulin (Ig)M in the neonate and a negative IgM serology on the mother.[120] A few infants with high viral concentrations developed severe manifestations such as meningoencephalitis and disseminated intravascular coagulation.[56,121,122]

In the CHIMERE cohort study of CHIldren Exposed to Perinatal MothEr-to-Child Chikungunya Virus Infections on the REunion Island,[54] 33 children with maternal–fetal transmission of CHIKV at birth and 135 uninfected controls during the Reunion outbreak were evaluated. Neurodevelopmental follow-up at 2 years showed that 51% of infected children had a global neurodevelopmental delay compared to 15% of controls. These findings suggested that there might be a causal relationship between perinatal CHIKV infection and neurocognitive outcomes. Both the encephalopathic and nonencephalopathic forms of CHIKV infections have been associated with early cytotoxic and late vasogenic cerebral edema along with the presence of viral genome in CSF.[55,123] Pregnant women who acquired CHIKV long before delivery delivered healthy neonates.[55,58,124,125] In 12 cases of CHIKV neonatal encephalopathy, 5 have been identified as having microcephaly and 4 matched the definition of cerebral palsy. The MRI scans showed severe restrictions of WM areas, predominant in the frontal lobes in these children.[54]

Eighteen months after the Reunion outbreak of CHIKV infections, a retrospective cohort TELECHIK survey was performed on a random representative sample of the SEROCHIK population-based sero survey.[126] The TELECHIK cohort study revealed that 10% of CHIKV patients had light cerebral disorders (headache, sleep, memory, and depression) on 18-month follow-up.[126] Preterms were at risk of severe neurologic damage,[92] as exemplified by brain swelling and WM injury on MRI.[55,123] Coordination and language skills were frequently affected followed by movement/posture and sociability. The CHIKV neonatal encephalopathy shows low *N*-acetyl aspartate peaks on magnetic resonance spectroscopy, indicating WM hypometabolism, especially in the frontal lobes, thereby affecting coordination and language centers.[127]

Case definitions used in perinatal chikungunya are summarized in Table 3.

Table 2: Pigmentary changes noted in chikungunya infection[117]

Generalized hyperpigmentation.
Striking pigmentation on the nose is called "brownie nose" or the "Chik" sign of CHIKV disease.
Macular type.
Freckle-like pigmented macules that tend to coalesce with each other Pinpoint confetti-like macules.
Irregular flagellate or whiplash pattern of brownish pigmentation seen over trunk and extremities.
Periorbital hypermelanosis.
Addisonian-type palmar pigmentation

Laboratory Diagnosis

The diagnosis of CHIKV is done by detection of chikungunya viral RNA via real-time RT-PCR or IgM- and IgG-specific serology.[128]

Reverse-transcription Polymerase Chain Reaction

The RT-PCR is usually positive during the viremic phase, which continues till 1 week after the onset of symptoms.[129] For individuals presenting 1–7 days following the onset of symptoms, a positive CHIKV RT-PCR is diagnostic of infection.[129] The RT-PCR has 100% sensitivity and 98% specificity.[40,50]

Serology

Serologic testing is done by enzyme-linked immunosorbent assay (ELISA) or indirect fluorescent antibody for those presenting ≥8 days following the onset of symptoms. Immunoglobulin M anti-CHIKV antibodies (detected by direct ELISA) are detected on the fifth day (range: 1–12 days) after disease onset and persist for several weeks to 3 months, whereas specific IgG antibodies begin to appear on the 15th day and persist for years.[4,130] A plaque-reduction neutralization test can help to quantitate virus-specific neutralizing antibodies and to discriminate between cross-reacting antibodies such as those reactive with the Mayaro and o'nyong'nyong viruses.[131] The absence of positive CHIKV serology at birth does not exclude neonatal CHIKV infection because the development of CHIKV IgG and IgM antibodies in infected infants can be delayed in the first 3–4 weeks of life.[132] Hence, serial serologic monitoring may be helpful in the follow-up of these infants.[133] Transplacentally transferred CHIKV-IgG antibodies disappear by around 8 months of age in uninfected neonates.[132,134,135] The time to neonatal seroconversion is inversely related to the time of maternal infection as evidenced by IgG positivity of approximately 75%, 30%, and <1% for maternal infection in the first, second, and third trimesters, respectively.[132] Uninfected neonates may achieve full seroconversion (IgG-negative status) by 24 months.[132] Infants who are vertically infected with CHIKV may be seronegative at birth but specific IgM and IgG antibodies may appear by 3–4 weeks later.[132]

Viral Culture

The CHIKV isolation has a high specificity and high sensitivity in early infection but reduces after day 5 of onset of illness. It is expensive and labor intensive, hence usually done for research purposes.[50,129,136] Virus isolation takes around 7–10 days.[137] However, it can help identify the viral strain which is of value in the assessment of risk and for collecting epidemiological data.[10] Immunohistochemical staining can detect specific viral antigens in fixed tissue.[138]

Laboratory Evaluation

The CHIKV-exposed neonates are not symptomatic at birth but become ill before day 7, thereby making observational care in the postnatal ward mandatory at least for a week with serial

Table 3: Definitions used in perinatal CHIKV

Neonatal CHIKV infection	Defined as RT-PCR detection of the viral genome in the neonate's serum and/or CSF during the first week of life and/or detection of serum anti-CHIKV IgM.[123]
Maternal CHIKV infection	Defined by RT-PCR detection of the viral genome in maternal serum and/or the presence of serum anti-CHIKV IgM.[123]
Prepartum maternal CHIKV infection	Maternal symptoms lasting between day 7 and day 3 before delivery and diagnosed by RT-PCR (or IgM seroconversion when RT-PCR not available).[55]
Intrapartum maternal CHIKV infection	Maternal symptoms between preceding 2 to subsequent 2 days after delivery and with positive RT-PCR (or IgM seroconversion when RT-PCR not available).[55]
CHIKV perinatal mother-to-infant infection (p-CHIKV infection) or exposed-infected (EI)	Diagnosed if infants of mothers infected during pregnancy have a positive RT-PCR result and/or presence of CHIKV-specific MAC-ELISA IgM antibodies before day 10 (or day 15 in the CSF).[56]
Exposed-uninfected (EU)	Neonates exposed to maternal CHIKV infection and testing negative for RT-PCR and CHIKV-specific IgM antibodies at birth, for whom CHIKV-specific IgG seroreversed during follow-up.[54]
Severe CHIKV infection	Presence of convulsions, coma requiring mechanical ventilation, or abnormal MRI scans indicative of cytotoxic or vasogenic cerebral edema during the acute phase of the disease.[55,56]
Mild, multisystem illness	CHIKV-infected neonates who have difficulty in feeding, tachypnea, and vomiting/diarrhea.[56]
Nonencephalopathic, moderate-to-severe multisystem illness	Moderate severity of illness. May need ventilatory support. There may be some alterations in the laboratory evaluation of liver and renal function. No encephalopathy.[56]
Encephalopathy	Newborns show encephalopathy-related neurologic signs during the acute phase of the disease, such as convulsions, altered sensorium, and abnormal MRI scans indicative of cytotoxic/vasogenic cerebral edema.[212]

measurements of white blood cell and platelet counts, with urgent transfer to the neonatal intensive care units upon the appearance of symptoms, lymphopenia, or thrombocytopenia.[55] Laboratory investigations to be done are complete blood count, metabolic parameters (blood sugar, calcium, sodium, and potassium), liver function tests, sepsis screen, cultures, and CSF analysis.[139] Thrombocytopenia, leukopenia or leukocytosis, hypoalbuminemia, and transaminitis with direct hyperbilirubinemia and altered coagulation are seen in symptomatic infants.[47] Lymphocytopenia has been noted in nearly 70% of neonates with CHIKV infection.[55,70] Thrombocytopenia has been seen in 89% of infected neonates and is a marker of disease severity. Steroids and intravenous Igs have been tried to reduce the risk of bleeding complications but the benefits remain unproven.[140,141] In Salvador-Brazil, sera and urine samples have tested positive on RT-PCR for CHIKV during the first postnatal week in neonates and their mothers.[142]

Neurological Evaluation

The infant may require the ultrasound of the skull or MRI of the brain, CSF analysis, RT-PCR of CSF, and basic metabolic workup (blood sugar, calcium, magnesium, and sodium) to rule out other causes of encephalopathy.[143,144] In cases with signs of meningeal involvement, lymphocytic pleocytosis with normal CSF glucose and proteins is seen.[95]

Histopathology of Placenta

The CHIKV RNA and antigens can be detected in the placental tissue seen as histopathological (deciduitis, fibrin deposition, edema, fetal vessel thickening, and chorioamnionitis) and ultrastructural alterations (cytotrophoblast with mitochondrial swelling and dilated cisterns in the endoplasmic reticulum, vesicles in syncytiotrophoblasts, and thickening of the basement membrane of the endothelium).[145,146]

Table 3 presents the case definitions of neonatal chikungunya.

Differential Diagnosis of CHIKV Infection

Dengue and CHIKV have similar clinical manifestations and geographic distribution.[147] The CHIKV is more likely to cause high fever, severe arthralgia, arthritis, rash, and lymphopenia, while neutropenia, thrombocytopenia, hemorrhage, shock, and death are commoner in dengue.[148] Chikungunya virus manifests with higher fevers and more intense joint pain than Zika.

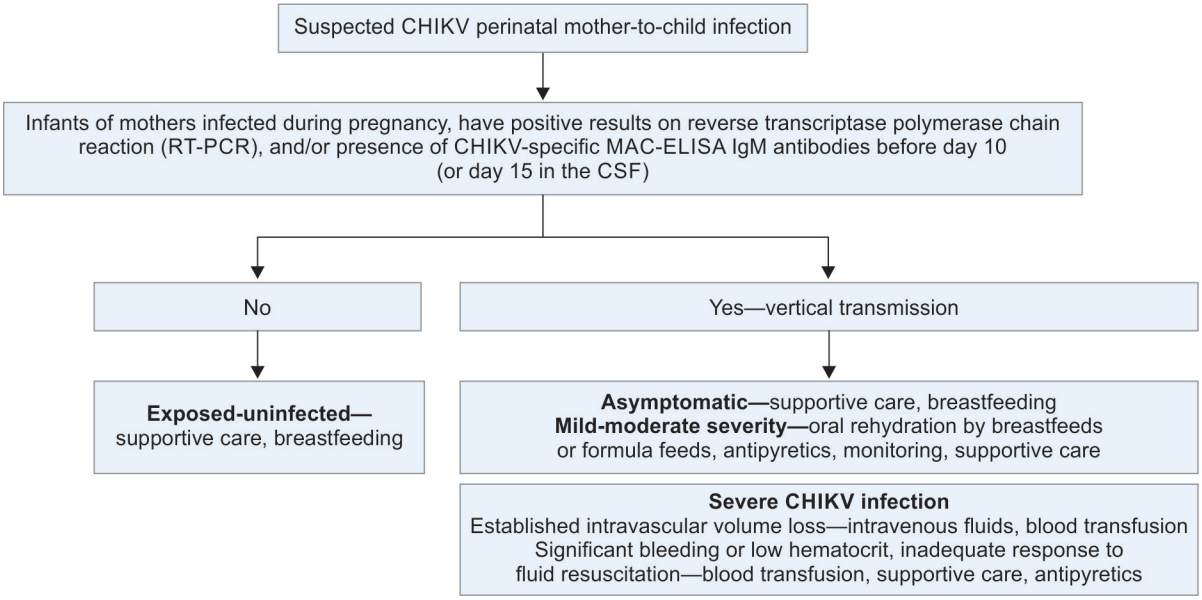
The CHIKV outbreaks have occurred concurrently with outbreaks of dengue, Zika virus,[149,150] and yellow fever.[150] Coinfection with CHIKV and other pathogens has been reported, namely, CHIKV, dengue, and Zika;[49] CHIKV and dengue;[151] and CHIKV and Zika,[152] and CHIKV and yellow fever.[153] Neonatal CHIKV infections can mimic meningoencephalitis, bacterial sepsis, or metabolic encephalopathy.[154]

Treatment

There is no antiviral treatment available for CHKV infections[155–157] and, therefore, primary treatment is supportive care by maintaining adequate intravascular volume (Flowchart 3). Oral rehydration by breastfeeds or formula feeding should be done. Acetaminophen (maximum 60 mg/kg/day) can be used for the management of fever. In a patient who could have a dengue virus infection, aspirin, and other non-steroidal anti-inflammatory drugs (NSAIDs) should not be used until dengue has been excluded in view of the bleeding complications associated with dengue and the potential risk of Reye's syndrome in children. Coinfection with dengue and CHIKV can occur. So, dengue infection needs to be excluded even if the diagnosis of CHIKV infection is confirmed.

Infants with CHKV infections should be monitored closely for vital signs, input–output, oxygen saturation, and sensorium. Administration of intravenous fluid is required in babies with established intravascular volume loss. Blood transfusion is

Flowchart 3: Management of perinatal chikungunya infection



warranted in patients with significant bleeding or low hematocrit and inadequate response to fluid resuscitation. There is no role for corticosteroids, intravenous Igs, or antivirals in the treatment of CHIKV.[24]

Pregnancy and Breastfeeding

Pregnant women should avoid travel to *Aedes* spp. endemic regions.[158] Post-travel laboratory testing should be reserved for symptomatic patients.[158] Whether CHIKV is secreted in human milk is uncertain, although CHIKV RNA has been detected in human milk.[45] Transmission by breastfeeding has not been reported and, therefore, breastfeeding should be continued even in areas with the circulation of the CHIKV.[159] Breast milk also contains antiviral antibodies that may provide protection.[160] Asymptomatic neonates may be discharged after a week; while in symptomatic cases, discharge can be considered when afebrile for 24–48 hours, hemodynamically stable, with good urine output and accepting feeds well.[120]

Outcomes

The case fatality rate in congenital CHIKV infection may vary between 0.8 and 37.5%.[58,59,123,161] Maternal–fetal transmission of CHIKV may result in severe neonatal complications. Among three symptomatic neonates with serologically confirmed, vertically transmitted CHIKV infection in Curaçao, two developed neurological complications, including convulsions and intracranial bleeding, while one newborn, in whom maternal infection occurred 7 weeks before delivery, had a fatal outcome after birth.[89] Exposed/infected children can have poor neurocognitive outcomes and must be monitored throughout childhood and early intervention therapy should be provided wherever feasible for "CHIKV-driven disability".[126,157,161,162] Long-term sequelae have been described including neurologic sequelae at 6 months of follow-up[56] or 2 years after CHIKV encephalitis.[159] CHIKV-infected pregnant women and neonates should be followed-up for sequelae such as chronic inflammatory rheumatism, which may persist for up to 6 years.[157,161] Children infected with CHIKV later during the first 2 years of life as determined by ELISA for antigens and/or specific antibody tests have 2-year neurodevelopmental outcomes similar to children who were not infected.[57,163–168]

Prevention

Approaches for the prevention of dengue virus (DENV) infection in endemic areas may include mosquito control, personal protective measures, and vaccination. Prevention of chikungunya virus infection consists of minimizing mosquito exposure through personal protection and environmental control measures.[169]

Vaccine Development

There are no licensed vaccines for CHIKV, but 15 candidate vaccines are currently under preclinical and clinical development.[113,170–175] In an randomised controlled trial (RCT), a live-attenuated, measles-vectored vaccine expressing CHIKV structural proteins (MV-CHIK) induced neutralizing antibodies against CHIKV after one to two immunizations.[175] Seroconversion rates varied between 50 and 93% after one and 86 and 100% after 2 doses. Immune responses lasted till 6 months of these doses, and the vaccine was safe and well-tolerated. Further studies are required for vaccine efficacy and cross-protection against multiple CHIKV strains.

Phase 2 RCTs of a CHIK virus-like particle (VLP) vaccine have revealed a 4-fold rise from baseline neutralization titers in 88% of recipients after an intramuscular dose.[176] The immune response lasts 72 weeks after vaccination, and the vaccine is safe and well-tolerated. Phase 3 trials are required. A messenger RNA (mRNA)-based vaccine (VLA-181388) is still in phase 1 clinical trials.[177,178]

Future Directions

Further efforts are needed to develop specific antiviral agents and vaccines for the management of chikungunya infections.[179] There is also a need for planned urbanization with efforts for mosquito control.[180] Public health agencies and clinicians should be aware of the existence of maternal–fetal transmission of chikungunya and be prepared to diagnose and treat these neonatal infections.[47,169]

Recent efforts to control mosquito populations through genetic strategies appear promising.[181,182] Several genetics-based approaches focused on male sterilization are being tried.[183–186] Recombinant DNA methods provide a step change in our ability to design and build specific genetic systems.[187,188] Several *Aedes*

species have now been transformed, either by recombinant DNA methods using transposon vectors or by artificial infections with various *Wolbachia*, a diverse group of intracellular bacteria.[189,190] These techniques may help control chikungunya and other vector-borne diseases.

References

1. Cunha MS, Costa PAG, Correa IA, et al. Chikungunya virus: An emergent arbovirus to the South American continent and a continuous threat to the world. Front Microbiol 2020;11:1297. DOI: 10.3389/fmicb.2020.01297.
2. Schwartz O, Albert ML. Biology and pathogenesis of chikungunya virus. Nat Rev Microbiol 2010;8(7):491–500. DOI: 10.1038/nrmicro2368.
3. Powers AM, Logue CH. Changing patterns of chikungunya virus: Re-emergence of a zoonotic arbovirus. J Gen Virol 2007;88(9):2363–2377. DOI: 10.1099/vir.0.82858-0.
4. Weaver SC, Lecuit M. Chikungunya virus and the global spread of a mosquito-borne disease. N Engl J Med 2015;372(13):1231–1239. DOI: 10.1056/NEJMra1406035
5. Monge P, Vega JM, Sapag AM, et al. Pan-American League of Associations for Rheumatology-Central American, Caribbean and Andean Rheumatology Association Consensus-Conference Endorsements and Recommendations on the diagnosis and treatment of chikungunya-related inflammatory arthropathies in Latin America. J Clin Rheumatol 2019;25(2):101–107. DOI: 10.1097/RHU.0000000000000868.
6. Ross RW. The Newala epidemic. III. The virus: Isolation, pathogenic properties and relationship to the epidemic. J Hyg (Lond) 1956;54(2):177–191. DOI: 10.1017/s0022172400044442.
7. Taksande A, Vilhekar KY. Neonatal chikungunya infection. J Prev Inf Cntrl 2015;1(1):8.
8. Gudo ES, Black JF, Cliff JL. Chikungunya in Mozambique: A forgotten history. PLoS Negl Trop Dis 2016;10(11):e0005001. DOI: 10.1371/journal.pntd.0005001.
9. Robinson MC. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. I. Clinical features. Trans R Soc Trop Med Hyg 1955;49(1):28–32. DOI: 10.1016/0035-9203(55)90080-8.
10. Silva LA, Dermody TS. Chikungunya virus: Epidemiology, replication, disease mechanisms, and prospective intervention strategies. J Clin Invest 2017;127(3):737–749. DOI: 10.1172/JCI84417.
11. Mourad O, Makhani L, Chen LH. Chikungunya: An emerging public health concern. Curr Infect Dis Rep 2022;24(12):217–228. DOI: 10.1007/s11908-022-00789-y.
12. Powers AM, Brault AC, Shirako Y, et al. Evolutionary relationships and systematics of the alphaviruses. J Virol 2001;75(21):10118–101131. DOI: 10.1128/JVI.75.21.10118-10131.2001.
13. Abere B, Wikan N, Ubol S, et al. Proteomic analysis of chikungunya virus infected microglial cells. PLoS One 2012;7(4):e34800. DOI: 10.1371/journal.pone.0034800.
14. Rezza G, Chen R, Weaver SC. O'nyong-nyong fever: A neglected mosquito-borne viral disease. Pathog Glob Health 2017;111(6):271–275. DOI: 10.1080/20477724.2017.1355431.
15. Laurent T, Kumar P, Liese S, et al. Architecture of the chikungunya virus replication organelle. Elife 2022;11:e83042. DOI: 10.7554/eLife.83042.
16. Griffin DE. Alphaviruses. In: Fields Virology, 6th edition. Lippincott-Raven: Philadelphia, 2015; pp. 651–686.
17. Kendall C, Khalid H, Muller M, et al. Structural and phenotypic analysis of chikungunya virus RNA replication elements. Nucleic Acids Res 2019;47(17):9296–9312. DOI: 10.1093/nar/gkz640.
18. Singh A, Kumar A, Uversky VN, et al. Understanding the intractability of chikungunya virus proteins via molecular recognition feature analysis. RSC Adv 2018;8(48):27293–27303. DOI: 10.1039/c8ra04760j.
19. Barr KL, Vaidhyanathan V. Chikungunya in infants and children: Is pathogenesis increasing? Viruses 2019;11(3):294. DOI: 10.3390/v11030294.
20. Kril V, Aiqui-Reboul-Paviet O, Briant L, et al. New insights into chikungunya virus infection and pathogenesis. Annu Rev Virol 2021;8(1):327–347. DOI: 10.1146/annurev-virology-091919-102021.
21. Schnierle BS. Cellular attachment and entry factors for chikungunya virus. Viruses 2019;11(11):1078. DOI:10.3390/v11111078.
22. Kim AS, Zimmerman O, Fox JM, et al. An evolutionary insertion in the Mxra8 receptor-binding site confers resistance to alphavirus infection and pathogenesis. Cell Host Microbe 2020;27(3):428–440e9. DOI: 10.1016/j.chom.2020.01.008.
23. Zhang R, Kim AS, Fox JM, et al. Mxra8 is a receptor for multiple arthritogenic alphaviruses. Nature 2018;557(7706):570–574. DOI: 10.1038/s41586-018-0121-3.
24. Fernandes AIV, Souza JR, Silva AR, et al. Immunoglobulin therapy in a patient with severe chikungunya fever and vesiculobullous lesions. Front Immunol 2019;10:1498. DOI: 10.3389/fimmu.2019.01498.
25. Pfeiffer JK, Kirkegaard K. A single mutation in poliovirus RNA-dependent RNA polymerase confers resistance to mutagenic nucleotide analogs via increased fidelity. Proc Natl Acad Sci USA 2003;100(12):7289–7294. DOI: 10.1073/pnas.1232294100.
26. Tsetsarkin KA, Vanlandingham DL, McGee CE, et al. A single mutation in chikungunya virus affects vector specificity and epidemic potential. PLoS Pathog 2007;3(12):e201. DOI: 10.1371/journal.ppat.0030201.
27. Holmes E. The RNA Virus Quasispecies. In: The Evolution and Emergence of RNA Viruses: Oxford Series in Ecology and Evolution. Harvey PH, May RM (eds). Oxford University Press: UK, 2009; pp. 87–103.
28. Stapleford KA, Rozen-Gagnon K, Das PK, et al. Viral polymerase-helicase complexes regulate replication fidelity to overcome intracellular nucleotide depletion. J Virol 2015;89(22):11233–11244. DOI: 10.1128/JVI.01553-15.
29. Kautz TF, Forrester NL. RNA virus fidelity mutants: a useful tool for evolutionary biology or a complex challenge? Viruses 2018;10(11):600. DOI: 10.3390/v10110600.
30. Fitzsimmons WJ, Woods RJ, McCrone JT, et al. A speed-fidelity trade-off determines the mutation rate and virulence of an RNA virus. PLoS Biol 2018;16(6):e2006459. DOI: 10.1371/journal.pbio.2006459.
31. Lee HY, Perelson AS, Park SC, et al. Dynamic correlation between intrahost HIV-1 quasispecies evolution and disease progression. PLoS Comput Biol 2008;4(12):e1000240. DOI: 10.1371/journal.pcbi.1000240.
32. Sullivan DG, Bruden D, Deubner H, et al. Hepatitis C virus dynamics during natural infection are associated with long-term histological outcome of chronic hepatitis C disease. J Infect Dis 2007;196(2):239–248. DOI: 10.1086/518895.
33. Schuffenecker I, Iteman I, Michault A, et al. Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. PLoS Med 2006;3(7):e263. DOI: 10.1371/journal.pmed.0030263.
34. Langsjoen RM, Haller SL, Roy CJ, et al. Chikungunya virus strains show lineage-specific variations in virulence and cross-protective ability in murine and nonhuman primate models. *mBio* 2018;9(2):e02449-17. DOI: 10.1128/mBio.02449-17.
35. Burt FJ, Rolph MS, Rulli NE, et al. Chikungunya: A re-emerging virus. Lancet 2012;379(9816):662–671. DOI: 10.1016/S0140-6736(11)60281-X.
36. Lumsden WH. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. II. General description and epidemiology. Trans R Soc Trop Med Hyg 1955;49(1):33–57. DOI: 10.1016/0035-9203(55)90081-x.
37. Pialoux G, Gauzere BA, Jaureguiberry S, et al. Chikungunya, an epidemic arbovirosis. Lancet Infect Dis 2007;7(5):319–327. DOI: 10.1016/S1473-3099(07)70107-X.
38. Santhosh SR, Dash PK, Parida MM, et al. Comparative full genome analysis revealed E1: A226V shift in 2007 Indian Chikungunya virus isolates. Virus Res 2008;135(1):36–41. DOI: 10.1016/j.virusres.2008.02.004.
39. de Souza TMA, Ribeiro ED, Correa VCE, et al. Following in the footsteps of the chikungunya virus in Brazil: The first autochthonous cases in Amapa in 2014 and its emergence in Rio de Janeiro during 2016. Viruses 2018;10(11):623. DOI: 10.3390/v10110623.

40. Edwards T, Del Carmen Castillo Signor L, Williams C, et al. Analytical and clinical performance of a chikungunya qRT-PCR for Central and South America. Diagn Microbiol Infect Dis 2017;89(1):35–39. DOI: 10.1016/j.diagmicrobio.2017.06.001.
41. Priya R, Patro IK, Parida MM. TLR3 mediated innate immune response in mice brain following infection with Chikungunya virus. Virus Res 2014;189:194–205. DOI: 10.1016/j.virusres.2014.05.010.
42. Gerardin P, Freitas ARR, Sissoko D, et al. Transmission dynamics and disease severity in children infected with East Central South African (ECSA) or ECSA-diverged clades of chikungunya virus. Clin Infect Dis 2019;68(1):171–172. DOI: 10.1093/cid/ciy534.
43. Gordon A, Gresh L, Ojeda S, et al. Differences in transmission and disease severity between 2 successive waves of chikungunya. Clin Infect Dis 2018;67(11):1760–1767. DOI: 10.1093/cid/ciy356.
44. Powers AM, Brault AC, Tesh RB, et al. Re-emergence of chikungunya and o'nyong'nyong viruses: Evidence for distinct geographical lineages and distant evolutionary relationships. J Gen Virol 2000;81(2):471–479. DOI: 10.1099/0022-1317-81-2-471.
45. Kimberlin DW. Chikungunya. In: Red Book: 2021 Report of the Committee on Infectious Diseases. Kimberlin DW (ed). American Academy of Pediatrics: USA, 2021; pp. 254–256.
46. Mohan A, Kiran DH, Manohar IC, et al. Epidemiology, clinical manifestations, and diagnosis of chikungunya fever: Lessons learned from the re-emerging epidemic. Indian J Dermatol 2010;55(1):54–63. DOI: 10.4103/0019-5154.60355.
47. Contopoulos-Ioannidis D, Newman-Lindsay S, Chow C, et al. Mother-to-child transmission of chikungunya virus: A systematic review and meta-analysis. PLoS Negl Trop Dis 2018;12(6):e0006510. DOI: 10.1371/journal.pntd.0006510.
48. Sharif N, Sarkar MK, Ferdous RN, et al. Molecular epidemiology, evolution and reemergence of chikungunya virus in South Asia. Front Microbiol 2021;12:689979. DOI: 10.3389/fmicb.2021.689979.
49. Silva MMO, Tauro LB, Kikuti M, et al. Concomitant transmission of dengue, chikungunya, and zika viruses in Brazil: Clinical and epidemiological findings from surveillance for acute febrile illness. Clin Infect Dis 2019;69(8):1353–1359. DOI: 10.1093/cid/ciy1083.
50. Panning M, Grywna K, van Esbroeck M, et al. Chikungunya fever in travelers returning to Europe from the Indian Ocean region, 2006. Emerg Infect Dis 2008;14(3):416–422. DOI: 10.3201/eid1403.070906.
51. Parola P, de Lamballerie X, Jourdan J, et al. Novel chikungunya virus variant in travelers returning from Indian Ocean islands. Emerg Infect Dis 2006;12(10):1493–1499. DOI: 10.3201/eid1210.060610.
52. Brouard C, Bernillon P, Quatresous I, et al. Estimated risk of chikungunya viremic blood donation during an epidemic on Reunion Island in the Indian Ocean, 2005 to 2007. Transfusion 2008;48(7):1333–1341. DOI: 10.1111/j.1537-2995.2008.01646.x.
53. Simmons G, Bres V, Lu K, et al. High incidence of chikungunya virus and frequency of viremic blood donations during epidemic, Puerto Rico, USA, 2014. Emerg Infect Dis 2016;22(7):1221–1228. DOI: 10.3201/eid2207.160116.
54. Gerardin P, Samperiz S, Ramful D, et al. Neurocognitive outcome of children exposed to perinatal mother-to-child chikungunya virus infection: the CHIMERE cohort study on Reunion Island. PLoS Negl Trop Dis 2014;8(7):e2996. DOI: 10.1371/journal.pntd.0002996.
55. Gerardin P, Barau G, Michault A, et al. Multidisciplinary prospective study of mother-to-child chikungunya virus infections on the island of La Reunion. PLoS Med 2008;5(3):e60. DOI: 10.1371/journal.pmed.0050060.
56. Lenglet Y, Barau G, Robillard PY, et al. Chikungunya infection in pregnancy: Evidence for intrauterine infection in pregnant women and vertical transmission in the parturient. Survey of the Reunion Island outbreak. J Gynecol Obstet Biol Reprod (Paris) 2006;35(6):578–583. DOI: 10.1016/s0368-2315(06)76447-x.
57. Waechter R, Ingraham E, Evans R, et al. Pre and postnatal exposure to chikungunya virus does not affect child neurodevelopmental outcomes at two years of age. PLoS Negl Trop Dis 2020;14(10):e0008546. DOI: 10.1371/journal.pntd.0008546.
58. Fritel X, Rollot O, Gerardin P, et al. Chikungunya virus infection during pregnancy, Reunion, France, 2006. Emerg Infect Dis 2010;16(3):418–425. DOI: 10.3201/eid1603.091403.
59. Torres JR, Falleiros-Arlant LH, Duenas L, et al. Congenital and perinatal complications of chikungunya fever: a Latin American experience. Int J Infect Dis 2016;51:85–88. DOI: 10.1016/j.ijid.2016.09.009.
60. Ramos R, Viana R, Brainer-Lima A, et al. Perinatal chikungunya virus-associated encephalitis leading to postnatal-onset microcephaly and optic atrophy. Pediatr Infect Dis J 2018;37(1):94–95. DOI: 10.1097/INF.0000000000001690.
61. McCarthy MK, Morrison TE. Persistent RNA virus infections: Do PAMPS drive chronic disease? Curr Opin Virol 2017;23:8–15. DOI: 10.1016/j.coviro.2017.01.003.
62. Chen W, Foo SS, Taylor A, et al. Bindarit, an inhibitor of monocyte chemotactic protein synthesis, protects against bone loss induced by chikungunya virus infection. J Virol 2015;89(1):581–593. DOI: 10.1128/JVI.02034-14.
63. Miner JJ, Cook LE, Hong JP, et al. Therapy with CTLA4-Ig and an antiviral monoclonal antibody controls chikungunya virus arthritis. Sci Transl Med 2017;9(375):eaah3438. DOI: 10.1126/scitranslmed.aah3438.
64. Teo TH, Chan YH, Lee WW, et al. Fingolimod treatment abrogates chikungunya virus-induced arthralgia. Sci Transl Med 2017;9(375):eaal1333. DOI: 10.1126/scitranslmed.aal1333.
65. Nikitina E, Larionova I, Choinzonov E, et al. Monocytes and macrophages as viral targets and reservoirs. Int J Mol Sci 2018;19(9):2821. DOI: 10.3390/ijms19092821.
66. Chirathaworn C, Chansaenroj J, Poovorawan Y. Cytokines and chemokines in chikungunya virus infection: Protection or induction of pathology. Pathogens 2020;9(6):415. DOI: 10.3390/pathogens9060415.
67. Gupta S, Gupta N. Short-term pregnancy outcomes in patients chikungunya infection: An observational study. J Family Med Prim Care 2019;8(3):985–987. DOI: 10.4103/jfmpc.jfmpc_274_18.
68. Escobar M, Nieto AJ, Loaiza-Osorio S, et al. Pregnant women hospitalized with chikungunya virus infection, Colombia, 2015. Emerg Infect Dis 2017;23(11):1777–1783. DOI: 10.3201/eid2311.170480.
69. Kazankov K, Barrera F, Moller HJ, et al. Soluble CD163, a macrophage activation marker, is independently associated with fibrosis in patients with chronic viral hepatitis B and C. Hepatology 2014;60(2):521–530. DOI: 10.1002/hep.27129.
70. Schliefsteiner C, Peinhaupt M, Kopp S, et al. Human placental Hofbauer cells maintain an anti-inflammatory M2 phenotype despite the presence of gestational diabetes mellitus. Front Immunol 2017;8:888. DOI: 10.3389/fimmu.2017.00888.
71. Shi Z, Long W, Zhao C, et al. Comparative proteomics analysis suggests that placental mitochondria are involved in the development of pre-eclampsia. PLoS One 2013;8(5):e64351. DOI: 10.1371/journal.pone.0064351.
72. Burton GJ, Yung HW, Murray AJ. Mitochondrial – Endoplasmic reticulum interactions in the trophoblast: Stress and senescence. Placenta 2017;52:146–155. DOI: 10.1016/j.placenta.2016.04.001.
73. Clemente O, Sandoval C. The placenta in a case of pregnant woman infected by chikungunya virus. J Virol Retrovirol 2016;2(1):1–4.
74. Kinder JM, Stelzer IA, Arck PC, et al. Immunological implications of pregnancy-induced microchimerism. Nat Rev Immunol 2017;17(8):483–494. DOI: 10.1038/nri.2017.38.
75. Lamothe J, Khurana S, Tharmalingam S, et al. Oxidative stress mediates the fetal programming of hypertension by glucocorticoids. Antioxidants (Basel) 2021;10(4):531. DOI: 10.3390/antiox10040531.
76. Schepanski S, Buss C, Hanganu-Opatz IL, et al. Prenatal immune and endocrine modulators of offspring's brain development and cognitive functions later in life. Front Immunol 2018;9:2186. DOI: 10.3389/fimmu.2018.02186.
77. Rajapakse S, Rodrigo C, Rajapakse A. Atypical manifestations of chikungunya infection. Trans R Soc Trop Med Hyg 2010;104(2):89–96. DOI: 10.1016/j.trstmh.2009.07.031.

78. Iosif RE, Ekdahl CT, Ahlenius H, et al. Tumor necrosis factor receptor 1 is a negative regulator of progenitor proliferation in adult hippocampal neurogenesis. J Neurosci 2006;26(38):9703–9712. DOI: 10.1523/JNEUROSCI.2723-06.2006.
79. von Ehrenstein OS, Neta GI, Andrews W, et al. Child intellectual development in relation to cytokine levels in umbilical cord blood. Am J Epidemiol 2012;175(11):1191–1199. DOI: 10.1093/aje/kwr393.
80. Semple BD, Kossmann T, Morganti-Kossmann MC. Role of chemokines in CNS health and pathology: a focus on the CCL2/CCR2 and CXCL8/CXCR2 networks. J Cereb Blood Flow Metab 2010;30(3):459–473. DOI: 10.1038/jcbfm.2009.240.
81. Kelland EE, Gilmore W, Weiner LP, et al. The dual role of CXCL8 in human CNS stem cell function: Multipotent neural stem cell death and oligodendrocyte progenitor cell chemotaxis. Glia 2011;59(12):1864–1878. DOI: 10.1002/glia.21230.
82. Stuart MJ, Singhal G, Baune BT. Systematic review of the neurobiological relevance of chemokines to psychiatric disorders. Front Cell Neurosci 2015;9:357. DOI: 10.3389/fncel.2015.00357.
83. Lima MC, de Mendonca LR, Rezende AM, et al. The transcriptional and protein profile from human infected neuroprogenitor cells is strongly correlated to zika virus microcephaly cytokines phenotype evidencing a persistent inflammation in the CNS. Front Immunol 2019;10:1928. DOI: 10.3389/fimmu.2019.01928.
84. Naveca FG, Pontes GS, Chang AY, et al. Analysis of the immunological biomarker profile during acute Zika virus infection reveals the overexpression of CXCL10, a chemokine linked to neuronal damage. Mem Inst Oswaldo Cruz 2018;113(6):e170542. DOI: 10.1590/0074-02760170542.
85. Puccioni-Sohler M, da Silva SJ, Faria LCS, et al. Neopterin and CXCL-10 in cerebrospinal fluid as potential biomarkers of neuroinvasive dengue and chikungunya. Pathogens 2021;10(12):1626. DOI: 10.3390/pathogens10121626.
86. Barbosa S, Khalfallah O, Forhan A, et al. Immune activity at birth and later psychopathology in childhood. Brain Behav Immun Health 2020;8:100141. DOI: 10.1016/j.bbih.2020.100141.
87. Venugopalan A, Ghorpade RP, Chopra A. Cytokines in acute chikungunya. PLoS One 2014;9(10):e111305. DOI: 10.1371/journal.pone.0111305.
88. Zhang YL, Luan B, Wang XF, et al. Peripheral blood MDSCs, IL-10 and IL-12 in children with asthma and their importance in asthma development. PLoS One 2013;8(5):e63775. DOI: 10.1371/journal.pone.0063775.
89. van Enter BJD, Huibers MHW, van Rooij L, et al. Perinatal outcomes in vertically infected neonates during a chikungunya outbreak on the Island of Curacao. Am J Trop Med Hyg 2018;99(6):1415–1418. DOI: 10.4269/ajtmh.17-0957.
90. Sevenoaks T, Wedderburn CJ, Donald KA, et al. Association of maternal and infant inflammation with neurodevelopment in HIV-exposed uninfected children in a South African birth cohort. Brain Behav Immun 2021;91:65–73. DOI: 10.1016/j.bbi.2020.08.021.
91. Kollmann TR, Levy O, Montgomery RR, Goriely S. Innate immune function by Toll-like receptors: Distinct responses in newborns and the elderly. Immunity 2012;37(5):771–783. DOI: 10.1016/j.immuni.2012.10.014.
92. Couderc T, Chretien F, Schilte C, et al. A mouse model for Chikungunya: young age and inefficient type-I interferon signaling are risk factors for severe disease. PLoS Pathog 2008;4(2):e29. DOI: 10.1371/journal.ppat.0040029.
93. Slaats J, Ten Oever J, van de Veerdonk FL, et al. IL-1beta/IL-6/CRP and IL-18/ferritin: Distinct inflammatory programs in infections. PLoS Pathog 2016;12(12):e1005973. DOI: 10.1371/journal.ppat.1005973.
94. Valero N, Mosquera J, Torres M, et al. Increased serum ferritin and interleukin-18 levels in children with dengue. Braz J Microbiol 2019;50(3):649–656. DOI: 10.1007/s42770-019-00105-2.
95. Torres MC, Di Maio F, Brown D, et al. In depth viral diversity analysis in atypical neurological and neonatal chikungunya infections in Rio de Janeiro, Brazil. Viruses 2022;14(9):2006. DOI: 10.3390/v14092006.
96. Lim SM, van den Ham HJ, Oduber M, et al. Transcriptomic analyses reveal differential gene expression of immune and cell death pathways in the brains of mice infected with West Nile virus and chikungunya virus. Front Microbiol 2017;8:1556. DOI: 10.3389/fmicb.2017.01556.
97. Verboon-Maciolek MA, Groenendaal F, Cowan F, et al. White matter damage in neonatal enterovirus meningoencephalitis. Neurology 2006;66(8):1267–1269. DOI: 10.1212/01.wnl.0000208429.69676.23.
98. Verboon-Maciolek MA, Groenendaal F, Hahn CD, et al. Human parechovirus causes encephalitis with white matter injury in neonates. Ann Neurol 2008;64(3):266–273. DOI: 10.1002/ana.21445.
99. Couderc T, Lecuit M. Focus on chikungunya pathophysiology in human and animal models. Microbes Infect 2009;11(14-15):1197–1205. DOI: 10.1016/j.micinf.2009.09.002.
100. Her Z, Malleret B, Chan M, et al. Active infection of human blood monocytes by chikungunya virus triggers an innate immune response. J Immunol 2010;184(10):5903–5913. DOI: 10.4049/jimmunol.0904181.
101. Ali M, Safriel Y, Sohi J, et al. West Nile virus infection: MR imaging findings in the nervous system. AJNR Am J Neuroradiol 2005;26(2):289–297. PMCID: PMC7974109.
102. Hauwel M, Furon E, Canova C, et al. Innate (inherent) control of brain infection, brain inflammation and brain repair: The role of microglia, astrocytes, "protective" glial stem cells and stromal ependymal cells. Brain Res Brain Res Rev 2005;48(2):220–233. DOI: 10.1016/j.brainresrev.2004.12.012.
103. Goedeke L, Fernandez-Hernando C. Regulation of cholesterol homeostasis. Cell Mol Life Sci 2012;69(6):915–930. DOI: 10.1007/s00018-011-0857-5.
104. Thio CL, Yusof R, Abdul-Rahman PS, et al. Differential proteome analysis of chikungunya virus infection on host cells. PLoS One 2013;8(4):e61444. DOI: 10.1371/journal.pone.0061444.
105. Houtman JJ, Fleming JO. Pathogenesis of mouse hepatitis virus-induced demyelination. J Neurovirol 1996;2(6):361–376. DOI: 10.3109/13550289609146902.
106. Fazakerley JK. Pathogenesis of Semliki Forest virus encephalitis. J Neurovirol 2002;8(2):66–74. DOI: 10.1080/135502802901068000.
107. Labadie K, Larcher T, Joubert C, et al. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. J Clin Invest 2010;120(3):894–906. DOI: 10.1172/JCI40104.
108. Kumar S, Agrawal G, Wazir S, et al. Experience of perinatal and Neonatal Chikungunya Virus (CHIKV) Infection in a Tertiary Care Neonatal Centre during Outbreak in North India in 2016: A case series. J Trop Pediatr 2019;65(2):169–175. DOI: 10.1093/tropej/fmy032.
109. Inamadar AC, Palit A, Sampagavi VV, et al. Cutaneous manifestations of chikungunya fever: Observations made during a recent outbreak in South India. Int J Dermatol 2008;47(2):154–159. DOI: 10.1111/j.1365-4632.2008.03478.x.
110. Prashant S, Kumar AS, Basheeruddin DD, et al. Cutaneous manifestations in patients suspected of chikungunya disease. Indian J Dermatol 2009;54(2):128–131. DOI: 10.4103/0019-5154.53186.
111. Bandyopadhyay D, Ghosh SK. Mucocutaneous features of Chikungunya fever: A study from an outbreak in West Bengal, India. Int J Dermatol 2008;47(11):1148–1152. DOI: 10.1111/j.1365-4632.2008.03817.x.
112. Centers for Disease Control and Prevention NCfEaZIDN, Division of Vector-Borne Diseases (DVBD). Chikungunya Virus: Clinical Evaluation & Disease. Centers for Disease Control and Prevention. Available from https://www.cdc.gov/chikungunya/hc/clinicalevaluation.html (Jan 12, 2023).
113. Evans-Gilbert T. Vertically transmitted chikungunya, Zika and dengue virus infections: The pathogenesis from mother to fetus and the implications of co-infections and vaccine development. Int J Pediatr Adolesc Med 2020;7(3):107–111. DOI: 10.1016/j.ijpam.2019.05.004.
114. Krutikov M, Manson J. Chikungunya virus infection: An update on joint manifestations and management. Rambam Maimonides Med J. Oct 31 2016;7(4):e0033. DOI: 10.5041/RMMJ.10260.

115. Gopakumar H, Ramachandran S. Congenital chikungunya. J Clin Neonatol 2012;1(3):155–156. DOI: 10.4103/2249-4847.101704.
116. Alvarado-Socarras JL, Ocampo-Gonzalez M, Vargas-Soler JA, et al. Congenital and neonatal chikungunya in Colombia. J Pediatric Infect Dis Soc 2016;5(3):e17–20. DOI: 10.1093/jpids/piw021.
117. Vasani R, Kanhere S, Chaudhari K, et al. Congenital Chikungunya--A cause of neonatal hyperpigmentation. Pediatr Dermatol 2016;33(2):209–212. DOI: 10.1111/pde.12650.
118. Srinivas SM, Pradeep GCM. Congenital chikungunya infection presenting with extensive dystrophic calcinosis cutis. Indian J Dermatol Venereol Leprol 2020;86(6):693–696. DOI: 10.4103/ijdvl.IJDVL_91_20.
119. Ferreira F, da Silva ASV, Recht J, et al. Vertical transmission of chikungunya virus: A systematic review. PLoS One 2021;16(4):e0249166. DOI: 10.1371/journal.pone.0249166.
120. Gupta V, Gupta N, Pandita A. Neonate with chikungunya. Clin Case Rep 2021;9(6):e04351. DOI: 10.1002/ccr3.4351.
121. Gerardin P, Couderc T, Bintner M, et al. Chikungunya virus-associated encephalitis: A cohort study on La Reunion Island, 2005-2009. Neurology 2016;86(1):94–102. DOI: 10.1212/WNL.0000000000002234.
122. Touret Y, Randrianaivo H, Michault A, et al. Early maternal-fetal transmission of the Chikungunya virus. Presse Med 2006;35(11 Pt 1):1656–1658.DOI: 10.1016/S0755-4982(06)74874-6.
123. Ramful D, Carbonnier M, Pasquet M, et al. Mother-to-child transmission of chikungunya virus infection. Pediatr Infect Dis J 2007;26(9):811–815. DOI: 10.1097/INF.0b013e3180616d4f.
124. Senanayake MP, Senanayake SM, Vidanage KK, et al. Vertical transmission in chikungunya infection. Ceylon Med J 2009;54(2):47–50. DOI: 10.4038/cmj.v54i2.865.
125. Shrivastava A, Waqar Beg M, Gujrati C, et al. Management of a vertically transmitted neonatal chikungunya thrombocytopenia. Indian J Pediatr 2011;78(8):1008–1009. DOI: 10.1007/s12098-011-0371-7.
126. Gerardin P, Fianu A, Malvy D, et al. Perceived morbidity and community burden after a Chikungunya outbreak: the TELECHIK survey, a population-based cohort study. BMC Med 2011;9:5. DOI: 10.1186/1741-7015-9-5.
127. Ratai EM, Annamalai L, Burdo T, et al. Brain creatine elevation and N-acetylaspartate reduction indicates neuronal dysfunction in the setting of enhanced glial energy metabolism in a macaque model of neuroAIDS. Magn Reson Med 2011;66(3):625–634. DOI: 10.1002/mrm.22821.
128. Prevention. CfDCa. Chikungunya Virus - Diagnostic testing. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Vector-Borne Diseases (DVBD). Available from https://www.cdc.gov/chikungunya/hc/diagnostic.html (Jan 11, 2023).
129. Lakshmi V, Neeraja M, Subbalaxmi MV, et al. Clinical features and molecular diagnosis of chikungunya fever from South India. Clin Infect Dis 2008;46(9):1436–1442. DOI: 10.1086/529444.
130. Kashyap RS, Morey SH, Chandak NH, et al. Detection of viral antigen, IgM and IgG antibodies in cerebrospinal fluid of chikungunya patients with neurological complications. Cerebrospinal Fluid Res 2010;7:12. DOI: 10.1186/1743-8454-7-12.
131. Azami NA, Moi ML, Takasaki T. Neutralization assay for chikungunya virus infection: Plaque reduction neutralization test. Methods Mol Biol 2016;1426:273–282. DOI: 10.1007/978-1-4939-3618-2_25.
132. Ramful D, Samperiz S, Fritel X, et al. Antibody kinetics in infants exposed to chikungunya virus infection during pregnancy reveals absence of congenital infection. J Infect Dis 2014;209(11):1726–1730. DOI: 10.1093/infdis/jit814.
133. Shenoy S, Pradeep GC. Neurodevelopmental outcome of neonates with vertically transmitted chikungunya fever with encephalopathy. Indian Pediatr 2012;49(3):238–240. PMID: 22484743.
134. Grivard P, Le Roux K, Laurent P, et al. Molecular and serological diagnosis of chikungunya virus infection. Pathol Biol (Paris) 2007;55(10):490–494. DOI: 10.1016/j.patbio.2007.07.002.
135. Watanaveeradej V, Endy TP, Simasathien S, et al. The study transplacental chikungunya virus antibody kinetics, Thailand. Emerg Infect Dis 2006;12(11):1770–1772. DOI: 10.3201/eid1211.051560.
136. Simon F, Savini H, Parola P. Chikungunya: A paradigm of emergence and globalization of vector-borne diseases. Med Clin North Am 2008;92(6):1323–1343, ix. DOI: 10.1016/j.mcna.2008.07.008.
137. Cunha RVD, Trinta KS. Chikungunya virus: Clinical aspects and treatment – A Review. Mem Inst Oswaldo Cruz 2017;112(8):523–531. DOI: 10.1590/0074-02760170044.
138. Chiam CW, Sam IC, Chan YF, et al. Immunohistochemical detection of chikungunya virus antigens in formalin-fixed and paraffin-embedded tissues. Methods Mol Biol 2016;1426:235–240. DOI: 10.1007/978-1-4939-3618-2_21.
139. Meena SS, Arya S, Meena D, et al. Neonatal chikungunya: A case series. Trop Doct 2021;51(1):103–105. DOI: 10.1177/0049475520977011.
140. Sumarmo, Talogo W, Asrin A, et al. Failure of hydrocortisone to affect outcome in dengue shock syndrome. Pediatrics 1982;69(1):45–49. PMID: 7054760.
141. Ascher DP, Laws HF, Hayes CG. The use of intravenous gammaglobulin in dengue fever, a case report. Southeast Asian J Trop Med Public Health 1989;20(4):549–554. PMID: 2484144.
142. Lyra PP, Campos GS, Bandeira ID, et al. Congenital chikungunya virus infection after an outbreak in Salvador, Bahia, Brazil. AJP Rep 2016;6(3):e299–300. DOI: 10.1055/s-0036-1587323.
143. Correa DG, Freddi TAL, Werner H, et al. Brain MR imaging of patients with perinatal chikungunya virus infection. AJNR Am J Neuroradiol 2020;41(1):174–177. DOI: 10.3174/ajnr.A6339.
144. Johnson BW, Russell BJ, Goodman CH. Laboratory diagnosis of chikungunya virus infections and commercial sources for diagnostic assays. J Infect Dis 2016;214(5):S471–S474. DOI: 10.1093/infdis/jiw274.
145. Salomao N, Araujo L, Rabelo K, et al. Placental alterations in a chikungunya-virus-infected pregnant woman: A case report. *Microorganisms* 2022;10(5):872. DOI: 10.3390/microorganisms10050872.
146. Salomao N, Rabelo K, Avvad-Portari E, et al. Histopathological and immunological characteristics of placentas infected with chikungunya virus. Front Microbiol 2022;13:1055536. DOI: 10.3389/fmicb.2022.1055536.
147. Rezza G. Dengue and chikungunya: Long-distance spread and outbreaks in naive areas. Pathog Glob Health 2014;108(8):349–355. DOI: 10.1179/2047773214Y.0000000163.
148. Goupil BA, Mores CN. A review of chikungunya virus-induced arthralgia: Clinical manifestations, therapeutics, and pathogenesis. Open Rheumatol J 2016;10:129–140. DOI: 10.2174/1874312901610010129.
149. Roth A, Mercier A, Lepers C, et al. Concurrent outbreaks of dengue, chikungunya and Zika virus infections – An unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012-2014. Euro Surveill 2014;19(41):20929. DOI: 10.2807/1560-7917.es2014.19.41.20929.
150. Ratsitorahina M, Harisoa J, Ratovonjato J, et al. Outbreak of dengue and Chikungunya fevers, Toamasina, Madagascar, 2006. Emerg Infect Dis 2008;14(7):1135–1137. DOI: 10.3201/eid1407.071521.
151. Nayar SK, Noridah O, Paranthaman V, et al. Co-infection of dengue virus and chikungunya virus in two patients with acute febrile illness. Med J Malaysia 2007;62(4):335–336. PMID: 18551940.
152. Waggoner JJ, Gresh L, Vargas MJ, et al. Viremia and clinical presentation in nicaraguan patients infected with zika virus, chikungunya virus, and dengue virus. Clin Infect Dis 2016;63(12):1584–1590. DOI: 10.1093/cid/ciw589.
153. Gould LH, Osman MS, Farnon EC, et al. An outbreak of yellow fever with concurrent chikungunya virus transmission in South Kordofan, Sudan, 2005. Trans R Soc Trop Med Hyg 2008;102(12):1247–1254. DOI: 10.1016/j.trstmh.2008.04.014.
154. Sreekanth R, Venugopal L, Arunkrishnan B, et al. Neonatal chikungunya encephalitis. Trop Doct 2022;52(1):199–201. DOI: 10.1177/00494755211063268.
155. Simon F, Parola P, Grandadam M, et al. Chikungunya infection: An emerging rheumatism among travelers returned from Indian Ocean islands. Report of 47 cases. Medicine (Baltimore) 2007;86(3):123–137. DOI: 10.1097/MD/0b013e31806010a5.

156. Simon F, Javelle E, Cabie A, et al. French guidelines for the management of chikungunya (acute and persistent presentations). November 2014. Med Mal Infect 2015;45(7):243–263. DOI: 10.1016/j.medmal.2015.05.007.
157. Javelle E, Ribera A, Degasne I, et al. Specific management of post-chikungunya rheumatic disorders: a retrospective study of 159 cases in Reunion Island from 2006-2012. PLoS Negl Trop Dis 2015;9(3):e0003603. DOI: 10.1371/journal.pntd.0003603.
158. Vouga M, Chiu YC, Pomar L, et al. Dengue, Zika and chikungunya during pregnancy: Pre- and post-travel advice and clinical management. J Travel Med 2019;26(8):taz077. DOI: 10.1093/jtm/taz077.
159. Prevention CfDCa. Chikungunya Virus: Transmission. Centers for Disease Control and Prevention. Available from https://www.cdc.gov/chikungunya/transmission/index.html (Jan 23).
160. de Paula Souza J, de Jesus BLS, Giusti AL, et al. Breastfeeding by chikungunya virus-infected dams confers resistance to challenge in the offspring. Transl Res 2022:S1931–5244(22)00280-8. DOI: 10.1016/j.trsl.2022.12.001.
161. Villamil-Gomez W, Alba-Silvera L, Menco-Ramos A, et al. Congenital chikungunya virus infection in Sincelejo, Colombia: A case series. J Trop Pediatr 2015;61(5):386–392. DOI: 10.1093/tropej/fmv051.
162. Ganesan K, Diwan A, Shankar SK, et al. Chikungunya encephalomyeloradiculitis: Report of 2 cases with neuroimaging and 1 case with autopsy findings. AJNR Am J Neuroradiol 2008;29(9):1636–1637. DOI: 10.3174/ajnr.A1133.
163. Robin S, Ramful D, Le Seach F, et al. Neurologic manifestations of pediatric chikungunya infection. J Child Neurol 2008;23(9):1028–1035. DOI: 10.1177/0883073808314151.
164. Sebastian MR, Lodha R, Kabra SK. Chikungunya infection in children. Indian J Pediatr 2009;76(2):185–189. DOI: 10.1007/s12098-009-0049-6.
165. Samra JA, Hagood NL, Summer A, et al. Clinical features and neurologic complications of children hospitalized with chikungunya virus in Honduras. J Child Neurol 2017;32(8):712–716. DOI: 10.1177/0883073817701879.
166. Ball JD, Elbadry MA, Telisma T, et al. Clinical and epidemiologic patterns of chikungunya virus infection and coincident arboviral disease in a School Cohort in Haiti, 2014–2015. Clin Infect Dis 2019;68(6):919–926. DOI: 10.1093/cid/ciy582.
167. Elenga N, Folin M, Vandamme YM, et al. Chikungunya infection in hospitalized febrile infants younger than 3 months of age. *Pediatr* Infect Dis J 2017;36(8):736–740. DOI: 10.1097/INF.0000000000001541.
168. Kumar A, Best C, Benskin G. Epidemiology, clinical and laboratory features and course of chikungunya among a cohort of children during the First Caribbean Epidemic. J Trop Pediatr 2017;63(1):43–49. DOI: 10.1093/tropej/fmw051.
169. Prevention CfDCa. Health Information for International Travel 2020. Centers for Disease Control and Prevention. Available from: https://wwwnc.cdc.gov/travel/page/yellowbook-home. 25th December 2022.
170. Edelman R, Tacket CO, Wasserman SS, et al. Phase II safety and immunogenicity study of live chikungunya virus vaccine TSI-GSD-218. Am J Trop Med Hyg 2000;62(6):681–685. DOI: 10.4269/ajtmh.2000.62.681.
171. Akahata W, Yang ZY, Andersen H, et al. A virus-like particle vaccine for epidemic chikungunya virus protects nonhuman primates against infection. Nat Med 2010;16(3):334–338. DOI: 10.1038/nm.2105.
172. Roy CJ, Adams AP, Wang E, et al. Chikungunya vaccine candidate is highly attenuated and protects nonhuman primates against telemetrically monitored disease following a single dose. J Infect Dis 2014;209(12):1891–1899. DOI: 10.1093/infdis/jiu014.
173. Chang LJ, Dowd KA, Mendoza FH, et al. Safety and tolerability of chikungunya virus-like particle vaccine in healthy adults: A phase 1 dose-escalation trial. Lancet 2014;384(9959):2046–2052. DOI: 10.1016/S0140-6736(14)61185-5.
174. Ramsauer K, Schwameis M, Firbas C, et al. Immunogenicity, safety, and tolerability of a recombinant measles-virus-based chikungunya vaccine: A randomised, double-blind, placebo-controlled, active-comparator, first-in-man trial. Lancet Infect Dis 2015;15(5):519–527. DOI: 10.1016/S1473-3099(15)70043-5.
175. Reisinger EC, Tschismarov R, Beubler E, et al. Immunogenicity, safety, and tolerability of the measles-vectored chikungunya virus vaccine MV-CHIK: A double-blind, randomised, placebo-controlled and active-controlled phase 2 trial. Lancet 2019;392(10165):2718–2727. DOI: 10.1016/S0140-6736(18)32488-7.
176. Chen GL, Coates EE, Plummer SH, et al. Effect of a chikungunya virus-like particle vaccine on safety and tolerability outcomes: A randomized clinical trial. JAMA 2020;323(14):1369–1377. DOI: 10.1001/jama.2020.2477.
177. Schrauf S, Tschismarov R, Tauber E, et al. Current efforts in the development of vaccines for the prevention of zika and chikungunya virus infections. Front Immunol 2020;11:592. DOI: 10.3389/fimmu.2020.00592.
178. ClinicalTrials.gov. Safety, Tolerability, and Immunogenicity of VAL-181388 in Healthy Subjects. Available from https://clinicaltrials.gov/ct2/show/NCT03325075?term=NCT03325075&draw=2 (Jan 11, 2022).
179. Hucke FIL, Bugert JJ. Current and promising antivirals against chikungunya virus. Front Public Health 2020;8:618624. DOI: 10.3389/fpubh.2020.618624.
180. Kolimenakis A, Heinz S, Wilson ML, et al. The role of urbanisation in the spread of Aedes mosquitoes and the diseases they transmit-A systematic review. PLoS Negl Trop Dis 2021;15(9):e0009631. DOI: 10.1371/journal.pntd.0009631.
181. Wang GH, Gamez S, Raban RR, et al. Combating mosquito-borne diseases using genetic control technologies. Nat Commun 2021;12(1):4388. DOI: 10.1038/s41467-021-24654-z.
182. Wilke AB, Marrelli MT. Paratransgenesis: A promising new strategy for mosquito vector control. Parasit Vectors 2015;8:342. DOI: 10.1186/s13071-015-0959-2.
183. Labbe GM, Nimmo DD, Alphey L. piggybac- and PhiC31-mediated genetic transformation of the Asian tiger mosquito, Aedes albopictus (Skuse). PLoS Negl Trop Dis 2010;4(8):e788. DOI: 10.1371/journal.pntd.0000788.
184. Coates CJ, Jasinskiene N, Miyashiro L, et al. Mariner transposition and transformation of the yellow fever mosquito, Aedes aegypti. Proc Natl Acad Sci USA 1998;95(7):3748–3751. DOI: 10.1073/pnas.95.7.3748.
185. Jasinskiene N, Coates CJ, Benedict MQ, et al. Stable transformation of the yellow fever mosquito, Aedes aegypti, with the Hermes element from the housefly. Proc Natl Acad Sci USA 1998;95(7):3743–3747. DOI: 10.1073/pnas.95.7.3743.
186. Rodrigues FG, Oliveira SB, Rocha BC, et al. Germline transformation of Aedes fluviatilis (Diptera:Culicidae) with the piggyBac transposable element. Mem Inst Oswaldo Cruz 2006;101(7):755–757. DOI: 10.1590/s0074-02762006000700008.
187. Alphey L, McKemey A, Nimmo D, et al. Genetic control of Aedes mosquitoes. Pathog Glob Health 2013;107(4):170–179. DOI: 10.1179/2047773213Y.0000000095.
188. Matthews BJ, Dudchenko O, Kingan SB, et al. Improved reference genome of Aedes aegypti informs arbovirus vector control. Nature 2018;563(7732):501–507. DOI: 10.1038/s41586-018-0692-z.
189. Ye YH, Woolfit M, Rances E, et al. Wolbachia-associated bacterial protection in the mosquito Aedes aegypti. PLoS Negl Trop Dis 2013;7(8):e2362. DOI: 10.1371/journal.pntd.0002362.
190. Bennett KL, Gomez-Martinez C, Chin Y, et al. Dynamics and diversity of bacteria associated with the disease vectors Aedes aegypti and Aedes albopictus. Sci Rep 2019;9(1):12160. DOI: 10.1038/s41598-019-48414-8.
191. Santos IA, Shimizu JF, de Oliveira DM, et al. Chikungunya virus entry is strongly inhibited by phospholipase A2 isolated from the venom of Crotalus durissus terrificus. Sci Rep 2021;11(1):8717. DOI: 10.1038/s41598-021-88039-4.
192. Kielian M, Rey FA. Virus membrane-fusion proteins: More than one way to make a hairpin. Nat Rev Microbiol 2006;4(1):67–76. DOI: 10.1038/nrmicro1326.
193. Brehin AC, Rubrecht L, Navarro-Sanchez ME, et al. Production and characterization of mouse monoclonal antibodies reactive to chikungunya envelope E2 glycoprotein. Virology 2008;371(1):185–195. DOI: 10.1016/j.virol.2007.09.028.

194. Voss JE, Vaney MC, Duquerroy S, et al. Glycoprotein organization of chikungunya virus particles revealed by X-ray crystallography. Nature 2010;468(7324):709–712. DOI: 10.1038/nature09555.
195. Ashbrook AW, Burrack KS, Silva LA, et al. Residue 82 of the Chikungunya virus E2 attachment protein modulates viral dissemination and arthritis in mice. J Virol 2014;88(21):12180–12192. DOI: 10.1128/JVI.01672-14.
196. Smith TJ, Cheng RH, Olson NH, et al. Putative receptor binding sites on alphaviruses as visualized by cryoelectron microscopy. Proc Natl Acad Sci USA 1995;92(23):10648–10652. DOI: 10.1073/pnas.92.23.10648.
197. Li L, Jose J, Xiang Y, et al. Structural changes of envelope proteins during alphavirus fusion. Nature 2010;468(7324):705–708. DOI: 10.1038/nature09546.
198. Asnet Mary J, Paramasivan R, Tyagi BK, et al. Identification of structural motifs in the E2 glycoprotein of chikungunya involved in virus-host interaction. J Biomol Struct Dyn 2013;31(10):1077–1085. DOI: 10.1080/07391102.2012.721496.
199. Yap ML, Klose T, Urakami A, et al. Structural studies of chikungunya virus maturation. Proc Natl Acad Sci USA 2017;114(52):13703–13707. DOI: 10.1073/pnas.1713166114.
200. Ye F, Zhang M. Structures and target recognition modes of PDZ domains: Recurring themes and emerging pictures. Biochem J 2013;455(1):1–14. DOI: 10.1042/BJ20130783.
201. Zhang K, Law YS, Law MCY, et al. Structural insights into viral RNA capping and plasma membrane targeting by chikungunya virus nonstructural protein 1. Cell Host Microbe 2021;29(5):757–764e3. DOI: 10.1016/j.chom.2021.02.018.
202. van Duijl-Richter MK, Hoornweg TE, Rodenhuis-Zybert IA, et al. Early events in chikungunya virus infection-from virus cell binding to membrane fusion. Viruses 2015;7(7):3647–3674. DOI: 10.3390/v7072792.
203. Cho B, Jeon BY, Kim J, et al. Expression and evaluation of Chikungunya virus E1 and E2 envelope proteins for serodiagnosis of Chikungunya virus infection. Yonsei Med J 2008;49(5):828–835. DOI: 10.3349/ymj.2008.49.5.828.
204. Tahir Ul Qamar M, Bari A, Adeel MM, et al. Peptide vaccine against chikungunya virus: Immuno-informatics combined with molecular docking approach. J Transl Med 2018;16(1):298. DOI: 10.1186/s12967-018-1672-7.
205. Hong EM, Perera R, Kuhn RJ. Alphavirus capsid protein helix I controls a checkpoint in nucleocapsid core assembly. J Virol 2006;80(18):8848–8855. DOI: 10.1128/JVI.00619-06.
206. Sharma R, Kesari P, Kumar P, et al. Structure-function insights into chikungunya virus capsid protein: Small molecules targeting capsid hydrophobic pocket. Virology 2018;515:223–234. DOI: 10.1016/j.virol.2017.12.020.
207. Strauss JH, Strauss EG. The alphaviruses: Gene expression, replication, and evolution. Microbiol Rev 1994;58(3):491–562. DOI: 10.1128/mr.58.3.491-562.1994.
208. Kallio K, Hellstrom K, Jokitalo E, et al. RNA replication and membrane modification require the same functions of alphavirus nonstructural proteins. J Virol 2016;90(3):1687–1692. DOI: 10.1128/JVI.02484-15.
209. Lemm JA, Rice CM. Roles of nonstructural polyproteins and cleavage products in regulating Sindbis virus RNA replication and transcription. J Virol 1993;67(4):1916–1926. DOI: 10.1128/JVI.67.4.1916-1926.1993.
210. Bartholomeeusen K, Utt A, Coppens S, et al. A Chikungunya virus trans-replicase system reveals the importance of delayed nonstructural polyprotein processing for efficient replication complex formation in mosquito cells. J Virol 2018;92(14): e00152-18. DOI: 10.1128/JVI.00152-18.
211. Chen MW, Tan YB, Zheng J, et al. Chikungunya virus nsP4 RNA-dependent RNA polymerase core domain displays detergent-sensitive primer extension and terminal adenylyltransferase activities. Antiviral Res 2017;143:38–47. DOI: 10.1016/j.antiviral.2017.04.001.
212. Bandeira AC, Campos GS, Sardi SI, et al. Neonatal encephalitis due to Chikungunya vertical transmission: First report in Brazil. IDCases 2016;5:57–59. DOI: 10.1016/j.idcr.2016.07.008.