

Review Article

The critical role of N-glycosylation in mediating arbovirus-host interactions

Li Wang^{1,2*}, Hu Zhou^{2*}, Xu Huang^{3*}, Jing Wang^{2,5*}, Yefei Cheng², Qili Yu², Xueyu Zhang², Fei Chen², Xiangjie Kong², Pu Wang^{4,5}, Baoming Wang², Lili Zhao⁴

¹College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing 163319, Heilongjiang, China; ²Hangzhou Xiaoshan Donghai Aquaculture Co., Ltd., Hangzhou 311200, Zhejiang, China; ³School of Medicine, Taizhou University (Jiaojiang Campus), No. 1139 Shifu Avenue, Jiaojiang District, Taizhou, Zhejiang, China; ⁴Zhejiang Jindakang Animal Health Co., Ltd., Jinhua 321000, Zhejiang, China; ⁵Zhejiang International Science and Technology Cooperation Base for Veterinary Medicine and Health Management, College of Veterinary Medicine of Zhejiang A&F University, Hangzhou, Zhejiang, China. *Equal contributors.

Received May 27, 2026; Accepted July 27, 2026; Epub October 15, 2026; Published October 30, 2026

Abstract: Glycosylation is a major post-translational modification that regulates protein folding, stability, trafficking, and recognition in biological systems. During viral replication, many viruses exploit host glycosylation machinery to modify their own proteins and thereby shape virus-host interactions. Among the major forms of protein glycosylation, N-linked glycosylation has a particularly important role in viral glycoprotein folding, virion assembly, receptor engagement, membrane fusion, tissue tropism, vector competence, and immune evasion. Current studies have revealed diverse functions of viral protein glycosylation in the infection processes of several pathogens, including Zika virus, dengue virus, West Nile virus, Japanese encephalitis virus, duck Tembusu virus, tick-borne encephalitis virus, and other medically or veterinary important arboviruses. Here, we review the biological significance of N-glycosylation in mediating interactions between arboviruses and their hosts, with emphasis on conserved mechanisms, virus-specific glycosylation sites and their contributions to viral replication, pathogenesis, host adaptation and immune escape. We also discuss unresolved questions and potential applications of glycosylation-targeted strategies in antiviral research and vaccine development.

Keywords: Glycosylation, arbovirus, virus-host interaction, *Flaviviridae* virus

Introduction

Arboviruses (arthropod-borne viruses) are a group of RNA viruses in biological taxonomy, mainly classified within the families *Flaviviridae*, *Togaviridae*, and *Bunyaviridae*. These pathogens are ecologically maintained through complex transmission cycles involving hematophagous arthropod vectors, such as mosquitoes, sandflies, fleas, and ticks. In recent decades, arboviral diseases have shown an accelerated global dissemination pattern, driven by a combination of factors including increased international travel and trade, unplanned urbanization, and the expansion of vector geographic ranges induced by climate change [1]. Therefore, clarifying the relationship between arboviruses and their vectors is crucial [2], which highlights the urgent need for

novel control strategies for mosquito-borne diseases [3].

As obligate intracellular parasites, viruses hijack the host cellular machinery to modify their own proteins and enhance survival and infectivity. Among all post-translational modifications (PTMs), glycosylation - the covalent attachment of glycans to proteins - is crucial for the pathogenic mechanisms of viruses [4]. This evolutionarily conserved process occurs across all domains of life (eukaryotes, eubacteria, archaea) and involves multiple glycosidic linkage types. The major forms of protein glycosylation are as follows: 1. O-linked glycosylation: glycan chains are covalently attached to the hydroxyl moieties of serine (Ser) or threonine (Thr) residues; this modification regulates protein stability and functional modulation [5].

2. N-linked glycosylation: glycan chains are attached to asparagine (Asn) residues of the proteins. This modification is critical for protein folding, cell-cell recognition, and signal transduction [6]. 3. C-linked glycosylation: a rare modification in which mannofuranose is attached to the C2 position of tryptophan's indole ring through a C-C bond, observed in only a limited number of organisms [7]. 4. Glycosylphosphatidylinositol (GPI) anchor linkage: a post-translational modification that tethers proteins to the cell membrane by a glycolipid anchor. The GPI core consists of a conserved carbohydrate structure linked to the C-terminus of target proteins, although its sugar composition varies across species [6]. These diverse glycosylation mechanisms enable viruses to manipulate host interactions, immune evasion, and infection efficiency, highlighting their significance for viral biology.

Successful viral invasion of host cells relies heavily on viral glycoproteins, many of which are folded, processed, and matured through the host glycosylation machinery [8]. During replication, structural and non-structural proteins undergo extensive N-glycosylation, which can influence receptor binding, membrane fusion, intracellular trafficking, protein secretion, virion assembly, and immune evasion [9]. For example, envelope-protein glycosylation in West Nile virus and Japanese encephalitis virus contributes to membrane fusion and viral infectivity [10], while Zika virus and dengue virus envelope glycans regulate entry, vector competence and antibody recognition [11, 12] (**Table 1**). Host-derived glycan-binding pathways also participate in the viral life cycle by shaping attachment to lectin receptors, infection of immune cells, endothelial barrier dysfunction and complement-mediated immune escape [13]. In this review, we focus on both individual glycosylation sites and the molecular mechanisms by which N-glycosylation mediates arbovirus-host interactions.

The role of N-glycosylation in the Flaviviridae family

The Flaviviridae family is distinguished by enveloped, spherical viruses (approximately 50 nm in diameter), possessing a positive-sense single-stranded RNA genome (9.5-12.5 kb) which has a 5' cap and a 3' hairpin structure

at its ends. The proteins encoded by the open reading frame are classified into three structural proteins and seven non-structural proteins. The structural proteins include capsid protein (C), pre-membrane protein (prM), and envelope protein (E), which mediate viral attachment, membrane fusion, and virion assembly. The non-structural proteins (NS1-NS5) are indispensable for replication and host immune modulation [14]. Comprising over 100 members divided into four genera, the Flavivirus genus encompasses zoonotic pathogens (e.g., Japanese encephalitis, dengue, and Zika viruses) and non-mammalian viruses (e.g., duck Tembusu virus) [15]. Most flaviviruses can infect humans through mosquito or tick bites, leading to a variety of diseases with clinical symptoms ranging from mild febrile illnesses to severe hemorrhagic fever or neurological manifestations [16].

During the replication and infection cycles of the Flaviviridae family, utilizing host cell machinery for the post-translational N-glycosylation of viral proteins represents a highly conserved and critical evolutionary strategy [4, 7, 8]. Although individual flaviviruses display distinct heterogeneity regarding specific amino acid positions, the N-glycosylation of the envelope (E), pre-membrane (prM), and non-structural protein 1 (NS1) consistently adheres to shared, foundational biological mechanisms that govern virus-host interactions [8, 13]. Specifically, glycans attached to the surface-exposed E protein predominantly mediate host cell attachment, receptor binding, membrane fusion, and tissue tropism, serving as key determinants for vector competence and neuroinvasiveness [9, 12, 17]. In contrast, prM protein glycosylation plays a critical role in structural quality control of the heterodimer; its presence is essential for stabilizing prM-E heterodimer folding, preventing premature protein aggregation and ER stress, and maintaining endoplasmic reticulum (ER) homeostasis to coordinate efficient virion assembly and release [10, 11, 22]. The highly conserved glycosylation of the multifunctional NS1 protein not only is essential for its dimeric/hexameric assembly and cellular secretion, but also directly drives viral replication and pathogenesis by interacting with host endothelial layers and immune components to modulate viremia and vascular leakage [13, 21, 25]. Comprehending these shared para-

N-glycosylation in arbovirus-host interactions

Table 1. N-glycosylation sites of viral proteins and their reported functions

Virus	Viral protein	N-glycosylation site	Reported function
ZIKV	Envelope	Asn-154	Replication, assembly, neurotoxicity and invasion
	prM	Asn-69	Virion production
	NS1	Asn-130, Asn-207	Regulation of NS1 internalization and endothelial barrier function
DENV	Envelope	Asn-67, Asn-153	Viral replication, cellular invasion, and survival in mosquitoes
	NS1	Asn-130	Viral replication, assembly, and secretion (essential for viral viability)
		Asn 207	Regulation of NS1 endocytosis and endothelial barrier disruption
JEV	Envelope	Asn-154	Viral replication and neurotoxicity
	prM	Asn-15	Virion release
WNV	Envelope	Asn-154	Particle assembly, mosquito infection, and neuroinvasion in mice
	prM	Asn-15	Assembly and release of virus particles
	NS1	Asn-130, Asn-175, Asn-207	Regulation of NS1 internalization and neuroinvasiveness in mice
DTMUV	Envelope (WT)	Asn-154	Viral proliferation, entry, infection, assembly, and neurotoxicity
	Envelope (MM1775)	Absent (S156P mutation)	Impaired viral attachment, entry, and propagation
	NS1	Asn-130, Asn-175, Asn-207	Viral proliferation, viremia, and virulence
YFV	Envelope	Asn-152, Asn-154	Not reported
	NS1	Asn-130, Asn-207	In vitro viral proliferation and neurotoxicity
TBEV	Envelope	Asn-154	Virion release and neurotoxicity
HCV	E1 and E2		Viral assembly, secretion, entry, and immune response
SFTSV	Gn	Asn-33, Asn-63	Immune response
	Gc	Asn-853, Asn-914, Asn-936	Immune response and membrane fusion
RVFV	Gn	Asn-438	Viral infection and induction of neutralizing antibodies
	Gc	Asn-794, Asn-829, Asn-1035, Asn-1077	Viral infection and induction of neutralizing antibodies

Note: N-linked glycosylation-site positions (e.g., Asn-154) are assigned according to representative reference strains for each virus (e.g., ZIKV MR766, DENV-2 16681, JEV Nakayama, WNV NY99, and DTMUV MM1775). Numbering may vary among isolates because of natural sequence variation or deletions. Where multiple glycosylation sites are listed in a single row (e.g., DENV NS1), the functions reflect the combined roles of those sites, which may vary in essentiality; individual site-specific contributions are discussed in the corresponding text.

digms provides a unified blueprint for dissecting specific flavivirus features and developing broad-spectrum antiviral strategies or targeted vaccine development [5, 8, 32]. The detailed individual glycosylation profiles of major family members are characterized below.

Zika virus (ZIKV) circulates predominantly between wild primates and arboreal mosquito species. Its prM and E proteins contain N-glycosylation sites that contribute to viral infectivity and pathogenesis [17]. The E protein N154 glycan differs among ZIKV lineages;

some early African isolates lack this site, whereas most Asian lineage isolates retain it. This modification can influence viral pathogenesis by modulating virion assembly and *in vitro* infectivity, enhancing viral entry through interactions with cell-surface lectins, and facilitating invasion of the mosquito midgut epithelium, a key determinant of vector competence [18, 19]. However, the biological effect of E N154 is strain- and host-dependent. For example, loss or mutation of this site may alter replication and neurovirulence in neonatal mouse models, indicating that glycosylation affects

ZIKV-host interactions in a context-dependent manner [20, 21].

All ZIKV strains contain an N-linked glycosylation site at Asn-69 in prM. Loss of prM N-glycosylation leads to protein aggregation in the endoplasmic reticulum, triggering ER stress and inducing CHOP nuclear translocation [22]. Both prM and E glycosylation are required for efficient E-protein secretion; ablation of prM glycosylation disrupts processing and causes abnormal accumulation in the endoplasmic reticulum, thereby impairing glycoprotein maturation and productive virion assembly. These findings indicate that prM and E glycans are functionally non-redundant and jointly maintain viral glycoprotein folding fidelity, ER homeostasis, and infectious particle production.

Structural analysis of the prM-E heterodimer indicates that exposed N-glycans can be positioned at sites accessible to host receptors and antibodies. Such surface accessibility supports a dual role in viral entry and immune evasion. For ZIKV NS1, the two commonly used mature-protein N-glycosylation sites are N130 and N207*Note: These positions are based on the mature NS1 protein sequence, corresponding to N924 and N1001 in the viral polyprotein, respectively. Some studies also describe the corresponding positions according to polyprotein numbering, but the mature NS1 numbering is used here for consistency with **Table 1** and common academic usage. Although ZIKV NS1 glycans have been implicated in secretion, endothelial barrier function and immune modulation, their site-specific contributions to host-pathway activation require further mechanistic clarification.

Classified under the Flavivirus genus of the Flaviviridae family, dengue virus encompasses four distinct serotypes, namely DENV-1 through DENV-4. This mosquito-borne pathogen spreads to humans chiefly through bites inflicted by *Aedes aegypti* and *Aedes albopictus*. *Aedes aegypti* serves as the principal urban vector owing to its anthropophilic feeding behavior and extensive distribution in tropical and subtropical regions.

Clinical symptoms appear 3-14 days after infection, including high fever, emesis, headache, myalgia, arthralgia, and a characteristic exan-

thema. The recovery from dengue fever generally takes place 2 to 7 days after infection [23, 24]. The fifth serotype of DENV was identified in 2013 [25, 26]. Presently, the four serotypes DENV-1, 2, 3, and 4 are predominantly prevalent, and there is a cross-immune reaction with ZIKV and JEV [27, 28].

The genome of the dengue virus encodes 3 structural proteins and 7 non-structural proteins as well. Antigenic determinants situated on the E protein can not only induce the host to produce protective neutralizing antibodies and hemagglutination-inhibiting antibodies but may also be involved in dengue shock syndrome and the onset of dengue hemorrhagic fever [29, 30]. Many studies have indicated that the E protein has a significant effect on membrane fusion, host receptor binding, and viral internalization within cells [19].

Dengue virus envelope protein harbors two potential N-linked glycosylation sites at asparagine residues 67 and 153. The Asn153 site is highly conserved among most flaviviruses, whereas Asn67 exhibits obvious specificity. Loss of the Asn67 glycosylation site restrains viral replication and blocks the formation of infectious virions, making this site indispensable for dengue virus transmission [31]. Glycosylation at Asn67 markedly facilitates viral entry into host cells.

Glycosylation modification at Asn153 contributes to viral survival in mammalian and mosquito cells. During viral infection, N-glycosylation of envelope protein and NS1 protein exerts vital effects on virus adsorption, internalization, secretion, assembly and maturation [32, 33]. Such modifications can shield neutralizing epitopes on viral proteins, participate in infection progression, and modulate viral pathogenicity. Two highly conserved N-linked glycosylation sites, N130 and N207, are identified on the NS1 protein.

N207 glycosylation site

Research indicates that NS1 damages the endothelial glycocalyx layer and triggers vascular leakage. Furthermore, NS1-induced endothelial hyperpermeability has been associated with cellular internalization processes and may be influenced by N-linked glycosylation [34]. Mutation at the N207 glycosylation site of

NS1 impairs its cellular internalization and endosomal trafficking, thereby attenuating its ability to disrupt the endothelial glycocalyx and compromise barrier integrity. This role differs from that reported for WNV and ZIKV NS1, in which glycosylation at the corresponding site contributes to NS1-mediated pathogenic effects through mechanisms that appear distinct from those in DENV. Notably, mutation at Asn-207 has only limited effects on viral replication.

N130 glycosylation site

In contrast, the N130 glycosylation site is essential for viral replication and particle production, since its mutation in DENV-1 and DENV-2 abolishes the generation of infectious virions in both mosquito and mammalian cells (Somnuk et al., 2019). Similarly, the N130 mutation of DENV-4 suppresses viral replication in C6/36 cells and mammalian cells.

Furthermore, NS1 is a critical diagnostic marker. It is found circulating in the blood of patients experiencing primary or secondary DENV infections [35].

Through reverse genetics approaches, researchers can generate N-glycosylation site deletion mutants of the NS1 protein. These engineered variants enable the selection of genetically stable dengue virus strains with attenuated virulence. This molecular strategy provides a critical basis for developing both: (1) attenuated dengue vaccine vectors and (2) chimeric dengue vaccine candidates, offering promising directions for vaccine development. Nevertheless, the function of prM glycosylation has not been extensively investigated.

The prM protein plays a key role in the proper folding, membrane localization, and final assembly of Japanese encephalitis virus E protein [36]. The biological function of prM was investigated in 1995, and it was found to be an important synergistic component of the virus in inducing protective immunity in the organism. The E protein serves as the primary structural protein of the Japanese encephalitis virus. It is generally accepted that the E protein promotes the fusion of the virus with the cell membrane and the entry of the virus. The virulence of the virus largely depends on this protein. Substituting some of its key amino acids may

lead to the loss of neurovirulence and invasiveness. In the Japanese encephalitis virus, the prM protein possesses an N-linked glycosylation site at Asn-15, while the E protein harbors an N-linked glycosylation site at Asn-154. [37, 38]. In animal experimental models, JEV has an N-glycosylation site N15-X16-T17 on prM. After removing this site, when JEV reinfects mice, the virus virulence was found to decline significantly. Through mutation of this site, prM mutants (N15A, T17A, and N15A/T17A) are obtained. The proliferation of these mutants is specifically restricted by cell type. In a JEV DNA vaccine study, immunization of mice with a prME protein carrying the N154A mutation increased IL-4 secretion, induced IgG1 isotype antibodies, yielded higher anti-JEV titers, and provided comprehensive protection against virus challenge. Moreover, the N-linked glycosylation pattern of the E protein might exert an influence on the interaction between the virus and cell lectins, and play a role in virus replication and pathogenesis. In comparison to the wild-type Japanese encephalitis virus (which possesses a native N-glycan at Asn-154), recombinant variants lacking this site (non-glycosylated), or those with an artificially introduced glycan at Asn-67 show sub-optimal growth in mice. Also, their neurovirulence and neuroinvasiveness are reduced. Compared to the wild-type strains, viruses with glycosylation at both N67 and N154 sites show replicability and neurovirulence in cell culture, but they exhibit diminished neuroinvasiveness during *in vivo* experiments. During the evolutionary process, the neurotropic flavivirus E protein, which has only one glycosylation site at N153, may confer a selective advantage in traversing the blood-brain barrier. The aforementioned study also confirms the significant role of polysaccharides on the E protein of flaviviruses during virus infection and their influence on virus tropism.

The West Nile virus (WNV), a member of the Flavivirus genus within the Flaviviridae family, was first isolated in Uganda in 1937 [39, 40]. Culex mosquitoes serve as its primary transmission vectors, while birds are susceptible to this virus and act as amplifiers. Following WNV infection, the majority of animals and humans do not display clinical symptoms. A small proportion of mild cases present with fever and headache, and severe cases manifest as encephalitis and may result in death.

The entire genome of WNV encodes 10 proteins, including three structural proteins and seven non-structural proteins. The prM protein has an N-linked glycosylation site; however, not all strains contain an N-linked site on the E protein. The N-linked glycosylation motif of the West Nile virus (WNV) envelope protein is situated between amino acid positions 154 and 156, with the glycosylation site being Asn-154 [41, 42]. WNV strains undergoing glycosylation on their E protein exhibit enhanced infectivity in both mammalian and mosquito hosts. Conversely, E protein-unglycosylated WNV variants demonstrate significantly impaired ability to replicate and spread effectively in mosquito cells [43]. This underscores the critical role of E protein glycosylation in the viral lifecycle within the arthropod vector [44]. Glycosylation of the E protein promotes the assembly of WNV and significantly enhances its infectivity.

Mice infected solely with West Nile virus harboring glycosylated E protein demonstrate neuroinvasiveness, suggesting that the glycosylation of the E protein serves as a molecular determinant of the virus's neuroinvasiveness [45]. Additionally, The N-linked glycosylation site within the WNV prM protein exerts a crucial influence on the regulation of viral assembly and release. However, the elimination of glycosylation on either the E or prM protein will lead to a decrease in the release of subviral particles [46].

The NS1 protein of WNV harbors three N-linked glycosylation sites at asparagine residues N130, N175, and N207. Prior studies have demonstrated that the elimination of these glycosylation sites in WNV, accomplished by substituting asparagine with alanine, leads to an attenuation of mouse neuroinvasiveness [47]. Nevertheless, complete attenuation is not attained, and the virus still manifests a neurovirulence phenotype. After the removal of all N-glycosylation sites in the NS1 protein of WNV, the attenuated virus completely loses its neurovirulence to mice while retaining beneficial immunogenicity.

Duck Tembusu virus (DTMUV) belongs to the genus *Flavivirus* and is an emerging avian pathogen [48]. Since its detection in duck farms in southeastern China in 2010, DTMUV has spread widely across several provinces [49]. Its natural hosts include *Culex* mosqui-

toes, avian species and waterfowl, and *Culex* mosquitoes are considered an important component of the DTMUV transmission cycle [50, 51]. DTMUV encodes three structural proteins and seven non-structural proteins. The E protein is the major surface glycoprotein involved in viral attachment, entry, assembly and dissemination [52, 53]. Most DTMUV isolates contain the E N154 glycosylation site; however, the prototype strain MM1775 lacks this glycan because the S156P substitution disrupts the N-linked glycosylation motif. Reverse-genetics studies have shown that disruption of E N154 glycosylation attenuates viral attachment, invasion, and infectivity in duck embryo fibroblast (DEF) cells and C6/36 mosquito cells, and reduces viral assembly and release [54-56]. Mutation of this site also decreases tissue viral loads and alleviates brain injury and neuroinflammation *in vivo* [57]. Thus, DTMUV E glycosylation appears to connect viral propagation in avian and mosquito cells with tissue tropism and virulence. DTMUV NS1 contains the same three mature-protein N-glycosylation sites as WNV NS1, namely N130, N175 and N207, and these glycans contribute to viral propagation, viremia and virulence [58, 59]. Nevertheless, the host determinants and signaling pathways linking DTMUV E/NS1 glycosylation to avian pathogenicity and neurovirulence remain incompletely defined.

Yellow fever virus (YFV) serves as the etiological agent of yellow fever, which stands as the first documented viral hemorrhagic fever in history [60]. In the seventeenth century, it spread from West Africa to the Americas and is transmitted through the bites of *Aedes* mosquitoes. Its clinical manifestations include high fever, headache, jaundice, and proteinuria [61, 62]. Analogous to other members of the *Flaviviridae* family, the yellow fever virus (YFV) encompasses 10 protein-encoding genes. The NS1 and E proteins of YFV feature glycosylation sites. Deletion of the N130 site or both N130 and N207 sites affects *in vitro* proliferation and substantially reduces neurovirulence in mice [63]. Moreover, the elimination of the initial glycosylation site in the NS1 of YFV also leads to a reduction in virus titer.

Based on studies of the E protein of the YFV 17DD strain and the 17D-213 strain, it was discovered that the primary disparity between

these two viral strains resides in their potential N-linked glycosylation sites [64].

Tick-borne encephalitis, a severe viral disorder attacking the human central nervous system, is caused by tick-borne encephalitis virus belonging to the *Flavivirus* genus of the *Flaviviridae* family. This virus consists of three structural and seven non-structural proteins. Its envelope protein carries only one N-glycosylation site at Asn-154. Glycosylation of envelope protein enhances viral infectivity and plays essential roles in virion assembly, virulence, and pathogenicity [65-69]. Deglycosylation of the envelope protein does not significantly affect the *in vitro* infectivity of TBEV; however, it leads to a marked reduction of *in vivo* virulence in mouse models, suggesting that the N-glycan at Asn-154 is critical for viral pathogenicity rather than for basic replication [70]. In-depth investigations have shown that in mutants with glycosylation at positions 66 and 154 of the E protein, the secretion level of the E protein is fourfold higher than that of the wild-type [71]. The glycosylation site Asn-154 of the TBEV E protein can promote the release of virus-like particles by TBEV in mammalian cells and plays a significant part in the process of inducing neurotoxicity [72]. According to the prediction by the NetNGlyc 1.0 server, there is another N-linked glycosylation site, N641, in TBEV; however, the residue following asparagine is proline. It is well-established that proline can hinder the glycosylation of the preceding residue. The specific function of this site has not been further clarified. Research on the glycosylation site of the TBEV may contribute to the understanding of the biological characteristics of TBEV in mammals and ticks. It may also contribute to the development of highly efficacious antiviral drugs against TBEV.

Although Hepatitis C Virus (HCV) belongs to the family *Flaviviridae*, it is a classic non-arthropod-borne virus that is primarily transmitted through blood exposure and contaminated medical products, distinguishing it from typical arboviruses [73-75]. Nevertheless, as a prominent member of the same viral family, the N-glycosylation profile of HCV envelope glycoproteins serves as an invaluable comparative model to understand the evolutionary and functional modification strategies within the *Flaviviridae* family. The HCV envelope glycoproteins, E1 and E2, are heavily glycosylated, typi-

cally possessing 4 and 11 N-linked glycans, respectively [76]. Mutations at specific glycosylation sites, including E1N1, E2N3, E2N7, E2N8, E2N10, and E2N11, are deeply detrimental to viral particle assembly, secretion, and cell entry [76]. Specifically, modification at the E2N7 site is critical for maintaining infectivity, while glycans at E2N2 and E2N4 are essential for the internalization mediated by envelope proteins [77]. Furthermore, glycans at positions E2N1, E2N2, E2N4, E2N6, and E2N11 function to shield key epitopes from neutralizing antibodies and modulate the binding affinity between E2 and the host receptor CD81, revealing a sophisticated mechanism of immune evasion [77]. Therefore, while HCV lacks an arboviral transmission route, its structural glycosylation studies offer essential reference value for elucidating conserved virus-host interactions across related pathogens. Alkhurma hemorrhagic fever virus (AHFV) is also classified within the genus *Flavivirus* of the family *Flaviviridae* [78]. AHFV is a relatively new virus, which was discovered merely 28 years ago. Initially, this virus was isolated from a butcher in Jeddah, Saudi Arabia [79]. Phylogenetic analyses based on the NS5 sequence have demonstrated that the virus clusters within the tick-borne encephalitis virus serocomplex. It shows a high level of similarity to another tick-borne virus, the Kyasanur forest disease virus. However, the latter was first identified in India in the late 1950s [80]. Analogous to other flaviviruses, the AHFV and KFDV viruses are cleaved by host proteases into three structural proteins and eight non-structural proteins [81]. In AHFV, two N-linked glycosylation sites (amino acids 145 and 862) are present. In Kyasanur Forest disease virus (KFDV), only one N-linked glycosylation site (located at the 145th amino acid position) has been recognized, which is located at the same position as that in Alkhurma hemorrhagic fever virus (AHFV) [82]. However, the functions of the glycosylation sites in AHFV and KFDV remain unreported. Additionally, certain other viruses, such as SLEV and MVEV, possess a third glycosylation site at amino acid position 175, in addition to the N103 and N207 sites on the NS1 protein [83].

The role of 3N-glycosylation in the *Bunyaviridae* family

The *Bunyaviridae* family is the most extensive virus family, comprising five genera and over 30

serogroups [84]. Among these, four are classified as animal virus genera, specifically Bunyavirus, Nairovirus, Phlebovirus, and Hantavirus [85]. One genus pertains to plant viruses, with a total of approximately 350 viruses [86]. Among the four animal virus genera, except that the primary reservoir of the Hantavirus genus is *Apodemus agrarius*, nearly all members of the Bunyaviridae family are arboviruses, mainly presenting three transmission modes: mosquito-borne, tick-borne, and sandfly-borne. Some viruses can be transmitted through eggs, mating, or during the embryonic stage in their arthropod vectors [87]. The majority of viruses in this family exhibit a spherical morphology, having a diameter in the range of 80 to 120 nm and an envelope. This virus has a genome comprising three negative-sense RNA segments (L, M and S) with unidirectional or bidirectional transcription, whose lengths vary among different genera. The L segment encodes the L protein, the M segment produces two glycoproteins and one non-structural protein, and the S segment expresses the nucleocapsid protein plus another non-structural protein [88].

The severe fever with thrombocytopenia syndrome virus (SFTSV), belonging to the Bunyavirus genus, is capable of being transmitted by *Haemaphysalis longicornis* [89]. SFTSV genome comprises L, M and S three segments [90]. Following cleavage and modification by proteases within host cells, it generates two envelope glycoproteins, namely Glycoprotein C (Gc) and Glycoprotein N (Gn). These two glycoproteins exert a significant influence on the formation of virus particles, virus assembly, and attachment to target cells. Certain research also indicates that the two glycoproteins Gn and Gc may play a crucial role in mediating phagocytosis and the invasion of SFTSV into the nervous system. In ferret SFTSV DNA vaccine experiments, these glycoproteins primarily induce SFTSV-specific neutralizing antibodies [91]. The subdomain of Gn encompasses two N-linked glycosylation sites, namely Asn-33 and Asn-63. The extracellular domain of Gc comprises three N-linked glycosylation sequences, specifically Asn-853, Asn-914, and Asn-936 [92].

Rift Valley fever (RVF), a mosquito-borne zoonotic viral disease, is widely prevalent across

Africa. It has the capacity to lead to increased abortion rates, teratogenic rates, and neonatal mortality in cattle, goats, and sheep [93]. Humans are infected with this disease through exposure to the bodily fluids of infected animals or bites from mosquitoes harboring the virus [94]. Rift Valley fever virus (RVFV) is a tripartite negative-sense RNA virus belonging to Phlebovirus of Bunyaviridae [95]. In addition to causing hemorrhagic fever and hepatitis, RVFV is also recognized for its potential to cause severe neurological complications in both humans and animals [96]. The viral genome includes L, M and S segments. The M segment encodes NSm, Gn and Gc proteins. Gn has one N-glycosylation site at N438, and Gc contains four such sites at N794, N829, N1035 and N1077. Mutagenesis replacing asparagine with glutamine revealed that N438Q and N1035Q mutants exhibited impaired infectivity. In contrast, the dual N438Q/N1035Q mutation had nearly no effect on viral infection in these cells. The experiment further demonstrated the presence of N-glycosylation at the N794, N1035, and N1077 sites of Gc, while the N829 site did not show such glycosylation. Notably, Gc N1077 displayed heterogeneous N-glycosylation patterns. Glycosylation modifications at the N438 site of the RVFV Gn protein or the N1077 site of the Gc protein are crucial for DC-SIGN-mediated viral infection. Additionally, the RVFV Gn and Gc glycoproteins play a significant part in inducing neutralizing antibodies and vaccine development [97, 98].

The role of 4N-glycosylation in Togaviridae

The Togaviridae family is primarily classified into the Alphavirus genus and the Rubivirus genus based on serological reactions [99]. The Alphavirus genus encompasses numerous members, and the transmission and spread links are more intricate. Nearly all alphaviruses are transmitted by mosquitoes and can infect arthropods and vertebrates, causing diseases in humans and livestock [100]. Except for the Getah virus reported in Taiwan, Province of China, currently, only some arboviruses of relatively less significance in veterinary medicine in China remain in this family [101]. The virus particles of this family are spherical, with a diameter spanning from 40 to 70 nm, and possess an envelope and fine membrane particles. The membrane particles consist of 80 trimers,

Table 2. Functional divergence of N-glycosylation across major arbovirus families

Virus family	Representative viruses	Dominant glycosylated proteins	Major functional implications
Flaviviridae	ZIKV, DENV, JEV, WNV, DTMUV, YFV	E, prM and NS1	Protein folding, virion maturation, receptor/lectin-mediated entry, vector competence, endothelial dysfunction, immune evasion and neurovirulence
Bunyaviridae	SFTSV and RVFV	Gn and Gc	Host entry, tissue tropism, membrane fusion, immune recognition and induction of neutralizing antibodies
Togaviridae	GETV, RRV, CHIKV, VEEV, MAYV and SINV	E1 and E2	Glycoprotein-capsid interaction, virion assembly, structural stability, pH-dependent fusion and pathogenicity

and each trimer is composed of two glycoproteins, E1 and E2 [102, 103].

Getah virus (GETV), a poorly studied mosquito-borne alphavirus in the Togaviridae family, encodes four non-structural proteins (nsP1-nsP4) and five structural proteins (CP, E3, E2, 6K, E1) [104, 105]. Structural proteins mainly mediate viral adsorption, assembly and budding, while non-structural proteins participate in viral RNA replication. Envelope glycoproteins E1 and E2 form viral spikes to bind cellular receptors and induce membrane fusion [106, 107]. The NetNGlyc 1.0 server forecasts that the E1 protein possesses one N-glycosylation site (residues 141-143, NQT), and the E2 protein has two N-glycosylation sites (200 NCT 202 and 262 NST 264). This is consistent with the presence of two N-glycosylation sites on the E2 protein (N200 and N262) [108]. The glycosylation modification of GETV glycoproteins E1 and E2 promotes the replication and assembly of the virus within cells. Once removed, the replication capacity and assembly efficiency of the virus will decline, and the virus titer will decrease by 10 to 100 times [109]. The glycosylation modification at the E1 N141 and E2 N200 sites facilitates the assembly of glycoproteins and capsids during the virus assembly process. After removal, the expression of structural protein E2 is reduced, resulting in a decrease in the interaction opportunities between the basically normally expressed CP protein and the E2 protein. Eventually, fewer viruses are assembled, which indirectly leads to a large accumulation of CP protein in the cytoplasm [110].

Furthermore, N-glycosylation at comparable sites has been reported in several other alphaviruses, such as RRV, CHIKV, VEEV, MAYV, and SINV [111]. The glycosylation at E1 N141 of

RRV and SINV has been demonstrated to be associated with virulence [112]. The glycan attached to E2 N200 may stabilize the fusion loop of E1 through the formation of hydrogen bonds and may participate in the pH-dependent conformational changes and membrane fusion of E1. E2 N262 shows a high level of conservation among multiple arthritogenic alphaviruses, and this site may be related to the pathogenicity of alphaviruses [113]. Among all alphaviruses, the E1 protein exhibits relative conservation, whereas arthritogenic and encephalitic alphaviruses display notable disparities in their E2 proteins. This implies that the E1 protein may serve as a target for the development of a universal subunit vaccine against all alphaviruses.

Table 2 summarizes the functional divergence of N-glycosylation across major arbovirus families. In flaviviruses, N-glycosylation is closely associated with protein folding, virion maturation and immune shielding; in bunyaviruses, it is more prominently linked to host entry and tissue tropism; and in togaviruses, it contributes to virion assembly, structural stability, and pathogenicity.

Molecular mechanisms of host-virus interplay mediated by N-glycosylation

Beyond their structural role in maintaining the integrity of viral glycoproteins, N-glycans serve as a critical molecular interface for host-virus interplay. The interaction between viral glycans and host factors orchestrates the viral life cycle through several refined mechanisms.

Arboviruses exploit host C-type lectin receptors (CLRs), such as DC-SIGN and L-SIGN, to enhance entry into dendritic cells and macrophages. The mannose-rich N-glycans on viral envelope proteins act as high-affinity ligands

for these receptors, effectively stabilizing viral attachment to the cell surface and facilitating receptor-mediated endocytosis [114]. This glycan-lectin interaction not only boosts infection efficiency but also determines the virus's specific tissue tropism, enabling the pathogen to selectively target immune cells that facilitate systemic dissemination.

Furthermore, N-glycans function as a “glycan shield” that physically masks conserved immunogenic epitopes from neutralizing antibody recognition. During acute or chronic viremia, the dense glycan coverage on envelope proteins limits the binding accessibility of host antibodies [115]. Simultaneously, certain viral N-glycans interact with host mannose-binding lectin (MBL) to modulate the activation of the complement system [116]. By hijacking these host regulatory pathways, arboviruses delay or suppress the early host innate immune response, providing a window for rapid replication.

The processing of viral N-glycans within the host endoplasmic reticulum (ER) also influences the host's unfolded protein response (UPR). The improper folding or premature processing of viral glycoproteins can trigger cellular stress responses, which subsequently modulate host inflammatory signaling pathways, such as the NF- κ B or MAPK pathways [117]. This crosstalk suggests that N-glycosylation is not an isolated post-translational modification but a dynamic trigger that signals the host cell to alter its internal environment, including lipid metabolism or redox balance, to favor viral assembly.

Finally, the variation in glycosylation sites observed among different strains, such as the loss of Asn-154 in the DTMUV MM1775 strain [118], highlights a rapid evolutionary adaptation. As host immune pressure increases, the virus may evolve to shed or gain specific glycan sites to recalibrate the balance between infectivity and immune evasion. This dynamic evolutionary process underscores the high-level dependence of arboviruses on host glycosylation machinery to maintain their ecological fitness across diverse host species.

Summary and outlook

Glycobiology is an essential area of cell biology and virology. N-linked glycosylation of viral pro-

teins contributes to viral survival, infectivity, tissue tropism, virulence, and immune evasion, and has therefore become an important focus in arbovirus research [119]. Enveloped viruses exploit the host glycosylation machinery to modify their own glycoproteins, thereby improving protein folding, facilitating viral entry, mediating membrane fusion, and modulating sensitivity to neutralizing antibodies. Because surface glycoproteins are also major antigens, their glycosylation patterns are highly relevant to vaccine design, antiviral development and interpretation of host-range adaptation.

Across flaviviruses, E-protein glycans mainly influence attachment, entry, fusion, vector competence and antibody recognition, whereas NS1 glycans regulate secretion, endothelial barrier dysfunction, complement interaction, inflammation and immune escape. These shared mechanisms explain why similar glycosylation motifs can have different biological consequences in different virus-vector-host systems. Future studies should move beyond cataloguing glycosylation sites and should define how individual glycans interact with specific host receptors, lectins, immune mediators and cellular trafficking pathways. Particular priorities include resolving strain-specific glycosylation differences, standardizing site numbering, clarifying the role of NS1 glycans in vascular and immune pathology, and evaluating whether glycosylation-targeted strategies can be safely used for vaccine attenuation or antiviral intervention.

Despite extensive research into the functional paradigms of viral glycosylation across families like Flaviviridae, Bunyaviridae, and Togaviridae, several critical knowledge gaps persist that warrant systematic investigation:

Structural and Non-Structural Glycoprotein Gaps: While the chaperone functions of prM glycosylation are recognized in major model pathogens, its specific role in viral assembly across multiple serotypes requires deeper biochemical exploration. Similarly, the potential N-glycosylation sites on the non-structural protein NS1—such as the N924 and N1001 sites in ZIKV, and the triple-site configurations in emerging flaviviruses—have established correlations with *in vivo* virulence and replication, yet the detailed downstream molecular mecha-

Table 3. Knowledge gaps concerning N-glycosylation in emerging and neglected arboviruses

Virus	Predicted or reported N-glycosylation sites	Current evidence	Key unresolved question
SFTSV	Gn: Asn-33 and Asn-63; Gc: Asn-853, Asn-914 and Asn-936	Mainly sequence prediction and limited functional inference	Whether these glycans directly regulate glycoprotein folding, particle assembly, cell entry and immune escape
AHFV	Putative sites including Asn-145 and Asn-862	Predominantly bioinformatic prediction	Whether the predicted sites influence replication fitness, virulence and host adaptation
KFDV	Putative site around Asn-145	Predominantly bioinformatic prediction	Whether this site has conserved or divergent functions compared with related tick-borne flaviviruses

Table 4. Potential applications of glycosylation engineering in antiviral and vaccine development

Strategy	Potential target	Glycosylation-based approach	Research or clinical relevance
Attenuated-vaccine design	DTMUV E protein	Removal or alteration of E-protein N-glycosylation	May reduce tissue viral load, neuroinflammation and virulence while preserving immunogenicity
Attenuated-vaccine design	WNV NS1 protein	Deglycosylation of NS1 sites	May reduce neuroinvasiveness and support rational vaccine-vector development
Broad-spectrum antiviral development	Flavivirus and bunyavirus glycoprotein maturation	Interference with host glycosylation machinery or glycan-dependent viral entry	May provide a host-oriented antiviral strategy, but safety and specificity require careful evaluation

nisms governing these interactions remain to be fully resolved.

Pathogenicity and Host Tropism Determinants: Although the fundamental roles of E and NS1 glycosylation in certain waterfowl pathogens like DTMUV have been described [56, 57, 59], the precise structural configurations and downstream molecular mechanisms through which specific glycan motifs drive pathogen-host interactions remain incompletely understood. Specifically, how these specific envelope glycan motifs cross highly selective anatomical barriers, such as the blood-brain barrier to induce neuroinvasiveness or neuroinflammation, remains ambiguous. This critical gap is particularly evident in specialized neurotropic variants of YFV and emerging avian flaviviruses. Resolving these mechanistic discrepancies requires further comparative structural and functional alignment with established viral models.

Emerging and Neglected Pathogens: For several newly isolated or geographically restricted arboviruses, including AHFV, KFDV, and highly pathogenic bunyaviruses like SFTSV, current understanding is limited to server-based sequence predictions. The physical presence, structural configurations, and functional contri-

butions of these predicted glycosylation sites (such as the Gn and Gc sites of SFTSV) to replication, virion assembly, and cellular invasion remain completely uncharacterized.

Future efforts must prioritize the deployment of reverse genetics systems, high-resolution cryo-EM glycoproteomics, and advanced *in vivo* imaging models to decipher these specific glycan-host interactions. Resolving these questions will not only enhance our understanding of arboviral evolutionary biology but will also accelerate the development of highly efficacious, next-generation antiviral therapies and attenuated vaccine candidates.

For several emerging or neglected arboviruses, available evidence remains insufficient to define the biological functions of predicted N-glycosylation sites. **Table 3** summarizes the main knowledge gaps for SFTSV, AHFV, and KFDV, highlighting the need for reverse-genetics systems, structural analyses, and infection models to clarify their roles in replication, virulence, particle assembly and host adaptation.

The translational relevance of viral N-glycosylation is further summarized in **Table 4**. Targeted manipulation of N-glycosylation sites may contribute to attenuated-vaccine design,

while interference with host glycosylation machinery or glycan-dependent viral processes may provide a strategy for broad-spectrum antiviral intervention.

Disclosure of conflict of interest

None.

Address correspondence to: Pu Wang, Zhejiang International Science and Technology Cooperation Base for Veterinary Medicine and Health Management, College of Veterinary Medicine of Zhejiang A&F University, Hangzhou, Zhejiang, China. Tel: +86-571-82629627; E-mail: wangpu110@zafu.edu.cn; Baoming Wang, Hangzhou Xiaoshan Donghai Aquaculture Co., Ltd., Hangzhou 311200, Zhejiang, China. Tel: +86-571-82629627; E-mail: wangbaoming0114@live.com; Lili Zhao, Zhejiang Jindakang Animal Health Co., Ltd., Jinhua 321000, Zhejiang, China. Tel: +86-571-82629627; E-mail: wuyefeng-8022@126.com

References

- [1] Alves RPDS and Amorim JH. Editorial: arboviruses: co-circulation, co-transmission, and co-infection. *Front Microbiol* 2023; 14: 1321166.
- [2] Madewell ZJ. Arboviruses and their vectors. *South Med J* 2020; 113: 520-523.
- [3] Jones RT, Ant TH, Cameron MM and Logan JG. Novel control strategies for mosquito-borne diseases. *Philos Trans R Soc Lond B Biol Sci* 2021; 376: 20190802.
- [4] Bagdonaite I and Wandall HH. Global aspects of viral glycosylation. *Glycobiology* 2018; 28: 443-467.
- [5] Evans DeWald L, Starr C, Butters T, Treston A and Warfield KL. Iminosugars: a host-targeted approach to combat Flaviviridae infections. *Antiviral Res* 2020; 184: 104881.
- [6] Chatham JC and Patel RP. Protein glycosylation in cardiovascular health and disease. *Nat Rev Cardiol* 2024; 21: 525-544.
- [7] Minakata S, Manabe S, Inai Y, Ikezaki M, Nishitsuji K, Ito Y and Ihara Y. Protein C-Mannosylation and C-Mannosyl tryptophan in chemical biology and medicine. *Molecules* 2021; 26: 5258.
- [8] Cheng N, Liu M, Li W, Sun B, Liu D, Wang G, Shi J and Li L. Protein post-translational modification in SARS-CoV-2 and host interaction. *Front Immunol* 2023; 13: 1068449.
- [9] Feng T, Zhang J, Chen Z, Pan W, Chen Z, Yan Y and Dai J. Glycosylation of viral proteins: implication in virus-host interaction and virulence. *Virulence* 2022; 13: 670-683.
- [10] Kim JM, Yun SI, Song BH, Hahn YS, Lee CH, Oh HW and Lee YM. A single N-linked glycosylation site in the Japanese encephalitis virus prM protein is critical for cell type-specific prM protein biogenesis, virus particle release, and pathogenicity in mice. *J Virol* 2008; 82: 7846-7862.
- [11] Gwon YD, Zusinaite E, Merits A, Överby AK and Evander M. N-glycosylation in the pre-membrane protein is essential for the Zika virus life cycle. *Viruses* 2020; 12: 925.
- [12] Mondotte JA, Lozach PY, Amara A and Gamarnik AV. Essential role of dengue virus envelope protein N glycosylation at asparagine-67 during viral propagation. *J Virol* 2007; 81: 7136-7148.
- [13] Vigerust DJ and Shepherd VL. Virus glycosylation: role in virulence and immune interactions. *Trends Microbiol* 2007; 15: 211-218.
- [14] Carbaugh DL, Baric RS and Lazear HM. Envelope protein glycosylation mediates Zika virus pathogenesis. *J Virol* 2019; 93: e00113-19.
- [15] Simmonds P, Becher P, Bukh J, Gould EA, Meyers G, Monath T, Muerhoff S, Pletnev A, Rico-Hesse R, Smith DB and Stapleton JT; ICTV Report Consortium. ICTV virus taxonomy profile: flaviviridae. *J Gen Virol* 2017; 98: 2-3.
- [16] Guo Y, Bao L, Xu Y, Li F, Lv Q, Fan F and Qin C. The ablation of envelope protein glycosylation enhances the neurovirulence of ZIKV and cell apoptosis in newborn mice. *J Immunol Res* 2021; 2021: 5317662.
- [17] Wen D, Li S, Dong F, Zhang Y, Lin Y, Wang J, Zou Z and Zheng A. N-glycosylation of viral E protein is the determinant for vector midgut invasion by flaviviruses. *mBio* 2018; 9: e00046-18.
- [18] Moudy RM, Zhang B, Shi PY and Kramer LD. West Nile virus envelope protein glycosylation is required for efficient viral transmission by Culex vectors. *Virology* 2009; 387: 222-228.
- [19] Fontes-Garfias CR, Shan C, Luo H, Muruato AE, Medeiros DBA, Mays E, Xie X, Zou J, Roundy CM, Wakamiya M, Rossi SL, Wang T, Weaver SC and Shi PY. Functional analysis of glycosylation of Zika virus envelope protein. *Cell Rep* 2017; 21: 1180-1190.
- [20] Crabtree MB, Kinney RM and Miller BR. Deglycosylation of the NS1 protein of dengue 2 virus, strain 16681: construction and characterization of mutant viruses. *Arch Virol* 2005; 150: 771-786.
- [21] Fan J, Liu Y and Yuan Z. Critical role of Dengue Virus NS1 protein in viral replication. *Virol Sin* 2014; 29: 162-169.
- [22] Gwon YD, Zusinaite E, Merits A, Överby AK and Evander M. N-glycosylation in the pre-membrane protein is essential for the Zika virus life cycle. *Viruses* 2020; 12: 925.
- [23] Pryor MJ, Gualano RC, Lin B, Davidson AD and Wright PJ. Growth restriction of dengue virus type 2 by site-specific mutagenesis of vi-

- rus-encoded glycoproteins. *J Gen Virol* 1998; 79: 2631-2639.
- [24] Tajima S, Takasaki T and Kurane I. Characterization of Asn130-to-Ala mutant of dengue type 1 virus NS1 protein. *Virus Genes* 2008; 36: 323-329.
- [25] Wang C, Puerta-Guardo H, Biering SB, Glasner DR, Tran EB, Patana M, Gomberg TA, Malvar C, Lo NTN, Espinosa DA and Harris E. Endocytosis of flavivirus NS1 is required for NS1-mediated endothelial hyperpermeability and is abolished by a single N-glycosylation site mutation. *PLoS Pathog* 2019; 15: e1007938.
- [26] Zhang Y, Chen P, Cao R and Gu J. Mutation of putative N-linked glycosylation sites in Japanese encephalitis virus premembrane and envelope proteins enhances humoral immunity in BALB/c mice after DNA vaccination. *Virol J* 2011; 8: 138.
- [27] Wang P, Hu K, Luo S, Zhang M, Deng X, Li C, Jin W, Hu B, He S, Li M, Du T, Xiao G, Zhang B, Liu Y and Hu Q. DC-SIGN as an attachment factor mediates Japanese encephalitis virus infection of human dendritic cells via interaction with a single high-mannose residue of viral E glycoprotein. *Virology* 2016; 488: 108-119.
- [28] Martina BE, Koraka P, van den Doel P, Rimmelzwaan GF, Haagmans BL and Osterhaus AD. DC-SIGN enhances infection of cells with glycosylated West Nile virus in vitro and virus replication in human dendritic cells induces production of IFN-alpha and TNF-alpha. *Virus Res* 2008; 135: 64-71.
- [29] Murata R, Eshita Y, Maeda A, Maeda J, Akita S, Tanaka T, Yoshii K, Kariwa H, Umemura T and Takashima I. Glycosylation of the West Nile Virus envelope protein increases in vivo and in vitro viral multiplication in birds. *Am J Trop Med Hyg* 2010; 82: 696-704.
- [30] Khetarpal N and Khanna I. Dengue fever: causes, complications, and vaccine strategies. *J Immunol Res* 2016; 2016: 6803098.
- [31] Shirato K, Miyoshi H, Goto A, Ako Y, Ueki T, Kariwa H and Takashima I. Viral envelope protein glycosylation is a molecular determinant of the neuroinvasiveness of the New York strain of West Nile virus. *J Gen Virol* 2004; 85: 3637-3645.
- [32] Whiteman MC, Wicker JA, Kinney RM, Huang CY, Solomon T and Barrett AD. Multiple amino acid changes at the first glycosylation motif in NS1 protein of West Nile virus are necessary for complete attenuation for mouse neuroinvasiveness. *Vaccine* 2011; 29: 9702-9710.
- [33] Peng C, Zhang Y, Chen L, Li Z, Lv P, Wang P, Li N and Wang F. *Bacillus subtilis* expressing duck Tembusu virus E protein induces immune protection in ducklings. *Microb Pathog* 2023; 185: 106419.
- [34] Puerta-Guardo H, Glasner DR and Harris E. Dengue virus NS1 disrupts the endothelial glycocalyx, leading to hyperpermeability. *PLoS Pathog* 2016; 12: e1005738.
- [35] Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V and Flamand M. Enzyme-linked immunosorbent assay specific to Dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. *J Clin Microbiol* 2002; 40: 376-381.
- [36] Lee E and Lobigs M. E protein domain III determinants of yellow fever virus 17D vaccine strain enhance binding to glycosaminoglycans, impede virus spread, and attenuate virulence. *J Virol* 2008; 82: 6024-6033.
- [37] Liang JJ, Chou MW and Lin YL. DC-SIGN binding contributed by an extra N-linked glycosylation on Japanese encephalitis virus envelope protein reduces the ability of viral brain invasion. *Front Cell Infect Microbiol* 2018; 8: 239.
- [38] Zai J, Mei L, Wang C, Cao S, Fu ZF, Chen H and Song Y. N-glycosylation of the premembrane protein of Japanese encephalitis virus is critical for folding of the envelope protein and assembly of virus-like particles. *Acta Virol* 2013; 57: 27-33.
- [39] Goffard A, Callens N, Bartosch B, Wychowski C, Cosset FL, Montpellier C and Dubuisson J. Role of N-linked glycans in the functions of hepatitis C virus envelope glycoproteins. *J Virol* 2005; 79: 8400-8409.
- [40] Kwak JE, Kim YI, Park SJ, Yu MA, Kwon HI, Eo S, Kim TS, Seok J, Choi WS, Jeong JH, Lee H, Cho Y, Kwon JA, Jeong M, Maslow JN, Kim YE, Jeon H, Kim KK, Shin EC, Song MS, Jung JU, Choi YK and Park SH. Development of a SFTSV DNA vaccine that confers complete protection against lethal infection in ferrets. *Nat Commun* 2019; 10: 3836.
- [41] Wu Y, Zhu Y, Gao F, Jiao Y, Oladejo BO, Chai Y, Bi Y, Lu S, Dong M, Zhang C, Huang G, Wong G, Li N, Zhang Y, Li Y, Feng WH, Shi Y, Liang M, Zhang R, Qi J and Gao GF. Structures of phlebovirus glycoprotein Gn and identification of a neutralizing antibody epitope. *Proc Natl Acad Sci U S A* 2017; 114: E7564-E7573.
- [42] Wright D, Allen ER, Clark MHA, Gitonga JN, Karanja HK, Hulswit RJG, Taylor I, Biswas S, Marshall J, Mwololo D, Muriuki J, Bett B, Bowden TA and Warimwe GM. Naturally acquired rift valley fever virus neutralizing antibodies predominantly target the Gn glycoprotein. *iScience* 2020; 23: 101669.
- [43] Totani M, Yoshii K, Kariwa H and Takashima I. Glycosylation of the envelope protein of West Nile virus affects its replication in chicks. *Avian Dis* 2011; 55: 561-8.
- [44] Zhang X, Jia R, Shen H, Wang M, Yin Z and Cheng A. Structures and functions of the enve-

- lope glycoprotein in flavivirus infections. *Viruses* 2017; 9: 338.
- [45] Gould EA, Coutard B, Malet H, Morin B, Jamal S, Weaver S, Gorbalenya A, Moureau G, Baronti C, Delogu I, Forrester N, Khasnatinov M, Gritsun T, de Lamballerie X and Canard B. Understanding the alphaviruses: recent research on important emerging pathogens and progress towards their control. *Antiviral Res* 2010; 87: 111-124.
- [46] Glass WG and Diamond MS. West Nile virus envelope glycosylation is a determinant of neuroinvasiveness. *J Virol* 2007; 81: 10309-10317.
- [47] Whiteman MC, Li L, Wicker JA, Kinney RM, Huang C, Beasley DW, Chung KM, Diamond MS, Solomon T and Barrett AD. Development and characterization of non-glycosylated E and NS1 mutant viruses as a potential candidate vaccine for West Nile virus. *Vaccine* 2010; 28: 1075-1083.
- [48] Su J, Li S, Hu X, Yu X, Wang Y, Liu P, Lu X, Zhang G, Hu X, Liu D, Li X, Su W, Lu H, Mok NS, Wang P, Wang M, Tian K and Gao GF. Duck egg-drop syndrome caused by BYD virus, a new Tembusu-related flavivirus. *PLoS One* 2011; 6: e18106.
- [49] Yun T, Zhang D, Ma X, Cao Z, Chen L, Ni Z, Ye W, Yu B, Hua J, Zhang Y and Zhang C. Complete genome sequence of a novel flavivirus, duck Tembusu virus, isolated from ducks and geese in China. *J Virol* 2012; 86: 3406-3407.
- [50] Sanisuriwong J, Yurayart N, Thontiravong A and Tiawsirisup S. Vector competence of *Culex tritaeniorhynchus* and *Culex quinquefasciatus* (Diptera: Culicidae) for duck Tembusu virus transmission. *Acta Trop* 2021; 214: 105785.
- [51] Sanisuriwong J, Yurayart N, Thontiravong A and Tiawsirisup S. Duck Tembusu virus detection and characterization from mosquitoes in duck farms, Thailand. *Transbound Emerg Dis* 2020; 67: 1082-1088.
- [52] Yan P, Zhao Y, Zhang X, Xu D, Dai X, Teng Q, Yan L, Zhou J, Ji X, Zhang S, Liu G, Zhou Y, Kawaoka Y, Tong G and Li Z. An infectious disease of ducks caused by a newly emerged Tembusu virus strain in mainland China. *Virology* 2011; 417: 1-8.
- [53] Tang Y, Diao Y, Gao X, Yu C, Chen L and Zhang D. Analysis of the complete genome of Tembusu virus, a flavivirus isolated from ducks in China. *Transbound Emerg Dis* 2012; 59: 336-343.
- [54] Yan D, Shi Y, Wang H, Li G, Li X, Wang B, Su X, Wang J, Teng Q, Yang J, Chen H, Liu Q, Ma W and Li Z. A single mutation at position 156 in the envelope protein of Tembusu virus is responsible for virus tissue tropism and transmissibility in ducks. *J Virol* 2018; 92: e00427-18.
- [55] Liu P, Lu H, Li S, Wu Y, Gao GF and Su J. Duck egg drop syndrome virus: an emerging Tembusu-related flavivirus in China. *Sci China Life Sci* 2013; 56: 701-710.
- [56] Yan D, Wang B, Shi Y, Ni X, Wu X, Li X, Liu X, Wang H, Su X, Teng Q, Yang J, Liu Q and Li Z. A single mutation at position 120 in the envelope protein attenuates Tembusu virus in ducks. *Viruses* 2022; 14: 447.
- [57] Li L, Zhang Y, Dong J, Zhang J, Zhang C, Sun M and Cao Y. The truncated E protein of DTMUV provide protection in young ducks. *Vet Microbiol* 2020; 240: 108508.
- [58] Qu Z, Wu X, Guo X, Han H, Zhang P, Wang M, Song Y, Jiao F, He S, Lu S and Zhang X. Self-assembled nanoparticle with E protein domain III of DTMUV based on ferritin as carrier can induce a more comprehensive immune response and against DTMUV challenge in duck. *Vet Microbiol* 2023; 284: 109820.
- [59] Cheng Y, Wang R, Wu Q, Chen J, Wang A, Wu Z, Sun F and Zhu S. Advancements in research on duck Tembusu virus infections. *Viruses* 2024; 16: 811.
- [60] Lu X, Xiao H, Li S, Pang X, Song J, Liu S, Cheng H, Li Y, Wang X, Huang C, Guo T, Ter Meulen J, Daffis S, Yan J, Dai L, Rao Z, Klenk HD, Qi J, Shi Y and Gao GF. Double lock of a human neutralizing and protective monoclonal antibody targeting the yellow fever virus envelope. *Cell Rep* 2019; 26: 438-446, e5.
- [61] Li Y, Chen Z, Wu L, Dai L, Qi J, Chai Y, Li S, Wang Q, Tong Z, Ma S, Duan X, Ren S, Song R, Liang M, Liu W, Yan J and Gao GF. A neutralizing-protective supersite of human monoclonal antibodies for yellow fever virus. *Innovation (Camb)* 2022; 3: 100323.
- [62] Wang H, Han M, Qi J, Hilgenfeld R, Luo T, Shi Y, Gao GF and Song H. Crystal structure of the C-terminal fragment of NS1 protein from yellow fever virus. *Sci China Life Sci* 2017; 60: 1403-1406.
- [63] Fioravanti R, Desideri N, Carta A, Atzori EM, Delogu I, Collu G and Loddo R. Inhibitors of yellow fever virus replication based on 1,3,5-Triphenyl-4,5-Dihydropyrazole scaffold: design, synthesis and antiviral evaluation. *Eur J Med Chem* 2017; 141: 15-25.
- [64] Post PR, Santos CN, Carvalho R, Cruz AC, Rice CM and Galler R. Heterogeneity in envelope protein sequence and N-linked glycosylation among yellow fever virus vaccine strains. *Virology* 1992; 188: 160-167.
- [65] Gritsun TS, Lashkevich VA and Gould EA. Tick-borne encephalitis. *Antiviral Res* 2003; 57: 129-146.

- [66] Elshuber S and Mandl CW. Resuscitating mutations in a furin cleavage-deficient mutant of the flavivirus tick-borne encephalitis virus. *J Virol* 2005; 79: 11813-11823.
- [67] Yang X, Qi J, Peng R, Dai L, Gould EA, Gao GF and Tien P. Molecular basis of a protective/neutralizing monoclonal antibody targeting envelope proteins of both tick-borne encephalitis virus and louping ill virus. *J Virol* 2019; 93: e02132-18.
- [68] Kaur H and Shishido AA. Tick-borne encephalitis: an update for the special operations forces provider. *J Spec Oper Med* 2023; 23: 110-113.
- [69] Phipps LP and Johnson N. Tick-borne encephalitis virus. *J Med Microbiol* 2022; 71: 001533.
- [70] Ličková M, Fumačová Havlíková S, Sláviková M and Klempa B. Alimentary infections by tick-borne encephalitis virus. *Viruses* 2021; 14: 56.
- [71] Goto A, Yoshii K, Obara M, Ueki T, Mizutani T, Kariwa H and Takashima I. Role of the N-linked glycans of the prM and E envelope proteins in tick-borne encephalitis virus particle secretion. *Vaccine* 2005; 23: 3043-3052.
- [72] Stone ET and Pinto AK. T cells in tick-borne flavivirus encephalitis: a review of current paradigms in protection and disease pathology. *Viruses* 2023; 15: 958.
- [73] Schlabe S and Rockstroh JK. Advances in the treatment of HIV/HCV coinfection in adults. *Expert Opin Pharmacother* 2018; 19: 49-64.
- [74] Tsutsumi Y, Ito S, Shiratori S and Teshima T. Hepatitis C Virus (HCV)-Ribonucleic Acid (RNA) as a biomarker for lymphoid malignancy with HCV infection. *Cancers (Basel)* 2023; 15: 2852.
- [75] Wang Y, Jie W, Ling J and Yuanshuai H. HCV core antigen plays an important role in the fight against HCV as an alternative to HCV-RNA detection. *J Clin Lab Anal* 2021; 35: e23755.
- [76] Helle F, Vieyres G, Elkrief L, Popescu CI, Wychowski C, Descamps V, Castelain S, Roingeard P, Duverlie G and Dubuisson J. Role of N-linked glycans in the functions of hepatitis C virus envelope proteins incorporated into infectious virions. *J Virol* 2010; 84: 11905-11915.
- [77] LeBlanc EV, Kim Y, Capicciotti CJ and Colpitts CC. Hepatitis C virus glycan-dependent interactions and the potential for novel preventative strategies. *Pathogens* 2021; 10: 685.
- [78] Alshehri AA and Irekeola AA. Global prevalence of alkhurma hemorrhagic fever virus infection: the first meta-analysis and systematic review. *J Infect Public Health* 2024; 17: 986-993.
- [79] Abdulhaq AA, Hershan AA, Karunamoorthi K and Al-Mekhlafi HM. Human alkhurma hemorrhagic fever: emergence, history and epidemiological and clinical profiles. *Saudi J Biol Sci* 2022; 29: 1900-1910.
- [80] Stapleford KA and Lindenbach BD. Hepatitis C virus NS2 coordinates virus particle assembly through physical interactions with the E1-E2 glycoprotein and NS3-NS4A enzyme complexes. *J Virol* 2011; 85: 1706-1717.
- [81] Khera T, Behrendt P, Bankwitz D, Brown RJP, Todt D, Doepke M, Khan AG, Schulze K, Law J, Logan M, Hockman D, Wong JAJ, Dold L, Gonzalez-Motos V, Spengler U, Viejo-Borbolla A, Ströh LJ, Krey T, Tarr AW, Steinmann E, Manns MP, Klein F, Guzman CA, Marcotrigiano J, Houghton M and Pietschmann T. Functional and immunogenic characterization of diverse HCV glycoprotein E2 variants. *J Hepatol* 2019; 70: 593-602.
- [82] Bhatia B, Feldmann H and Marzi A. Kyasanur forest disease and alkhurma hemorrhagic fever virus-two neglected zoonotic pathogens. *Microorganisms* 2020; 8: 1406.
- [83] Flint M, McMullan LK, Dodd KA, Bird BH, Khristova ML, Nichol ST and Spiropoulou CF. Inhibitors of the tick-borne, hemorrhagic fever-associated flaviviruses. *Antimicrob Agents Chemother* 2014; 58: 3206-3216.
- [84] Briesse T, Calisher CH and Higgs S. Viruses of the family Bunyaviridae: are all available isolates reassortants? *Virology* 2013; 446: 207-216.
- [85] Horne KM and Vanlandingham DL. Bunyavirus-vector interactions. *Viruses* 2014; 6: 4373-4397.
- [86] Zhang Y, Liu X, Wu Z, Feng S, Lu K, Zhu W, Sun H and Niu G. Oropouche virus: a neglected global arboviral threat. *Virus Res* 2024; 341: 199318.
- [87] Lima RN, Faheem M, Barbosa JA, Polêto MD, Verli H, Melo FL and Resende RO. Homology modeling and molecular dynamics provide structural insights into tospovirus nucleoprotein. *BMC Bioinformatics* 2016; 17 Suppl 18: 489.
- [88] Junglen S. Evolutionary origin of pathogenic arthropod-borne viruses-a case study in the family Bunyaviridae. *Curr Opin Insect Sci* 2016; 16: 81-86.
- [89] Casel MA, Park SJ and Choi YK. Severe fever with thrombocytopenia syndrome virus: emerging novel phlebovirus and their control strategy. *Exp Mol Med* 2021; 53: 713-722.
- [90] Wang T, Xu L, Zhu B, Wang J and Zheng X. Immune escape mechanisms of severe fever with thrombocytopenia syndrome virus. *Front Immunol* 2022; 13: 937684.
- [91] Li J, Li S, Yang L, Cao P and Lu J. Severe fever with thrombocytopenia syndrome virus: a highly lethal bunyavirus. *Crit Rev Microbiol* 2021; 47: 112-125.

- [92] Shimojima M, Sugimoto S, Taniguchi S, Maeki T, Yoshikawa T, Kurosu T, Tajima S, Lim CK and Ebihara H. N-glycosylation of viral glycoprotein is a novel determinant for the tropism and virulence of highly pathogenic tick-borne bunyaviruses. *PLoS Pathog* 2024; 20: e1012348.
- [93] Wilson LR and McElroy AK. Rift valley fever virus encephalitis: viral and host determinants of pathogenesis. *Annu Rev Virol* 2024; 11: 309-325.
- [94] McMillen CM and Hartman AL. Rift valley fever: a threat to pregnant women hiding in plain sight? *J Virol* 2021; 95: e01394-19.
- [95] Ahmed Zehairy A, Sartaj Sohrab S, Al-Judaibi AA and Ibraheem Azhar E. Rift valley fever virus: an update on current status and future prospects. *Rev Argent Microbiol* 2026; 58: 183-193.
- [96] Connors KA and Hartman AL. Advances in understanding neuropathogenesis of Rift Valley fever virus. *Annu Rev Virol* 2022; 9: 437-450.
- [97] Ihara T, Smith J, Dalrymple JM and Bishop DN. Complete sequences of the glycoproteins and M RNA of Punta Toro phlebovirus compared to those of Rift Valley fever virus. *Virology* 1985; 144: 246-259.
- [98] Kakach LT, Suzich JA and Collett MS. Rift Valley fever virus M segment: phlebovirus expression strategy and protein glycosylation. *Virology* 1989; 170: 505-510.
- [99] Chen R, Mukhopadhyay S, Merits A, Bolling B, Nasar F, Coffey LL, Powers A and Weaver SC; Ictv Report Consortium. ICTV virus taxonomy profile: togaviridae. *J Gen Virol* 2018; 99: 761-762.
- [100] DuBois RM, Vaney MC, Tortorici MA, Kurdi RA, Barba-Spaeth G, Krey T and Rey FA. Functional and evolutionary insight from the crystal structure of rubella virus protein E1. *Nature* 2013; 493: 552-556.
- [101] Mori Y, Otsuki N, Sakata M and Okamoto K. Virology of the family Togaviridae. *Uirusu* 2011; 61: 211-219.
- [102] Stubbs SH, Cornejo Pontelli M, Mishra N, Zhou C, de Paula Souza J, Mendes Viana RM, Lipkin WI, Knipe DM, Arruda E and Whelan SPJ. Vesicular stomatitis virus chimeras expressing the oropouche virus glycoproteins elicit protective immune responses in mice. *mBio* 2021; 12: e0046321.
- [103] Simão EP, Silva DBS, Cordeiro MT, Gil LHV, Andrade CAS and Oliveira MDL. Nanostructured impedimetric lectin-based biosensor for arboviruses detection. *Talanta* 2020; 208: 120338.
- [104] Wang N, Zhai X, Li X, Wang Y, He WT, Jiang Z, Veit M and Su S. Attenuation of Getah virus by a single amino acid substitution at residue 253 of the E2 protein that might be part of a new heparan sulfate binding site on alphaviruses. *J Virol* 2022; 96: e0175121.
- [105] Voss JE, Vaney MC, Duquerroy S, Vonnrhein C, Girard-Blanc C, Crublet E, Thompson A, Bricogne G and Rey FA. Glycoprotein organization of Chikungunya virus particles revealed by X-ray crystallography. *Nature* 2010; 468: 709-712.
- [106] Miao Q, Nguyen W, Zhu J, Liu G, van Oers MM, Tang B, Yan K, Larcher T, Suhrbier A and Pijlman GP. A getah virus-like-particle vaccine provides complete protection from viremia and arthritis in wild-type mice. *Vaccine* 2024; 42: 126136.
- [107] Jiao H, Yan Z, Zhai X, Yang Y, Wang N, Li X, Jiang Z and Su S. Transcriptome screening identifies TIPARP as an antiviral host factor against the Getah virus. *J Virol* 2023; 97: e00591-23.
- [108] Shi N, Qiu X, Tian J, Cao X, Zhu X, Liu B, Zhang X, Zhang H, Wang H and Lu H. Glycosylation of GETV E2 promotes pathogenesis in animal models. *Proc Natl Acad Sci U S A* 2026; 123: e2516425123.
- [109] Bannai H, Nemoto M, Tsujimura K and Ohta M. Establishment of an enzyme-linked immunosorbent assay for Getah virus infection in horses using a 20-mer synthetic peptide for the E2 glycoprotein as an antigen. *Arch Virol* 2020; 165: 377-385.
- [110] Li X, Han C, Song W, Jiang Z, Lu Y, Wang S, Chen J, Deng Y, Merits A, He WT, Su S and Veit M. DHHHC enzymes in alphavirus glycoprotein acylation: a target for broad-spectrum antiviral therapy. *Cell Rep* 2025; 44: 116540.
- [111] Lancaster C, Pristatsky P, Hoang VM, Casimiro DR, Schwartz RM, Rustandi R and Ha S. Characterization of N-glycosylation profiles from mammalian and insect cell derived chikungunya VLP. *J Chromatogr B Analyt Technol Biomed Life Sci* 2016; 1032: 218-223.
- [112] Nelson MA, Herrero LJ, Jeffery JAL, Hoehn M, Rudd PA, Supramaniam A, Kay BH, Ryan PA and Mahalingam S. Role of envelope N-linked glycosylation in Ross River virus virulence and transmission. *J Gen Virol* 2016; 97: 1094-1106.
- [113] Wang M, Sun Z, Cui C, Wang S, Yang D, Shi Z, Wei X, Wang P, Sun W, Zhu J, Li J, Du B, Liu Z, Wei L, Liu C, He X, Wang X, Zhang X and Wang J. Structural insights into alphavirus assembly revealed by the Cryo-EM structure of Getah virus. *Viruses* 2022; 14: 327.
- [114] Alen MM, Kaptein SJ, De Burghgraeve T, Balzarini J, Neyts J and Schols D. Antiviral activity of carbohydrate-binding agents and the role of DC-SIGN in dengue virus infection. *Virology* 2009; 387: 67-75.
- [115] Seabright GE, Doores KJ, Burton DR and Crispin M. Protein and glycan mimicry in HIV vaccine design. *J Mol Biol* 2019; 431: 2223-2247.
- [116] Thielens NM, Tacnet-Delorme P and Arlaud GJ. Interaction of C1q and mannan-binding lectin

- with viruses. *Immunobiology* 2002; 205: 563-574.
- [117] Bartolini D, Stabile AM, Vacca C, Pistilli A, Rende M, Gioiello A, Cruciani G and Galli F. Endoplasmic reticulum stress and NF- κ B activation in SARS-CoV-2 infected cells and their response to antiviral therapy. *IUBMB Life* 2022; 74: 93-100.
- [118] Liu D, Xiao X, Zhou P, Zheng H, Li Y, Jin H, Jongkaewwattana A and Luo R. Glycosylation on envelope glycoprotein of duck Tembusu virus affects virus replication *in vitro* and contributes to the neurovirulence and pathogenicity *in vivo*. *Virulence* 2021; 12: 2400-2414.
- [119] Cavada BS, Osterne VJS, Oliveira MV, Ferreira WP, Neto CC, Nascimento KS and Pinto-Junior VR. Bridging viral Glycobiology and lectin biotechnology for antiviral and diagnostic strategies. *ACS Bio Med Chem Au* 2025; 5: 792-814.