Bulletin of Biological and Allied Sciences Research
ISSN: **2521-0092**
www.bbasr.org
DOI: https://doi.org/10.64013/bbasr.v2026i1.129
*Bull. Biol. All. Sci. Res., Volume, **11**: 129*
Original Research Article ***Open Access***

UNRAVELING THE STRUCTURAL AND IMMUNOLOGICAL MECHANISMS OF CROSS-REACTIVITY IN DENGUE VIRUS AND ZIKA VIRUS CO-INFECTION

YOUNAS S[1*], ZAHOOR U[1], MANZOOR S[1], SALEEM MZ[1], ALI Q[2]

[1]Centre for Applied Molecular Biology, University of the Punjab, Lahore, Pakistan
[2]Department of Plant Breeding and Genetics, Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan
**Correspondence Author Email Address: saima.younas@pu.edu.pk*

(Received, 24th January 2026, Accepted 29th June 2026, Published 9th July 2026)

Abstract *Citrus belongs to the family Rutaceae and comprises different plants and fruits. The Citrus limon peel consists of rich nutrient values, i.e., essential oil and compounds, which can be used as anticancer and antimicrobial agents. The present study has explored the concept of nutraceutical, antileishmanial, and anticancer activities of Citrus limon peel. The samples of local and hybrid Citrus limon were collected from the district of Mardan. The lemon peel was extracted and dried at room temperature. After drying, the lemon peel was ground to obtain fine powder. Powders of both types of lemon peel were soaked in methanol at room temperature for 15-21 days. Powdered extracts were analyzed for the presence of different active ingredients, i.e., alkaloids, steroids, flavonoids, and tannins. The result of digestibility revealed that hybrid lemon was high in dry matter, Ash, crude proteins, fiber, and lower fat. The preliminary phytochemical study revealed that alkaloids, steroids, flavonoids, and tannins were found in both local and hybrid lemon. The findings of EDX represent that the CK was 65.82 % and 67.51 % of the atomic weights for the CK, while 32.54% and 30.62 had been taken in the OK. The FTIR results showed that functional groups are present in both local and hybrid lemon peels. The alkane and amines are present in local and hybrid lemon peels, while ketones and tertiary alcohols are present in local lemon peels, and carboxylic acid and amine groups are present in hybrid lemon peels. The MTT assay showed that the methanolic extracts of hybrid and local Citrus limon peel were more efficacious than aqueous and chloroform extracts in suppressing promastigotes L. major and L. tropica, while the aqueous extracts showed the lowest activity against promastigotes L. major and L. tropica in both cases. Overall, the activity of berberine and hybrid Citrus limon peel was much higher than the activity of local Citrus limon peel. The anticancer potential of different solvent-based lemon peel extracts against the HepG2 cell line was evaluated after 48 hours. The extracts of lemon peel in four different solvents, including methanol, ethanol, ethyl acetate, and chloroform, were compared. In the case of lemon peel chloroform extract, the highest inhibition (65.93 percent) was seen at a concentration of 100ug/ml, but in the case of lemon peel ethyl acetate extract, it was 61.75 percent at a concentration of 200ug/ml. On the other hand, it is quite evident that hybrid lemon peel extract dissolved in various solvents (methanol, ethanol, ethyl acetate, and chloroform) showed significantly reduced cytotoxicity against HeLa cancer cells in comparison to wild-type lemon peel extract. The study concludes that C. lemon extract can be used for better treatment of different anticancer and antileishmaniasis assays.*

[Citation: Younas, S., Zahoor, U., Manzoor, S., Saleem, M.Z., Ali, Q. (2026). Unraveling the structural and immunological mechanisms of cross-reactivity in dengue virus and zika virus co-infection. *Bull. Biol. All. Sci. Res.* **11***: 129.* https://doi.org/10.64013/bbasr.v2026i1.129]

Keywords: *Citrus limon; antileishmanial; anticancer; Hela cell lines; Fourier Transform Infrared (FTIR) spectrophotometer; Scanning Electron Microscope*

Introduction

Mosquito-borne viral diseases have produced a large number of epidemics worldwide, particularly in tropical and subtropical regions. These diseases are caused by viruses transmitted to humans through the bites of infected mosquitoes, which serve as vectors for a range of pathogens. The incidence of these viruses has dramatically increased due to factors such as reduced mosquito control, increased travel, their ability to adapt to new environments and hosts, deforestation, climate change, and high population density in tropical and subtropical regions. Most of these viruses belong to the Flaviviridae family; Flaviviruses affect 400 million people each year, posing a severe threat to global health and the economy (Zhao et al., 2021). The Flaviviridae family includes several medically essential viruses, such as tick-borne encephalitis virus (TBEV), Japanese encephalitis virus (JEV), West Nile virus (WNV), yellow fever virus (YFV), dengue virus (DENV), and Zika Virus (ZIKV). Except for TBEV, which is transmitted by ticks, the other viruses in this family are transmitted by blood-sucking mosquitoes. The most common flaviviruses and their transmission vectors are represented in Table 1.

Table 1. Major Flaviviruses, Their Vectors, and Associated Diseases (Zhao et al., 2021)

Flavivirus	Vector	Associated Disease(s)z
Dengue Virus (DENV)	Aedes aegypti, Aedes albopictus	Dengue Fever, Dengue Hemorrhagic Fever, Dengue Shock Syndrome
Zika Virus (ZIKV)	Aedes aegypti, Aedes albopictus	Zika Fever, Microcephaly, Guillain-Barré Syndrome
West Nile Virus (WNV)	Culex species	West Nile Fever, West Nile Neuroinvasive Disease
Yellow Fever Virus (YFV)	Aedes aegypti, Haemagogus species	Yellow Fever
Japanese Encephalitis Virus (JEV)	Culex tritaeniorhynchus	Japanese Encephalitis
Tick-Borne Encephalitis Virus (TBEV)	Ixodes species	Tick-Borne Encephalitis
St. Louis Encephalitis Virus (SLEV)	Culex species	St. Louis Encephalitis
Murray Valley Encephalitis Virus (MVEV)	Culex annulirostris	Murray Valley Encephalitis
Powassan Virus (POWV)	Ixodes cookei, Ixodes marxi	Powassan Encephalitis

Flaviviridae family is divided into four genera: Flavivirus, Pestivirus, Pegivirus, and Hepacivirus (Bamford et al., 2022). Flavivirus is significant since it consists of substantial viruses like the Dengue Virus (DENV) and the Zika Virus (ZIKV). These two viruses deserve attention because of their similarities in structure and genome, which are important in the viruses' virulence and distribution. As with many Flaviviruses, both viruses are transmitted mainly through the *Aedes aegypti* mosquito (Figure 1). Dengue virus (DENV) has long been a significant cause of morbidity and mortality. Zika virus (ZIKV) has recently gained global attention due to its linkage with severe neurological complications. Recently, Zika virus (ZIKV) has been in the news worldwide because of its link to severe neurological complications. The expansion of disease overlap is a concern because it could lead to co-infections with people infected with both viruses at the same time. Co-infections may lead to difficulties in diagnosis, disease severity, and clinical management.

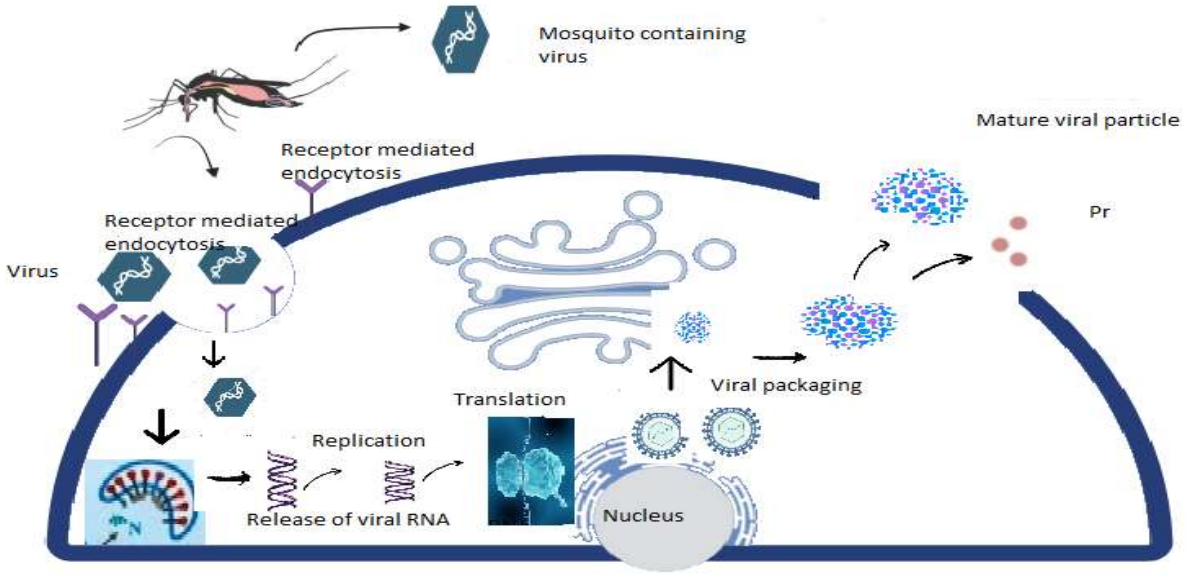

Figure 1 Lifecycle of common Flaviviruses

Phylogenetic relations of DENV and ZIKV

The relationship between Dengue virus (DENV) and Zika virus (ZIKV) is thought to likely be highly similar because the two appear to have split from a common ancestor only recently (Figure 2). It seems that DENV evolved from sylvatic strains that infect non-human primate populations and forest-associated Aedes mosquitoes; historical molecular phylogenetic studies have postulated that DENV originated in West Africa one thousand years ago. The migration of people, their dwellings in cities and towns, and the transportation of goods contributed to the dissemination and diversification of this ataxia. Uganda identified ZIKV in 1947; it is believed to have emerged from a natural cycle of transmission that occurred with several animals in Africa. It is evident from genetic analysis that ZIKV evolved from the Spondweni virus by means of an early branch point in the nineteenth century, and the virus became globalized in the mid-twentieth century. No precise period is provided for their development; however,

according to the present data, DENV and ZIKV may have probably diverged not earlier than 1000 years ago, and some of the hypotheses indicate that ZIKV may have evolved from DENV-4. This is because the two viruses have had a short evolutionary time, and this has made them genetically related, which is evidenced by molecular phylogenetic trees where strains of ZIKV such as ZIKV ArD41519 or Senegal, ZIKV IbH 30656 or Nigeria, and ZIKV MR766 or Uganda are closely related to DENV-4 strain H241 and DENV-2 strain New Guinea (Gaspar-Castillo et al., 2023).

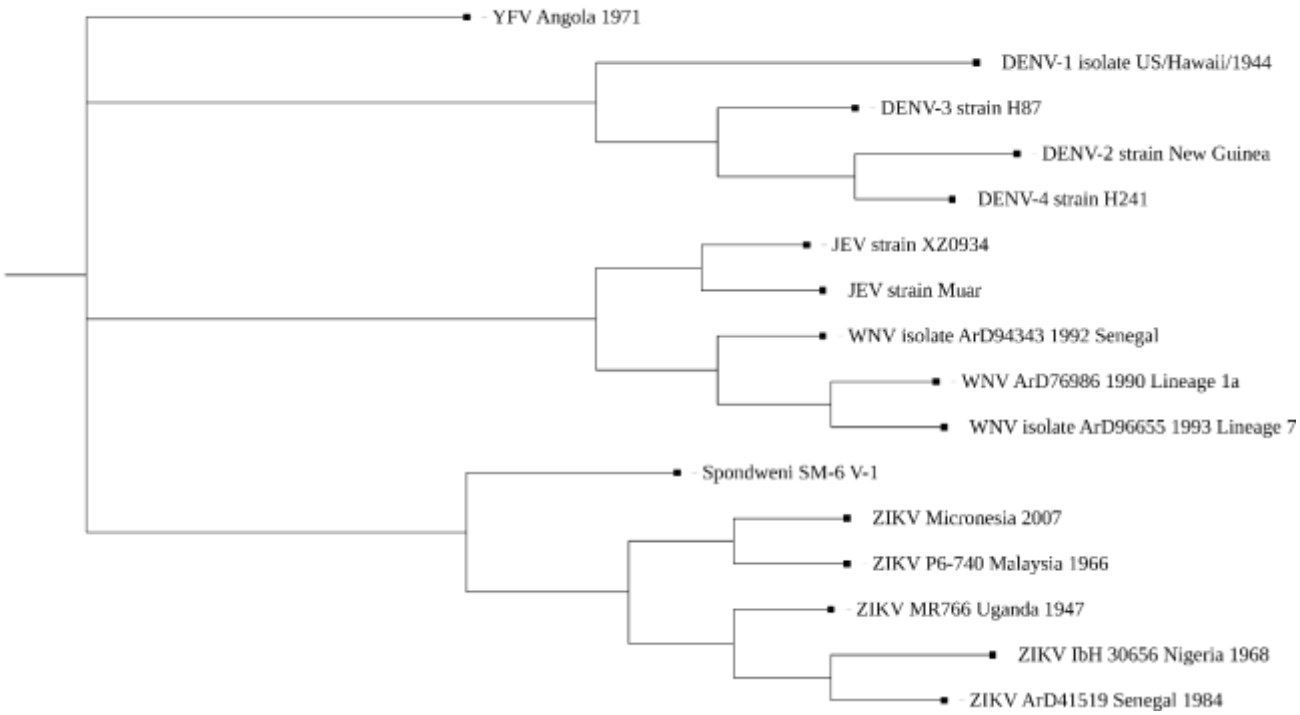

Figure 2. Phylogenetic tree illustrating the evolutionary relationships among ZIKV and DENV strains. The tree was constructed using genetic distance data based on the E gene (envelope gene), which is common for phylogenetic analysis in flaviviruses. Branch lengths correspond to the number of nucleotide substitutions per site, with a tree scale of 0.1 substitutions/site. Key ZIKV strains, such as ZIKV ArD41519 (Senegal), ZIKV IbH 30656 (Nigeria), and ZIKV MR766 (Uganda), are closely related to DENV strains such as DENV-4 strain H241 and DENV-2 strain New Guinea. (iTol)

Basic Structure of DENV and ZIKV

The members of this family are enveloped positive-sense RNA viruses with an approximately 10 to 11 kb RNA genome encapsulated in an icosahedral nucleocapsid (Salem et al., 2024). The viral RNA contains a 5' and a 3' untranslated region (5' and 3' UTR) and one ORF encoding a single polyprotein consisting of three structural proteins (C, prM/M, and E) and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) (Ou et al., 2020). Both species, DENV and ZIKV, are closely related structurally; their proteins, which focus on virus-host adapters and immune reactions, are almost identical (Figure 3). Such similarities in the Flaviviridae members explain the cross-reactivity usually evident in immunity or detection procedures. The genome organization of DENV and ZIKV looks quite similar, highlighting their taxonomical position in the Flaviviridae family. The viruses are spherical and enveloped, with a diameter of approximately 50 nm. This is in line with other members of the family and is indicative of the conservation of this adaptation throughout evolution. The envelope (E) and membrane (M) proteins form a herring-bone pattern when they are inserted into the lipid bilayer and are highly important in the structure of the icosahedron characteristic of the flaviviruses. Particular emphasis is given to the envelope proteins (E proteins) in the context of the biology of DENV and ZIKV. These proteins aggregate to form homodimers situated on the outer shell of the virion and act as the major immunogenic components called Major Antigens. Besides their role in immunogenicity, they also play vital roles in viral attachment, membrane fusion, and viral entry into the target host cells. This makes the E proteins highly essential in the functions of the virus as they infect and replicate in the host. However, the membrane (M) protein is necessary at the virus maturation stage; this protein alters conformation and shape, which makes the virus infectious.

Surrounded by a lipid envelope (L), the capsid (C) protein surrounds the RNA viral genome, offering it protection and shape. The C protein plays a critical role in the process of morphogenesis, whereby it guarantees the enclosure of an RNA genome into new budding virions (Montecillo-Aguado et al., 2019).

Despite the recently recognized differences in their amino acid sequences, the structural organization of DENV and ZIKV is closely similar. It is pertinent to discuss that they both fall under the Flavivirus genus to explain the structural conservancy, highlighting how these viruses, during the evolutionary course, have evolved multiple functional roles while occupying various ecological habitats.

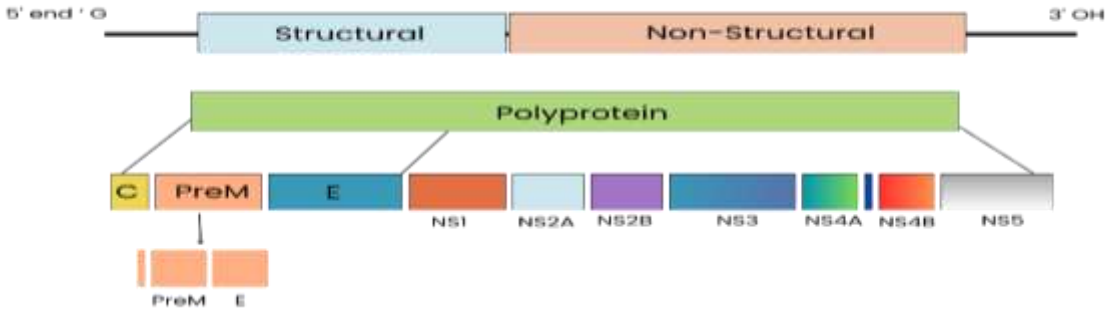

Figure 3 Diagrammatic illustration of the Flavivirus genome structure and the processing of the polypeptide into fully developed viral proteins.

Genomic and Protein Structure

Comparison of Genetic Sequences

Some differences between DENV and ZIKV are genetic and are essential for pathogenicity and immune responses to diseases. Phylogenetic studies have shown that there was a genealogical relationship between DENV and ZIKV, whereby DENV first branched into four different serotypes while ZIKV emerged as a separate lineage, which accounts for the fact that the two viruses had similar genomic relatedness. Another factor that results in the high degree of relatedness between the two viruses is the small time that the two have existed apart to be able to have different strains. This is evident in the sequences responsible for encoding the E protein, which is most vital in the antigenic properties. (Gaspar-Castillo et al., 2023. This sequence conservation within the E protein is important because the consistency of the amino acid sequence (Table 2) at these sites directly modulates cross-reactivity and antibody responses during infections and often results in the development of intricate immune interactions, including ADE in cases of sequential infections with different flaviviruses (Sevvana et al., 2020).

The genomic RNA of both DENV and ZIKV generates a single polyprotein and, after cleavage, into three structural proteins and seven non-structural proteins (Chan et al., 2022. The similarities do not only lie in the structural proteins of the two viruses are similar; research has amply proven that ZIKV and DENV are nearly 45 – 55% identical in protein sequence. Low levels of transgenic positives were observed during natural infections, including the envelope protein (EP) and structural proteins NS1, NS3, and NS5 (Cerutti et al., 2022). However, it is important to know that the E protein is highly conserved among flaviviruses, which explains why immune responses to one flavivirus cross-react with other flaviviruses. This factor complicates vaccine development. However, the presence of a shared set of antigens means that there are minute genetic differences in certain parts, for instance, prM and NS1 proteins that explain differences in virulence and antigenic drifts. Altogether, ZIKV and DENV are proven to be highly homologous, and at the genomic level, the nucleotide identity is more than 90% for most of the conserved regions (Singh et al., 2022). Such overlaps are significant on a diagnostic level since cross-reactivity may confuse serological analysis. It is essential to know these similarities and differences in sequences at the primary level in the fight against these new bugs and in the quest to develop the best treatment and vaccination mechanisms to distinguish between the effects of these similar viruses on the human immune system.

Table 2. Amino Acid Similarities and Differences between Dengue Virus (DENV) and Zika Virus (ZIKV) key Proteins (Cerutti et al., 2022)

Protein	Amino Acid Similarity (%)	Key Similarities	Key Differences
Envelope Protein (E)	35% - 55%	Conserved domains are critical for viral entry and fusion.	Variations in the glycan loop affect immune recognition.
NS1 Protein	~50%	Conserved regions are essential for immune response evasion.	Differences in antigenic sites influence cross-reactivity.
NS5 Protein	~70%	Highly conserved catalytic domains are involved in replication.	Sequence variations in the C-terminal region affect replication efficiency.

Structural Proteins: E Protein, prM/M, C Protein

Structural proteins of DENV and ZIKV, like E, M/M, and C proteins, play an important role in the virus life cycle and viral interactions with the host immune system (Table 3).

E Protein

Envelope (E) protein is the most prominent protein on the surface of flaviviruses, playing a critical role in mediating the virus's entry into host cells. In DENV and ZIKV, the E protein comprises three distinct domains: EDI, EDII, and EDIII.

- The EDI domain features a central β-barrel structure consisting of 130 amino acid residues arranged in three segments (residues 1–51, 132–192, and 280–295). A glycan loop (GL) is also located within this domain between residues 147 and 161, playing a role in viral attachment and immune evasion.
- The EDII domain adopts a finger-like conformation formed by two segments (residues 52–131 and 193–279) and comprises a highly hydrophobic fusion loop (FL) within residues 98–109, vital for fusion of viral and host cell membranes.
- The EDIII domain, characterized by an immunoglobulin-like fold, comprises residues 296–403 and is involved in receptor binding (Bamford et al., 2022; Gaspar-Castillo et al., 2023).

These E proteins are organized into 60 trimeric protrusions, anchored to the precursor membrane protein (preM) in the endoplasmic reticulum (ER). This assembly process forms immature virions with spiky surfaces, which undergo further maturation to become infectious. The E protein is highly conserved among flaviviruses, with some differences that may affect antigenicity and immune recognition. For instance, the ZIKV E protein has extra amino acids in its glycan loop that may influence the immune system's recognition of the virus and the efficacy of vaccines. However, the overall quaternary structure of the E protein is similar between DENV and ZIKV, with EDI, EDII and EDIII domains having 35%, 51% and 29% homology, respectively, between the two virus types. This structural similarity highlights the evolutionary relationship between these viruses and also the challenge of cross-protective vaccines.

M/M Protein

prM protein is a precursor membrane protein that protects the virus particle during virion formation by blocking premature fusion within a host cell. After maturation, the prM protein is cleaved to the M protein that remains attached to the viral coat. This cleavage is important in virion morphogenesis, where the E protein undergoes a conformational change allowing it to form a fusogenic conformation required for the virus to enter the host cell. Due to their closely related maturation mechanisms, the amino acid sequence identity of the prM/M protein of DENV and ZIKV is high. Nonetheless, variation concerning cleavage efficiency and subsequent structural reorganization may affect the virulence as well as immune evasion proficiency of these viruses (Sevvana et al., 2020).

C Protein

Capsid (C) protein is of immense significance in RNA genome packaging and forms the nucleocapsid core of the virion. The C protein assists in the binding of the viral RNA and the prM protein such that the genome of the virus can be enclosed well when new viruses are being produced. However, the C protein is much less conserved than E and prM/M proteins, which could be associated with some replication and pathogenic properties of DENV and ZIKV. That is why it is possible to postulate that even minor distinctions in the structure of the C protein in the given viruses can impact the assembly efficiency and the stability of the virion particles (Tan et al., 2020).

Table 3. Comparison of Structural Proteins (E, prM/M, C) and Their Roles in the Life Cycle of Dengue Virus (DENV) and Zika Virus (ZIKV) (Sevvana et al., 2020)

Structural Protein	Dengue Virus (DENV)	Zika Virus (ZIKV)	Role in Viral Life Cycle
Envelope Protein (E)	Highly conserved; forms trimeric protrusions on the virus surface.	Similar to DENV with additional amino acids in the glycan loop.	Mediates viral entry by facilitating attachment, membrane fusion, and immune evasion.
prM/M Protein	prM prevents premature fusion during virion formation; cleaved to M upon maturation.	Similar to DENV, there are slight differences in cleavage efficiency.	Assists in virion morphogenesis by protecting the E protein during assembly and enabling fusion.
Capsid Protein (C)	Less conserved; binds with viral RNA to make nucleocapsid core.	Similar to DENV but with minor structural differences.	Encapsulates the viral RNA genome, assisting in genome packaging and virion stability.

Implications of Structural Similarities

Dengue (DENV) and Zika virus (ZIKV) share a high degree of antigenic relatedness, which results in antibody-dependent enhancement (ADE). This phenomenon is even more alarming in areas with both viruses, for getting infected by the two viruses successively will result in increased disease severity owing to ADE. (Dejnirattisai et al., 2016)

Antibody-dependent Enhancement (ADE):

ADE (Antibody-dependent enhancement) is a critical mechanism that contributes to the severity of flavivirus infection, particularly Dengue Virus

(DENV) infections, mainly during secondary infections with a heterologous serotype (Langerak et al., 2019). Dengue (DENV) and Zika virus (ZIKV) share a high degree of antigenic relatedness, which results in antibody-dependent enhancement (ADE), whereby exposure to an infected individual with antibodies from a prior DENV infection may enhance ZIKV infection

Components of ADE

There are two components to ADE: extrinsic and intrinsic.

Extrinsic ADE

The increased virus entry due to the enhanced interaction between the virus-antibody complexes and FcγR on host cells occurs when non-neutralizing antibodies produced during a primary infection with one DENV serotype bind to a different DENV serotype during a secondary infection. These antibodies do not neutralize the virus but instead form immune complexes that facilitate the virus's entry into host cells via Fc gamma receptors (FcγRs) on mononuclear phagocytic cells (MPCs) such as macrophages and dendritic cells. This process is known as extrinsic ADE. (Halstead, 1970).

Intrinsic ADE

Instead of acting against viruses, these antibodies' receptors lead to more virus replication, more extensive infections, and possibly worsening disease outcomes such as Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) (Halstead, 1970; Katzelnick et al., 2017; Slon Campos et al., 2018). It involves more complex mechanisms within the host cell that favour viral replication. Specifically, intrinsic ADE modulates the host's innate immune responses, suppressing antiviral signalling pathways, such as those mediated by type I interferons (IFNs). During ADE, DENV can evade these immune responses by promoting the production of anti-inflammatory cytokines like interleukin-10 (IL-10), which inhibits the activation of Th1 immune responses and promotes a Th2 immune response, further dampening the host's ability to control the infection. (Chan et al., 2019; Tirado and Yoon, 2003). Furthermore, antibody-dependent DENV entry prevented the decrease in ribosomal genes provoked by canonical receptor-mediated endocytosis to enhance viral translation. (Chan et al., 2019). The modulation of the immune response promotes viral replication in ADE and plays a crucial role in developing severe dengue syndromes. For example, the downregulation of NO production and dysregulation of the JAK-STAT pathway due to a higher level of SOCS3 are some of the reasons that enable the virus to persist and progress the disease during ADE. (Flipse et al., 2013)

Experimental analysis

Various experimental studies, especially in mice, support this, showing that reinfection with DENV augments viremia and pathogenesis attributable to ADE.

Epidemiological study

Scientific evidence also provides proof of the existence of the ADE process in DENV, showing that infants who had maternal antibodies to dengue and were followed by secondary infection with a different serotype of DENV are likely to develop severe dengue disease (Tirado and Yoon, 2003). This is even more true in places where people get infected with both viruses; the chances of suffering from severe disease due to ADE will be high when one is infected with both viruses consecutively. However, the latest scientific findings show that when antibodies for one specific virus develop in the body, it only worsens the other virus, which is the case between dengue and Zika viruses. Some research shows that DENV antibodies could worsen ZIKV disease, while others do not. This variability provides more evidence of the complexity of ADE and the need for more research to better understand the meaning of ADE (Halstead, 2017)

Mechanisms of Immunological Cross-Reactivity

Immune Response to Flaviviruses

Innate Immune Response

The first line of defense against flaviviruses, including dengue (DENV) and Zika (ZIKV), is the activation of the innate immune response system. The former response is one of the first: the production of type I interferons (IFN-α and IFN-β) that engage specific receptors on host cells. This binding activates antiviral signal transduction pathways, including PKR and 2'5'-OAS, to curb viral replication. However, flaviviruses have developed strategies for overcoming these barriers. For example, the NS2A protein of West Nile Virus (WNV) can bind to STAT1 and STAT2, which in turn cannot be phosphorylated and transcribe antiviral genes, hence promoting viral replication while the host's antiviral mechanism is activated (Chong et al., 2019).

Humoral Immune Response

Humoral immunity, especially neutralizing antibodies, is considered to be highly effective in preventing flavivirus infections. The most notable of these antibodies focus on the virus's E glycoprotein, which hampers it from binding to the host cells. It also develops antibodies against nonstructural proteins such as NS1, which improve the immune system's performance. However, these antibodies do not neutralize the virus but can be protective indirectly through Fc-gamma receptor recruitment and complement activation. However, there is a process called Antibody-Dependent Enhancement (ADE), which raises the problem of immune response. ADE is a process in which low levels of antibodies produced in response to a previous flavivirus infection immobilize the virus and target it to phagocytic cells via Fc receptors. It can also stimulate more viral replication and disease in the host. This plays out given that cross-reactive antibodies can increase the virulence of both DENV and ZIKV in affected areas (Hurtado-Monzón et al., 2020).

Cellular Immune Response

CD8+ T cells have been found to control flavivirus infections by directly recognising and eliminating the infected cells. These T cells can identify viral peptides on the surface of infected cells and activate an apoptosis response to destroy these cells and prevent viral reproduction. Concerning DENV and ZIKV infections, it has been shown that the advantages of CD8+ T cells' targets include the non-structural proteins NS3, NS4B, and NS5. Although T-cell responses are important in managing viral load, they were also proven to cause immunopathology in severe dengue cases. For example, when their activity is too high, T cells can cause tissue injury, especially in the CNS, by producing pro-inflammatory cytokines and destroying diseased cells (Diamond, 2003).

Cross-Reactive Immunity

Antigenic relatedness or cross-reactivity is an essential feature of immunity to flaviviruses in that the immune response to one flavivirus influences the reaction to another. This is especially the case with DENV and ZIKV, which are very closely related, genetically speaking, and, therefore, very similar in their RNA sequences. They can remember another similar virus, sometimes with beneficial effects like immunity or detrimental effects like antigenic sin. This phenomenon happens when the immune system uses memory T cells from a previous infection to respond to a new and related virus, and this can result in a poor reaction (Rathore and St John, 2020)

Immune Evasion Strategies

Flaviviruses have also adopted diverse ways of avoiding the host's immune response, causing the disease to persist. One such tactic is the suppression of antigen presentation by dendritic cells, which play the role of an APC and are needed to induce adaptive immunity. DENV inhibits the effectiveness of DCs in presenting viral antigens to T cells through the induction of apoptosis, thereby escaping immune recognition. Furthermore, it has been discovered that flaviviruses can interfere with host miRNA pathways to inhibit any responses against the virus. For instance, DENV can evoke the production of miRNA-155 that suppresses viral replication by stimulating the HO-1 pathway to augment the antiviral properties of interferon (Lee et al., 2022).

Specific Immunity on Flavivirus Infections

T-cell responses are well documented to contribute to protective immunity and immunopathogenesis during flavivirus infections. However, what is unique is that the CD8+ T cells directly contact the target cell and destroy it, while the CD4+ T cells coordinate the whole affair. On the other hand, cross-reactive T cell responses, including the prior exposed individuals, may lead to the severity of the disease during the subsequent ZIKV encounter, called T cell original antigenic sin. This can lead to an undesirable immune response in which the body directs its response against the wrong viral epitopes, thus resulting in inefficient viral elimination and more tissue damage (Elong Ngono and Shresta, 2018).

Cross-reactive antibodies

The reason for the emergence of cross-reactive antibodies in the context of dengue virus (DENV) and Zika virus (ZIKV) is that these two viruses share a significant amount of protein homology (45–55%) and are members of the Flaviviridae family. The primary surface antigens of both viruses, the envelope proteins (EPs) of DENV and ZIKV, exhibit this homology, contributing to the cross-reactivity of antibodies.

Formation of cross-reactive antibodies

Antibodies are produced against multiple viral proteins during spontaneous infections, such as the envelope protein (EP), non-structural protein 1 (NS1), NS3, and NS5. Specifically, the EP features structural regions exposed on the viral surface that aid receptor binding on the host cell membrane and support the immune response. The EPs for ZIKV and DENV have comparable amino acid sequences but different glycosylation patterns. For example, Asn 154 is the only glycosylation site in ZIKV, yet Asn 67 and Asn 153 are found in DENV. Notwithstanding these variations, the EPs' structural closeness enables them to function as essential surface antigens, frequently detecting cross-reactive antibodies using the enzyme-linked immunosorbent test (Balmaseda et al.). Because the antibody profiles induced by ZIKV and DENV are similar, cross-reactivity can make it more difficult to differentiate between the two infections and confuse the immune response to one as a reaction to the other. Correct diagnosis is made more difficult, particularly in areas where both viruses are co-circulating and prevalent. Accurate detection of ZIKV and DENV antibodies in the acute and convalescent phase of infection requires the development of susceptible and specific serological tests. Creating monoclonal antibodies (mAbs) directed against the envelope protein (EP) of DENV and the NS1 protein of ZIKV is an important step in developing antigens that trigger an immune response against both viruses (Cerutti et al., 2022)

Function of cross-reactive antibodies

Cross-reactivity occurs when antibodies generated against one flavivirus, such as dengue virus (DENV), yellow fever virus (YFV), or Japanese encephalitis virus (JEV), can recognize and bind to other related flaviviruses. This cross-reactivity is significant because it can lead to either protection or enhancement of infection, depending on the specific circumstances. In particular, cross-reactive antibodies, especially those at sub-neutralizing concentrations, can enhance infections of related flaviviruses (Uhuami et al., 2024). For example, immune serum from YFV or JEV can enhance DENV infection in vitro. In DENV infections, secondary infection with a different serotype is associated with severe disease, and infants born to dengue-immune mothers are at increased risk of severe disease during primary infection due to waning maternal antibodies. (Byrne and Talarico, 2021). Cross-reactive antibodies

can have dual effects. On the one hand, they can neutralize the virus, providing protection. On the other hand, if the antibody levels are sub-neutralizing, they can facilitate ADE, where the virus-antibody complex enhances viral entry into host cells, potentially leading to more severe disease. Preexisting immunity for DENV can modulate the course of ZIKV infections. For example, some studies have shown that prior DENV immunity can reduce the severity of ZIKV infections. In contrast, others suggest that previous ZIKV infections may increase the risk of severe outcomes in subsequent DENV infections (Figure 4). The concept of "original antigenic sin" is discussed in DENV infections, where the immune system preferentially recalls the immune response from the first encounter with a DENV serotype when exposed to a different serotype. This phenomenon can also apply to interactions between ZIKV and DENV. For instance, if an individual previously infected with DENV encounters ZIKV, their immune system may produce antibodies against DENV that are not fully effective against ZIKV, potentially leading to enhanced disease severity. The cross-reactivity between DENV and ZIKV complicates serological testing. Because of the shared antigenic sites, serological assays like ELISA may yield false-positive results, making it challenging to accurately diagnose the specific flavivirus responsible for an infection. Neutralization tests, such as the plaque reduction neutralization test (PRNT), are essential for determining the difference between ZIKV and DENV infections. Since these tests assess an antibody's ability to neutralize viruses, they are more precise and less likely to result in a mistaken diagnosis due to cross-reactive antibodies (Gomes da Silva et al., 2023).

In addition to producing antibodies specific to DENV, patients infected with other flaviviruses may also develop antibodies cross-reactive with DENV. Serological testing may become more challenging because these cross-reactive antibodies may identify comparable epitopes on other flaviviruses. Although cross-reactive antibodies can occasionally provide protective immunity, they can also cause problems with diagnosis and unfavorable results, such as antibody-dependent enhancement (ADE), in which the antibodies strengthen the virus rather than kill it, thereby making it worsening the disease. A meaningful way to determine the precise flavivirus causing infection and differentiate between primary and secondary infections is to compare the antibody levels over time in paired serum samples, usually obtained during the acute and convalescent phases of the disease.

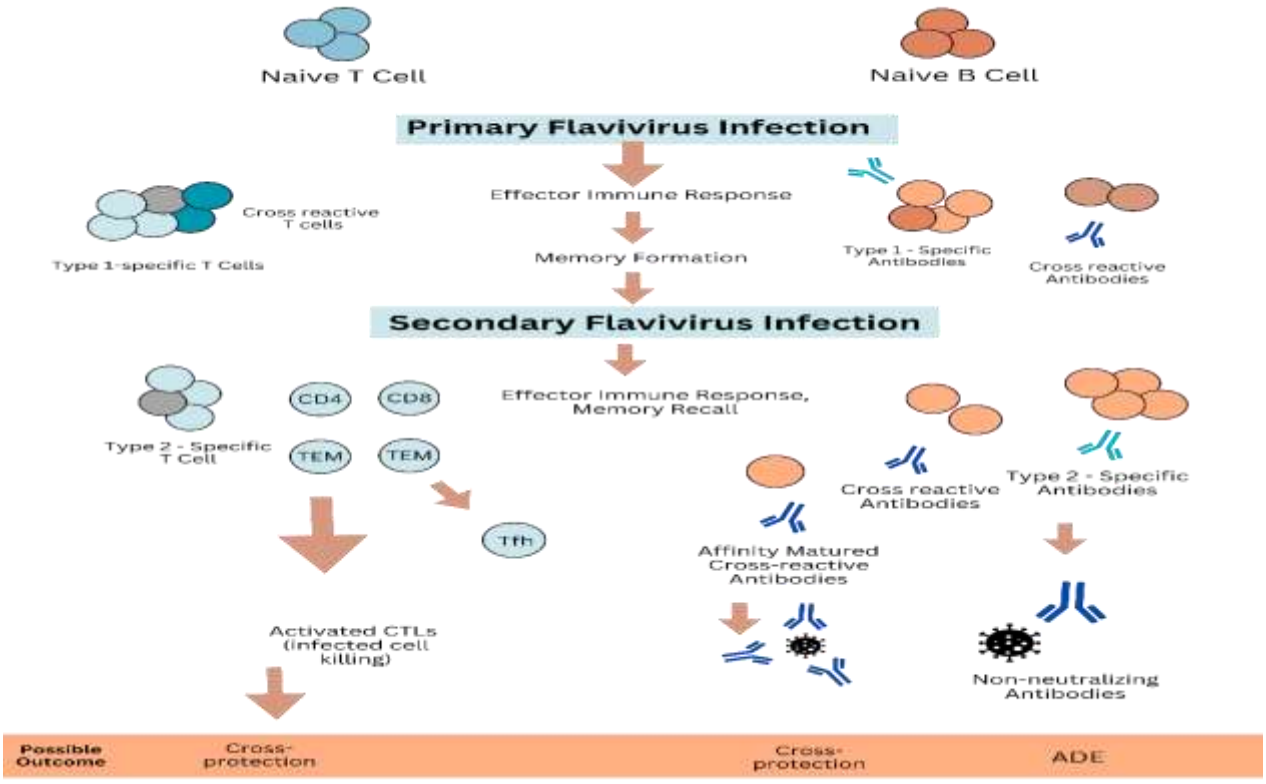

Figure 4 Flavivirus Cross-Reactive Immune Response

Types of Antibodies

- **Species-Specific Antibodies:** These antibodies are directed against particular species of flaviviruses and offer protection against the specific virus that initially produced them.

- **Cross-Reactive Antibodies:** Because distinct flavivirus species share structural and sequence similarities, these antibodies can identify and bind to diverse flavivirus species. Nonetheless, they might not adequately destroy the virus, which could cause antibody-dependent enhancement (ADE) of the illness.
- **Neutralizing Antibodies:** These antibodies prevent the virus from infecting host cells. They specifically target the viral envelope (E) protein. (Gomes da Silva et al., 2023)
- **Monoclonal Antibody 3B6:** Attaches itself to the EXE/DPPFG epitope in the E protein domain III. This epitope is highly conserved in flaviviruses, including the West Nile Virus (WNV), Zika Virus (ZIKV), Japanese Encephalitis Virus (JEV), Murray Valley Encephalitis Virus (MVEV), and Saint Louis Encephalitis Virus (SLEV). Among these viruses, this epitope is regarded as immunodominant.
- **Monoclonal antibody 2A10G6**: Effectively neutralizes a variety of flaviviruses, such as WNV, JEV, DENV, TBEV, and Yellow Fever Virus (YFV). A highly conserved flavivirus fusion loop peptide, the 98DRXW101 motif, is broadly cross-reactive with this antibody **(**Gaspar-Castillo et al., 2023**)**
- **ZIKV 8-8-11**: This monoclonal antibody identifies Zika virus non-structural protein 1 (NS1).
- **DENV 8G2-12-21:** This monoclonal antibody targets the Dengue virus type 2 envelope protein. (Cerutti et al., 2022)

Impact of cross-reactivity on serological testing:

- **Neutralization** assays gauge how well antibodies neutralize viruses and stop them from infecting cells. When assessing immunity against flaviviruses, one of the more precise techniques is the neutralization test.
- **Enzyme-Linked Immunosorbent Assay (**Balmaseda et al.**):** This test looks for particular antibodies in the blood, such as cross-reactive and species-specific antibodies. However, because of cross-reactivity, ELISA might not always be able to pinpoint the precise flavivirus that is infecting a person.
- **Plaque Reduction Neutralization Test (PRNT):** This more focused neutralization test counts the number of viral plaques produced in cell cultures following exposure to neutralizing antibodies. It is regarded as the gold standard for differentiating between various flavivirus infections (Gomes da Silva et al., 2023)
- **Hemagglutination-Inhibition Test:** This test assesses an antibody's capacity to prevent a virus from agglutinating red blood cells. It helps identify antibodies to the flavivirus, but cross-reactivity may also impact it.
- **Western Blot Test:** This assay uses antibodies to identify particular viral proteins. It can help confirm the existence of certain antibodies against flaviviruses.
- **Immunofluorescence Test:** This test uses fluorescent-labelled antibodies to look for flavivirus antigens in infected cells. It is an additional technique for identifying antibodies but needs specific tools and knowledge (Gaspar-Castillo et al., 2023).

Cellular Level Mechanisms involved in cross-reactivity

T-cell-mediated immunity is essential to the host's defense against viral pathogens. It is characterized chiefly by T-cells and B-cells, which identify and kill infected cells. Helping T-cells and cytotoxic T-lymphocytes (CTLs) are crucial in immune surveillance and controlling damaged cells. However, B-cells are tasked with producing antibodies that act on pathogens and immobilize them to reduce the possibility of reinfection. The coordination between these immune cells is critical for shaping an efficient and sustainable immune response. This is particularly the case regarding viral diseases like dengue virus disease (DVD) and Zika virus disease (ZVD). These two viruses are indeed immunologically closely related, and this biological interaction can influence immune effectiveness and disease severity. Knowledge of the cellular processes that contribute to cross-reactivity between these viruses is an essential aspect of disclosing the intricacies of the immune response to these two related viruses.

Interaction between DENV and ZIKV at the cellular level

Structural Similarity and its Implications

DENV and ZIKV come from the Flaviviridae family, and the two diseases are closely related genetically and molecularly. The viruses share over 90% of their genomic sequences, leading to changes in the structure of the envelope proteins. Such high homology creates opportunities for cross-interference of the two viruses regarding immunity. There is always cross-reactivity, mainly during secondary infections in which the reaction is quite noticeable. For example, if a person has been exposed to DENV and later infected with ZIKV, the immune response might cross-react with ZIKV. This misidentification can lead to false positive results in the serological tests, and the true seroprevalence and incidence of Zika virus outbreaks are difficult to estimate, especially in regions co-endemic for dengue virus (Gaspar-Castillo et al., 2023).

1. Role of Cross-Reactive Antibodies

An exciting and dynamic relationship between cross-reactive antibodies and subsequent ZIKV infection remains. These antibodies can increase the sensitization of ZIKV by expanding the type I IFN in pDCs, which plays a vital role in the immune response. Fc receptors on pDCs bound with these cross-reactive antibodies lead to improvement in the identification and combating of both viruses, strengthening the specificity of the immune response.

This mechanism may also afford protection against worse outcomes in secondary heterotypic infections with DENV or ZIKV, thus emphasizing the role of cross-reactive antibodies. Nevertheless, antibodies, memory T-cells, and B-cells are involved in cross-reactivity. T-cells can differentiate conserved epitopes of DENV and ZIKV, while B-cells present antigens that, when stimulated, promote activation of T-cells. Discussing this interaction, it is possible to emphasize the importance of cellular immunity in forming cross-reactive responses between these two viruses (Aisenberg et al., 2022)

Impact of Prior Immunity on Vaccine Responses

It has been found that the level of previous exposure to DENV can directly affect the response to the ZIKV vaccination. A cross-sectional study conducted on Puerto Rico participants showed that those who had past exposure to DENV had higher neutralizing antibody titer values against both DENV and ZIKV before the vaccination. This immunity can produce divergent outcomes after a person receives the ZIKV vaccine. It can improve the vaccine's efficacy in specific scenarios or cause antibody-dependent enhancement (ADE) of the disease, depending on precisely what kind of DENV serotype is at hand. These dynamics underscore the need to factor prior immunity into developing an approach to the immunization of ZIKV (Shan et al., 2017)

Broad Neutralization Potential

Some previous studies have established that envelope cross-reactivity exists between ZIKV and other flaviviruses whereby antibodies from people whom other Flaviviruses have infected, like Japanese encephalitis virus (JEV), can neutralize ZIKV. This observation indicates that previous sensitization with flaviviruses helps modulate humoral immunity to enable cross-neutralization. In support of this concept, human monoclonal antibodies with enhanced somatic hypermutation have been detected, which possess the capacity for wide-ranging neutralization from various flaviviruses. This was evident in illustrating the interactions between multiple flaviviruses and immune response systems to viral infections. (Salem et al., 2024)

Cellular Stress Responses and Their Role in Cross-Reactivity

The infection dynamics of these two viruses and the subsequent stress responses that occur in host cells are also different. For instance, ZIKV triggers a redox program that is essential in maintaining the balance of oxidants and antioxidants. In contrast, DENV triggers endoplasmic reticulum stress, which results in an unfolded protein response. Stress responses can, therefore, impact the overall immunological status and possibly even the cellular immunological responses to these viruses. As such, these responses can affect the disease and its progression. (Singh et al., 2022)

Besides, both cellular stress responses are connected with cross-reactivity in the immune system. There is information about T-cells that develop reactivity to the epitopes of the DENV and ZIKV and may be misdirected occasionally to provide less than the optimal response. ER stress elicited by DENV and redox signaling mediated by ZIKV can also modulate other immune responses and immunopathology, either promoting or suppressing the cross-reactive immune response. Altogether, these cellular factors contribute to determining cross-reactivity and the development of infections by these viruses.

These interactions complicate handling these infections in locations where both are prevalent and arguably make the case for customized vaccination plans. However, the cross-reactivity can also have disadvantageous effects, including immune enhancement, which might work in conjunction with ADC (antibody-dependent enhancement), which refers to cases when existing antibodies increase disease severity following subsequent infections. It is a complex process that presents significant issues in developing vaccines and infection prevention measures in areas affected by DENV and ZIKV. It is essential to better comprehend these cellular-level mechanisms to implement various interventions that could reduce the effects of these two genetically closely related viruses.

Challenges in Assessing Co-Infection Prevalence

Dengue Virus (DENV) and Zika Virus (ZIKV) infections are clinically diagnosed despite differences in symptoms and complications and cross-reactivity between their serological tests. On the same note, diagnostic technologies that have developed over the years can effectively increase and improve the distinction.

Diagnostic Challenges

Symptom Overlap

DENV and ZIKV manifest similar symptoms, so it is not easy to diagnose the two at first instance (Sekaran et al., 2022).

Serological Cross Reactivity:

Cross-reactivity is due to an extraordinary homological relationship between DENV and ZIKV, especially among NS1 and E proteins, and cross-over between them is a severe challenge to serological testing (Balmaseda et al., 2017; Pereira et al., 2024; Sittikul et al., 2022). This issue is followed by an improper diagnosis of the diseases, leading to difficulties in monitoring and controlling the disease outbreak. Improved diagnostic methods that enable the identification of these viruses with a lot of ease and clarity are essential for better public health. (Rabe, 2016). Also, since DENV and ZIKV share several identical and similar symptoms in their early stages, confirmation of the clinical diagnosis of the diseases is challenging; therefore, there is a need to develop unparalleled diagnostic tools (Priyamvada et al., 2016)

Overcoming Challenges

- Multiplex PCR Testing: TaqMan™ Arbovirus Triplex Kit as a screening test provides 100 %

sensitivity and 95. 1% specificity for DENV identification with distinction from other arboviruses (Panmei et al., 2024).

- Combined Immunoassays: It would be better if both NS1 IgG ELISA and blockade-of-binding ELISA were used in syntheses to increase the specificity rate to 94%. 59%, enhancing the detection of ZIKV in DENV-affected regions (Sittikul et al., 2022)
- Multi-Epitope Proteins: Emerging novel multi-epitope proteins for serological diagnosis also distinguish ZIKV from DENV due to the issues arising from cross-reactivity (Pereira et al., 2024)

Vaccine Development

This cross-reactivity poses huge challenges to preventing the development of vaccines since a person can be affected by both viruses. It has been proven that a ZIKV vaccine could increase the severity of DENV, as the generated antibodies are cross-reactive to both viruses, which is the same case for ZIKV (Dejnirattisai et al., 2016). This challenge needs a well-designed vaccine with the potential to produce various immunotypes to counteract the flaviviruses without causing ADE. More research has been done on the likelihood of administering different vaccine doses or producing a flavivirus vaccine that will use cross-reactive T-cells in the body to guard against the two viruses (Shan et al., 2017). Moreover, it was mentioned that there is a need to know the specific immunogenic triggers that can cause ADE so as not to further aggravate the severity of the disease in vaccinated individuals (Pantoja et al., 2017). Dengvaxia does not work equally well against all four serotypes of the dengue virus and worked well for those who had already been exposed but not for those who had not (Slon Campos et al., 2018)

Public Health Impact

Competitive interactions between DENV and ZIKV are not only germane to the clinical consequences for people infected by these viruses but also greatly concern public health. ADE results in more severe infection and diagnostic complications, and both viruses significantly stress healthcare systems in areas where both viruses are endemic (Tirado and Yoon, 2003). This is due to high morbidity and mortality caused by mass outbreaks of the diseases if accurate diagnostics or effective vaccines are not utilized. Furthermore, the cost implications to healthcare facilities and the fear that they might spread from one country to another are why there is a need to continue researching this field.

Conclusion

This chapter discusses the aspects of the DENV–ZIKV relationship with a focus on co-infection as well as immunological interactions. Other than having an almost comparable structure and genetic material, both viruses have similar clinical presentations and consequences to public health. ADE is one reason why infections become severe and shows aspects of immunity when there are multiple infections with flaviviruses. Moreover, instead of affording protection, cross-reactive antibodies can also cause diagnostic problems with the diagnosis of infections with these two viruses, particularly in regions where both exist. The chapter shows that better diagnostic techniques and killing vaccines are needed to differentiate between those viruses and prevent the disease from being made worse. To effectively overcome these hurdles and create vaccine and diagnostic bioassays relevant to risk reduction of cross-co-infection factors in public health, further analysis of cross-reactivity of cellular and molecular components is required.

References

Aisenberg, L. K., Rousseau, K. E., Cascino, K., Massaccesi, G., Aisenberg, W. H., Luo, W., Muthumani, K., Weiner, D. B., Whitehead, S. S., and Chattergoon, M. A. (2022). Cross-reactive antibodies facilitate innate sensing of dengue and Zika viruses. *JCI insight* **7**. e151782. https://doi.org/10.1172/jci.insight.151782

Balmaseda, A., Stettler, K., Medialdea-Carrera, R., Collado, D., Jin, X., Zambrana, J. V., Jaconi, S., Cameroni, E., Saborio, S., and Rovida, F. (2017). Antibody-based assay discriminates Zika virus infection from other flaviviruses. *Proceedings of the National Academy of Sciences* **114**, 8384-8389. https://doi.org/10.1073/pnas.1704984114

Bamford, C. G., de Souza, W. M., Parry, R., and Gifford, R. J. (2022). Comparative analysis of genome-encoded viral sequences reveals the evolutionary history of flavivirids (family Flaviviridae). *Virus Evolution* **8**, veac085. https://doi.org/10.1093/ve/veac085

Byrne, A. B., and Talarico, L. B. (2021). Role of the complement system in antibody-dependent enhancement of flavivirus infections. *International Journal of Infectious Diseases* **103**, 404-411. https://doi.org/10.1016/j.ijid.2020.11.183

Cerutti, H., Tesi, G., Soldatini, C., Bandini, T., Castria, M., and Brogi, A. (2022). Development of an Anti-Zika and Anti-Dengue IgM ELISA Assay: Evaluation of cross reactivity and validation. *Tropical Medicine and Infectious Disease* **7**, 348. https://doi.org/10.3390/tropicalmed7110348

Chan, C. Y., Low, J. Z. H., Gan, E. S., Ong, E. Z., Zhang, S. L.-X., Tan, H. C., Chai, X., Ghosh, S., Ooi, E. E., and Chan, K. R. (2019). Antibody-Dependent dengue virus entry modulates cell intrinsic responses for enhanced infection. *Msphere* **4**, 10.1128/msphere. 00528-19. https://doi.org/10.1128/mSphere.00528-19

Chan, K. R., Ismail, A. A., Thergarajan, G., Raju, C. S., Yam, H. C., Rishya, M., and Sekaran, S. D. (2022). Serological cross-reactivity among common flaviviruses. *Frontiers in Cellular and*

Infection Microbiology **12**, 975398. https://doi.org/10.3389/fcimb.2022.975398

Chong, H. Y., Leow, C. Y., Majeed, A. B. A., and Leow, C. H. (2019). Flavivirus infection—A review of immunopathogenesis, immunological response, and immunodiagnosis. *Virus research* **274**, 197770. https://doi.org/10.1016/j.virusres.2019.197770

Dejnirattisai, W., Supasa, P., Wongwiwat, W., Rouvinski, A., Barba-Spaeth, G., Duangchinda, T., Sakuntabhai, A., Cao-Lormeau, V.-M., Malasit, P., and Rey, F. A. (2016). Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus. *Nature immunology* **17**, 1102-1108. https://doi.org/10.1038/ni.3515

Diamond, M. S. (2003). Evasion of innate and adaptive immunity by flaviviruses. *Immunology and cell biology* **81**, 196-206. https://doi.org/10.1046/j.1440-1711.2003.01157.x

Elong Ngono, A., and Shresta, S. (2018). Immune response to dengue and Zika. *Annual review of immunology* **36**, 279-308. https://doi.org/10.1146/annurev-immunol-042617-053217

Flipse, J., Wilschut, J., and Smit, J. M. (2013). Molecular mechanisms involved in antibody-dependent enhancement of dengue virus infection in humans. *Traffic* **14**, 25-35. https://doi.org/10.1111/tra.12012

Gaspar-Castillo, C., Rodríguez, M. H., Ortiz-Navarrete, V., Alpuche-Aranda, C. M., and Martinez-Barnetche, J. (2023). Structural and immunological basis of cross-reactivity between dengue and Zika infections: Implications in serosurveillance in endemic regions. *Frontiers in Microbiology* **14**, 1107496. https://doi.org/10.3389/fmicb.2023.1107496

Gomes da Silva, P., Seixas dos Reis, J. A., Nogueira Rodrigues, M., da Silva Ardaya, Q., and Mesquita, J. R. (2023). Serological cross-reactivity in zoonotic flaviviral infections of medical importance. *Antibodies* **12**, 18. https://doi.org/10.3390/antibodies12010018

Halstead, S. (1970). Observations related to pathogensis of dengue hemorrhagic fever. VI. Hypotheses and discussion. *The Yale journal of biology and medicine* **42**, 350.

Halstead, S. B. (2017). Biologic evidence required for Zika disease enhancement by dengue antibodies. *Emerging Infectious Diseases* **23**, 569-573. https://doi.org/10.3201/eid2304.161878

Hurtado-Monzón, A. M., Cordero-Rivera, C. D., Farfan-Morales, C. N., Osuna-Ramos, J. F., De Jesús-González, L. A., Reyes-Ruiz, J. M., and Del Ángel, R. M. (2020). The role of anti-flavivirus humoral immune response in protection and pathogenesis. *Reviews in medical virology* **30**, e2100. https://doi.org/10.1002/rmv.2100

Katzelnick, L. C., Gresh, L., Halloran, M. E., Mercado, J. C., Kuan, G., Gordon, A., Balmaseda, A., and Harris, E. (2017). Antibody-dependent enhancement of severe dengue disease in humans. *Science* **358**, 929-932. https://doi.org/10.1126/science.aan6836

Langerak, T., Mumtaz, N., Tolk, V. I., van Gorp, E. C., Martina, B. E., Rockx, B., and Koopmans, M. P. (2019). The possible role of cross-reactive dengue virus antibodies in Zika virus pathogenesis. *PLoS pathogens* **15**, e1007640. https://doi.org/10.1371/journal.ppat.1007640

Lee, M. F., Voon, G. Z., Lim, H. X., Chua, M. L., and Poh, C. L. (2022). Innate and adaptive immune evasion by dengue virus. *Frontiers in Cellular and Infection Microbiology* **12**, 1004608. https://doi.org/10.3389/fcimb.2022.1004608

Montecillo-Aguado, M. R., Montes-Gómez, A. E., García-Cordero, J., Corzo-Gómez, J., Vivanco-Cid, H., Mellado-Sánchez, G., Muñoz-Medina, J. E., Gutiérrez-Castañeda, B., Santos-Argumedo, L., and González-Bonilla, C. (2019). Cross-reaction, enhancement, and neutralization activity of dengue virus antibodies against zika virus: a study in the Mexican population. *Journal of immunology research* **2019**, 7239347. https://doi.org/10.1155/2019/7239347

Ou, T. P., Yun, C., Auerswald, H., In, S., Leang, R., Huy, R., Choeung, R., Dussart, P., and Duong, V. (2020). Improved detection of dengue and Zika viruses using multiplex RT-qPCR assays. *Journal of Virological Methods* **282**, 113862. https://doi.org/10.1016/j.jviromet.2020.113862

Panmei, K., Hakeem, S. A., Okafor, O., Mammen, S., and Abraham, A. M. (2024). Performance evaluation of TaqMan™ Arbovirus Triplex Kit (ZIKV/DENV/CHIKV) for detection and differentiation of Dengue and Chikungunya viral RNA in serum samples of symptomatic patients. *medRxiv*, 2024.06. 12.24308802. https://doi.org/10.1101/2024.06.12.24308802

Pantoja, P., Pérez-Guzmán, E. X., Rodríguez, I. V., White, L. J., González, O., Serrano, C., Giavedoni, L., Hodara, V., Cruz, L., and Arana, T. (2017). Zika virus pathogenesis in rhesus macaques is unaffected by pre-existing immunity to dengue virus. *Nature communications* **8**, 15674. https://doi.org/10.1038/ncomms15674

Pereira, S. H., Sá Magalhães Serafim, M., Moraes, T. d. F. S., Zini, N., Abrahão, J. S., Nogueira, M. L., Coelho dos Reis, J. G. A., Bagno, F. F., and da Fonseca, F. G. (2024). Design, development, and validation of multi-epitope proteins for serological diagnosis of Zika virus infections and discrimination from dengue virus seropositivity. *PLOS Neglected Tropical*

Diseases **18**, e0012100. https://doi.org/10.1371/journal.pntd.0012100

Priyamvada, L., Quicke, K. M., Hudson, W. H., Onlamoon, N., Sewatanon, J., Edupuganti, S., Pattanapanyasat, K., Chokephaibulkit, K., Mulligan, M. J., and Wilson, P. C. (2016). Human antibody responses after dengue virus infection are highly cross-reactive to Zika virus. *Proceedings of the National Academy of Sciences* **113**, 7852-7857. https://doi.org/10.1073/pnas.1607931113

Rabe, I. B. (2016). Interim guidance for interpretation of Zika virus antibody test results. *MMWR. Morbidity and mortality weekly report* **65**. 543-546. https://doi.org/10.15585/mmwr.mm6521e1

Rathore, A., and St John, A. (2020). Cross-Reactive Immunity Among Flaviviruses. Front Immunol. 2020 11: 334. https://doi.org/10.3389/fimmu.2020.00334

Salem, G. M., Galula, J. U., Wu, S.-R., Liu, J.-H., Chen, Y.-H., Wang, W.-H., Wang, S.-F., Song, C.-S., Chen, F.-C., and Abarientos, A. B. (2024). Antibodies from dengue patients with prior exposure to Japanese encephalitis virus are broadly neutralizing against Zika virus. *Communications Biology* **7**, 15. https://doi.org/10.1038/s42003-023-05680-w

Sekaran, S. D., Ismail, A. A., Thergarajan, G., Chandramathi, S., Rahman, S. H., Mani, R. R., Jusof, F. F., Lim, Y. A., and Manikam, R. (2022). Host immune response against DENV and ZIKV infections. *Frontiers in cellular and infection microbiology* **12**, 975222. https://doi.org/10.3389/fcimb.2022.975222

Sevvana, M., Rogers, T. F., Miller, A. S., Long, F., Klose, T., Beutler, N., Lai, Y.-C., Parren, M., Walker, L. M., and Buda, G. (2020). Structural basis of Zika virus specific neutralization in subsequent flavivirus infections. *Viruses* **12**, 1346. https://doi.org/10.3390/v12111346

Shan, C., Muruato, A. E., Nunes, B. T., Luo, H., Xie, X., Medeiros, D. B., Wakamiya, M., Tesh, R. B., Barrett, A. D., and Wang, T. (2017). A live-attenuated Zika virus vaccine candidate induces sterilizing immunity in mouse models. *Nature medicine* **23**, 763-767. https://doi.org/10.1038/nm.4343

Singh, K., Martinez, M. G., Lin, J., Gregory, J., Nguyen, T. U., Abdelaal, R., Kang, K., Brennand, K., Grünweller, A., and Ouyang, Z. (2022). Transcriptional and translational dynamics of Zika and dengue virus infection. *Viruses* **14**, 1418. https://doi.org/10.3390/v14071418

Sittikul, P., Sriburin, P., Rattanamahaphoom, J., Limkittikul, K., Sirivichayakul, C., and Chatchen, S. (2022). Combining immunoassays to identify Zika virus infection in dengue-endemic areas. *Tropical Medicine and Infectious Disease* **7**, 254. https://doi.org/10.3390/tropicalmed7100254

Slon Campos, J. L., Mongkolsapaya, J., and Screaton, G. R. (2018). The immune response against flaviviruses. *Nature immunology* **19**, 1189-1198. https://doi.org/10.1038/s41590-018-0210-3

Tan, T. Y., Fibriansah, G., Kostyuchenko, V. A., Ng, T.-S., Lim, X.-X., Zhang, S., Lim, X.-N., Wang, J., Shi, J., and Morais, M. C. (2020). Capsid protein structure in Zika virus reveals the flavivirus assembly process. *Nature communications* **11**, 895. https://doi.org/10.1038/s41467-020-14647-9

Tirado, S. M. C., and Yoon, K.-J. (2003). Antibody-dependent enhancement of virus infection and disease. *Viral immunology* **16**, 69-86. https://doi.org/10.1089/088282403763635465

Uhuami, A. O., Bello, M. B., and Imam, M. U. (2024). Flavivirus cross-reactivity: insights into e-protein conservancy, pre-existing immunity, and co-infection. *The Microbe*, 100105. https://doi.org/10.1016/j.microb.2024.100105

Zhao, R., Wang, M., Cao, J., Shen, J., Zhou, X., Wang, D., and Cao, J. (2021). Flavivirus: from structure to therapeutics development. *Life* **11**, 615. https://doi.org/10.3390/life11070615

Declaration

Ethical Approval and Consent to Participate

Ethical approval is not needed for this review article.

Consent for publication

Not Applicable here

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding statement:

No specific funding or grants were utilized for the accomplishment of this review work.

Authorship contribution statement

Saima Younas: Writing – review & editing, Writing – original draft, Validation, Supervision.

Umaira Zahoor, Muhammad Zfar Saleem, Qurban Ali, Sadia Manzoor: Writing – review & editing, All authors approved final version of manuscript to publish.

Declaration of Generative AI and AI-assisted Technologies in the Writing Process

During the preparation of this work, the author(s) have not used any ChatGPT (OpenAI) tool.

Acknowledgments: None.