RESEARCH PAPER

Deciphering Codon Usage Bias in Nipah Virus: Genome Evolutionary Insights and Vaccine Design Implications

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ABSTRACT

Nipah virus (NiV) an emerging zoonotic pathogen belongs to genus Henipavirus, poses a significant threat to public health due to its high case fatality rate and recurrent out-breaks across Southeast Asia and Asia. In this study, we performed a comprehensive codon usage analysis of complete coding sequences from Nipah virus strains focusing on the evolutionary pressures that shape its genetic structure and the implications for vaccine design which originating from Malaysia, India, and Bangladesh, employing multiple bioinformatics approaches. The nucleotide composition analysis revealed that Nipah Virus genes are predominantly AT-rich, with an average GC content of ap-proximately 42%. The overall Codon usage bias across the genomes was found to be low, the overall codon usage bias was low, as reflected by relative synonymous codon usage (RSCU) values. Furthermore, codon adaptation index (CAI) analysis indicated that translational selection exerts a stronger influence than mutational pressure in shaping codon usage patterns. These findings suggest that the Nipah virus has evolved codon usage strategies that enhance replication efficiency and facilitate immune evasion in human hosts. The results provide critical insights for codon optimization in synthetic vaccine design and support the rational development of effective, host-adapted antiviral vaccines.

Keywords: Nipah Virus, Codon usage, CAI, RSCU, Nipah vaccine.

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INTRODUCTION

The death-dealing pathogenic Nipah virus, classified under the Paramyxoviridae family, belongs to the order Mononegavirales and includes the Nipah Virus genus with another representative virus of the genus, Hendra virus [1-6]. Nipah virus is transmitted zoonotically (from bats to humans, bats to palm dates, or from bats to pigs, and then passes into humans) as well as through human-to-human routes [7-9]. The Nipah virus is known for its ability to infect a wide range of hosts, including humans, pigs, and various bat species [10-15]. After ingesting pig carcasses, domestic dogs and cats became infected [16]. The Pteropus species, a fruit eating largest bat in the world, is a genus of megabats that is a natural host of Nipah Virus. The genus Pteropus acts as the intermediate host. Human beings are infected through close contact with pigs, by exposure to contaminated bat feces, urine, and saliva, and date palms [17].

Nipah virus is the causative agent of Nipah (Nee-pa) viral disease [18]. The Nipah virus zoonosis may also become the next emerging infection after the coronavirus infection. The Nipah virus contains one of the largest genomes of approximately 18,252 nucleotides. It is a non-segmented, negative-sense, single-stranded RNA virus enclosed within a lipid envelope. The genome follows a 3′–5′ orientation and encodes six major structural proteins—nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), attachment glycoprotein (G), and large polymerase (L). In addition, the P gene gives rise to three non-structural proteins (V, W, and C) through RNA editing and alternative translation, which contribute to immune evasion and viral pathogenesis. [19,20]. The emergence of zoonosis infectious diseases (e.g., Nipah virus, Hantavirus, West Nile virus, Zika virus) is an imminent danger to human and animal life, leading to substantial economic loss. Globally, 70%—75 % of zoonotic infections originated in animals and passed into humans, leading to substantial economic losses [21,22].

It is a negative sense, single-stranded, non-segmented, enveloped RNA virus possessing helical symmetry. The RNA genome, from the 3´-5´, contains consecutive arrangement of six genes, *viz*., nucleocapsid (N), phosphoprotein (P), matrix (M), fusion glycoprotein (F), attachment glycoprotein (G) and long polymerase (L). The N, P and L attached to the viral RNA, forming the virus ribonucleoprotein (vRNP). F and G proteins are responsible for the cellular attachment of the virion and subsequent host cell entry

The Nipah virus disease outbreak has been documented in various regions of the world, including countries in South and Southeast Asia [23,24], including Bangladesh [25], Cambodia [26], East Timor, Indonesia [27], India [28], Malaysia [29], Papua New Guinea [30], Vietnam, and Thailand [31,32]. The heart of South East Asia came down with acute febrile encephalitis that was associated with high mortality [33-35].

Outbreak in Malaysia: The initial outbreak was reported from the encephalitic patient's cerebrospinal fluid from the Kampung Sungai Nipah, a small village located near the city of Ipoh within the Kinta district of Perak state in Peninsular, West Malaysia (1998–1999), causing 1100 deaths linked to pig exposure, with high mortality and brain-stem involvement [36-40]. During the same period, the outbreak was documented in Bukit Pelandok, which is known as the biggest pig farming community in Southeast Asia, and Sikamat (state of Negeri Sembilan), Malaysia [41,42]. A serological survey of 1,469 people in Singapore found 22 (1.5%) Nipah virus infections, all in male abattoir workers, with no human-to-human transmission [43,44]. The outbreak in Malaysia of Japanese Encephalitis (JE) was endemic with sporadic occurrences [45,46]. There was an outbreak of febrile encephalitis in several pig-farming villages in Malaysia [47,48].

Outbreak in Bangladesh: In the winter of 2001, the Nipah virus infection was documented in the district of Meherpur, Bangladesh. In 2003 against the infection was documented in Nagaon. Rajbari, Faridpur, Golapgonj, Manikganj, Joypurhat. In January 2004, an outbreak of Nipah virus occurred in the Rajbari district of Bangladesh, where four confirmed cases of Nipah encephalitis were reported, resulting in a high mortality rate of approximately 90% [49,50].

Outbreaks in India: A similar pattern was observed in India, where an outbreak of Nipah virus infection emerged during the winter of 2001 in Siliguri, West Bengal. Another wave of cases was identified in the Nadia district of West Bengal in 2007, with a striking fatality rate of 100%. In 2018, Kerala witnessed a resurgence of Nipah virus infections, primarily affecting the districts of Kozhikode and Malappuram. The following year, in 2019, another case was identified in Ernakulam district, underscoring the continuing risk posed by the virus in this southern Indian state [51-53]. Recently, in September 2021, the Nipah virus infection was reported in the Kozhikode district of Kerala, India [54,55]. The infections occurred from the consumption of infected date palms by fruit bats. Transmission is reported from animal to human and from human to human. There is evidence that both Hendra and Nipah viruses are natural pathogens of Pteropus bats [56,57]. Further outbreaks are to be expected as a result of ecological disturbances due to climate or manmade changes to the environment.

The outbreak of febrile encephalitis in humans was preceded by the occurrence of respiratory illness and encephalitis in pigs in the same district [58]. The clinical manifestations of Nipah virus infection can be severe, with acute encephalitis and pulmonary syndromes often leading to high mortality rates. The virus targets the central nervous system, as well as other major organs, causing extensive vascular damage and cellular infection. The virus is categorized under biosafety level 4(BSL4), as it causes subclinical to severe respiratory infections and fatal encephalitis [59,60].

The Nipah virus, as a global health concern, has prompted extensive research efforts to understand its molecular mechanisms and develop effective countermeasures [61,62]. Proteins, essential macromolecules in all forms of life, are composed of 20 standard amino acids, each encoded by codons: triplets of nucleotides. Although 61 codons code for amino acids, most amino acids are encoded by multiple synonymous codons, with the exceptions of Methionine and Tryptophan [63-65]. This redundancy in the genetic code, known as degeneracy, leads to a phenomenon called codon usage bias (CUB), a non-random preference for certain synonymous codons over others. The pattern of CUB in viral genomes is influenced by a variety of factors, including mutational biases, natural selection, host adaptation, and evolutionary history [66]. Viruses with multiple open reading frames (ORFs) often exhibit gene-specific codon preferences, which can be shaped by pressures such as geographical distribution and host interactions. Studies have shown that such biases can reflect how well a virus is adapted to its host's translational machinery [67,68].

The current study investigates the codon usage patterns of Nipah virus genomes obtained from different regions, including Malaysia, India, and Bangladesh. The goal is to identify codon biases that could be leveraged in synthetic attenuated vaccine design. By identifying underutilized codons, scientists can design a de-optimized version of the viral genome, reduce its replication efficiency while preserve immunogenicity, a strategy that aligns with SAVE (Synthetic Attenuated Virus Engineering) [69].

This investigation not only sheds light on Nipah virus molecular evolution but also contributes valuable data to support rational vaccine design, enabling the development of attenuated strains optimized for immunogenicity and safety in human hosts. Furthermore, the biological implications of codon usage extend to the virus's ability to evade host immune responses, adapt to new hosts, and maintain its pathogenic potential. In this study, we conduct an in-silico immunoinformatic analysis of the Nipah virus genome to identify potential therapeutic targets and vaccine candidates. We analyse the Complete coding DNA Sequence of Nipah virus isolates from different geographical locations, viral genomic variations in context to the complete genome, which will contribute to evaluating their potential for eliciting a robust immune response, and assessing their suitability as targets for vaccine development.

MATERIALS AND METHODS

1.Retrieval of Genomic Data and Validation: The complete coding DNA sequence of the Nipah virus protein in FASTA format from Indian, Malaysian, and Bangladeshi origins, isolated from the human host, was downloaded from the BV-BRC (Bacterial and Viral Bioinformatics Resource Centre) https://www.bv-brc.org/ [70]. The GenBank accession numbers for the selected isolates are MK673562 (Malaysia), MH396625, MH523640, MH523641 and FJ513078 (India) and JN808857, JN808863, MK673592 and MK673571 (Bangladesh) respectively. To ensure accuracy in codon usage analysis, all coding sequences (CDS) underwent a validation process. The integrity of each sequence was assessed using the ORF finder tool provided by NCBI (https://www.ncbi.nlm.nih.gov/orffinder), which detects open reading frames (ORFs) by identifying the proper start and stop codons. Only sequences with complete and valid ORFs were included in the study [71-74].

2.Analysis of Nucleotide Composition: To understand the molecular characteristics and translational efficiency of Nipah virus (NiV) genes, a comprehensive nucleotide composition analysis was performed on their coding sequences. This analysis quantified the relative abundance of the four nucleotide bases: adenine (A), thymine (T), guanine (G), and cytosine (C) and calculated the overall **GC content** (G + C) and AT content (A + T) within the viral genome [75]. Several tools were employed for this analysis. EMBOSS GeeCee was used to calculate the GC content of the Nipah virus coding sequences (https://www.bioinformatics.nl/cgi-bin/emboss/geecee) [76,77]. Additionally, local platforms such as BioEdit, MEGA, and SnapGene facilitated a more granular exploration of base composition, while NCBI sequence analysis tools provided supplementary statistics upon sequence submission. The nucleotide content (G%, C%, A%, and U%) of the selected genes coding region was calculated using the CAIcal (http://genomes.urv.es/CAIcal/) [78].

3.Codon usage frequency: - Codon usage frequency refers to how often specific codons are used in the coding sequences of a gene or genome. Since multiple codons can encode the same amino acid (known as synonymous codons), organisms often show preferences for certain codons over others—a phenomenon called codon usage bias. The range of Nc values is from 20-61. If the value is less than 35, the genome is highly biased [79]. Understanding codon usage frequency is crucial for studying gene expression efficiency and understanding evolutionary adaptation, and designing codon-optimized genes for heterologous expression (e.g., vaccines, recombinant proteins). The SMS2 codon usage tool is used for https://www.bioinformatics.org/sms2/codon_usage.html [80].

4.Relative Synonymous Codon Usage (RSCU) Analysis of the Nipah virus Human Isolates: Measuring codon preference among synonyms is a widely used metric in codon usage studies, providing insight into the selection and preference of synonymous codons. Those that encode the same amino acid but differ in nucleotide sequence. The RSCU values are calculated by the ratio of the observed value to the expected value of given amino acids [81]. RSCU values facilitate the identification of codon usage bias independent of amino acid composition or gene length. An RSCU value of 1.0 indicates uniform usage of synonymous codons, while values greater than 1.0 reflect codon preference or overrepresentation, and values less than 1.0 indicate underutilization or avoidance. A codon with an RSCU value of 0 suggests a complete absence in the sequence under analysis. In this study, RSCU values were computed for all protein-coding genes of *Nipah virus* human isolates using the software package. The RSCU value of the envelope protein of the considered Nipah virus strains was calculated by https://jamiemcgowan.ie/bioinf/rscu.html. Codons with RSCU values ranging between 0.6 and 1.6 are considered unbiased or randomly used [82].

5.Estimation of the CAI (Codon Adaptation Index) Value: The CAIcal server is a powerful tool for codon usage analysis. The CAIcal serves as an indispensable resource for genome annotation, gene expression analysis, and studies exploring codon usage optimization across different organisms. The codon usage pattern of a set of highly expressed reference genes. The CAI index for any gene set ranges from 0 to 1, where a higher value indicates the increased efficiency of the virus in adapting to the host environment. CAIcal http://genomes.urv.es/CAIcal is an openly accessible web-based platform developed to evaluate codon usage bias through the calculation of the **Codon Adaptation Index (CAI)** [83,84].

6.Comparative Assessment of Nipah Virus Protein Translation Efficiency in the Human The translational efficiency of the envelope protein from Nipah virus strains in the human host system was evaluated by comparing the most preferred codons of the Nipah virus strains with the tRNA isotypes in humans. The tRNA isotope abundance data was retrieved to analyze the data. https://gtrnadb.ucsc.edu/ https://gtrnadb.ucsc.edu/genomes/eukaryota/Hsapi19/Hsapi19-summary.html [85-87].

RESULTS

1.Retrieval of Genomic Data: The complete coding DNA sequences (CDS) in FASTA form of the Nipah henipavirus were obtained from multiple geographic origins to assess genomic variability and codon usage patterns. The isolates analyzed include strains from Malaysia, Bangladesh, and India. These sequences were derived from the host species, humans. The sequence from the human host was specifically retrieved from the Bacterial and Viral Bioinformatics Resource Centre (BV-BRC), ensuring high-quality curated data. The GenBank accession numbers for the selected isolates are as follows: MK673562 (Malaysia), MH396625, MH523640, MH523641, and FJ513078 (India), and JN808857, JN808863, MK673592, and MK673571 (Bangladesh), respectively. These representative sequences were used to perform comparative analyses of codon usage bias, explore evolutionary trends, and evaluate their implications for host adaptation and vaccine design. The High-quality sequences ensure reliable analysis of codon usage patterns and viral evolution (Table 1).

Table 1: Demographic Details of Selected Nipah Virus Isolates

Genome Name	GenBank Accession number	Size	CDS	Year of isolation	Origin of the country	Host
Nipah henipavirus08651	MK673562	18231	9	1999	Malaysia	Homo sapiens
Nipah henipavirus MCL-18-H-1088	MH396625	18210	9	2018	India	Homo sapiens
Nipah henipavirus MCL-18-H-1197	MH523640	18132	9	2018	India	Homo sapiens
Nipah henipavirus MCL-18-H-1209	MH523641	18027	9	2018	India	Homo sapiens
Nipah henipavirus Ind-Nipah-07-FG	FJ513078	18252	9	2007	India	Homo sapiens
Nipah henipavirus NIVBGD2008MANIKGONJ	JN808857	18252	9	2008	Bangladesh	Homo sapiens
Nipah henipavirus NIVBGD2008RAJBARI	JN808863	18252	9	2008	Bangladesh	Homo sapiens
Nipah henipavirus 201601241	MK673592	18234	9	2014	Bangladesh	Homo sapiens
Nipah henipavirus 201200903	MK673571	18240	9	2011	Bangladesh	Homo sapiens
Nipah henipavirus	AY988601	18252	9	2004	Bangladesh	Homo sapiens

2.Validation and Preprocessing: To ensure accuracy in codon usage analysis, all coding sequences (CDS) underwent a validation process to eliminate incomplete or redundant entries, as these can distort analytical outcomes. The integrity of each sequence was assessed using the ORFfinder tool provided by NCBI (https://www.ncbi.nlm.nih.gov/orffinder), which detects open reading frames (ORFs) by identifying the proper start and stop codons. Only sequences with complete and valid ORFs were included in the study (Table 2).

Table 2: Comparative Analysis of Genome Architecture Among Nipah Virus Isolates Based on GenBank Accession Data

GenBank Accession number	Size	CDS	Year of isolation	Origin of the country	Number of ORFs	Genetic code	Start codon
MK673562	18231	9	1999	Malaysia	83	1	ATG
MH396625	18210	9	2018	India	89	1	ATG
MH523640	18132	9	2018	India	89	1	ATG
MH523641	18027	9	2018	India	87	1	ATG

FJ513078	18252	9	2007	India	88	1	ATG
JN808857	18252	9	2008	Bangladesh	91	1	ATG
JN808863	18252	9	2008	Bangladesh	91	1	ATG
MK673592	18234	9	2014	Bangladesh	91	1	ATG
MK673571	18240	9	2011	Bangladesh	86	1	ATG

3.Analysis of Nucleotide composition of Nipah virus strains: The diverse nucleotide compositional properties were calculated for the coding sequences of Nipah virus genomes. These compositional properties comprise the frequencies of occurrence of each nucleotide (A%, U%, G%, and C%); AU and GC contents via **EMBOSSGeeCee** https://www.bioinformatics.nl/cgibin/emboss/geecee?_pref_hide_optional With each nucleotide at the third position of the synonymous codons (A3%, U3%, G3%, and C3%); nucleotides G + C at the first (GC1), second (GC2), and third codon positions (GC3); mean frequencies of nucleotides G + C at the first and the second positions (GC12) average of GC1and GC2.(Table 3) The codons for Met (AUG), Trp (UGG), and termination codons (UAA, UGA, UAG) are unlikely to contribute to the codon usage bias, and therefore, these were excluded from the analysis. The low GC contents suggest that the mutational pressure plays a dominant role in shaping of Nipah virus genome. The GC1 and GC2 values range from 38.06 to 40.26% and 37.23-39.69% respectively. The third codon range, 56.9%-64.4% shows the highest variability as compared to GC3. The effective number of codons values 51.7-53.0 % confirms that the Nipah virus has low codon usage bias. The adaptation to human host codon usage may thus rely on matching A/U-ending codons to abundant human tRNAs, optimizing translation without the need for strong codon bias. The nucleotide composition data of Nipah virus reflects a genome evolution pattern primarily shaped by mutational tendency towards A and U, with only modest effects from translational selection, allowing the virus to maintain compatibility with the human host's translation machinery.

Table 3: Comparative Analysis of Nucleotide and Codon Positional Bias Across Three Human-Isolated Nipah Virus Strains

Nucleotide composition	MK673562	MH396625	MH523640	MH523641	FJ513078	JN808857	JN808863	MK673592	MK673571
Length	18231	18210	18132	18027	18252	18252	18252	18234	18240
%A	33.82	33.62	33.61	33.61	33.6	33.57	33.57	33.57	33.63
%C	18.25	18.37	18.37	18.4	18.42	18.35	18.35	18.43	18.36
%U	27.96	27.86	27.87	27.78	27.89	27.92	27.92	27.83	27.94
%G	19.97	20.15	20.15	20.21	20.09	20.16	20.16	20.17	20.07
%A3	34.67	34.74	34.94	34.5	34.71	34.75	34.75	34.75	33.73
%C3	17.46	17.02	16.88	18.29	17.21	17.19	17.19	17.21	20
%T3	23.17	23.57	24.52	27.03	23.36	23.36	23.36	23.31	30.79
%G3	24.7	24.66	23.66	20.19	24.72	24.7	24.7	24.73	15.48
%AU	61.78	61.48	61.48	61.39	61.49	61.49	61.49	61.4	61.57
%GC	38.22	38.52	38.52	38.61	38.51	38.51	38.51	38.6	38.43
GC 1	40	40.12	38.85	39.51	40.09	40.15	40.15	40.26	38.06
GC2	37.23	38.27	39.69	38.14	38.03	38.03	38.03	38.17	38.16

GC12	32.84	33.82	38.68	35.03	33.53	33.45	33.45	33.56	37.07
AU3%	56.99	57.19	58.13	60.64	56.96	56.93	56.93	56.88	64.42
GC3%	37.43	37.17	37.03	38.5	37.3	37.35	37.35	37.38	40.07
%GC3	42.2	41.7	40.5	38.5	41.9	41.9	41.9	41.9	35.5
Nc	52.8	52.8	51.8	52.7	53	53	53	53	51.7

4.Codon usage frequency of Nipah virus strains: The codon usage analysis of nine Nipah virus genomes from Malaysia, India, and Bangladesh revealed a clear preference for specific synonymous codons across amino acids, indicating a biased codon usage pattern. Codon usage frequency refers to some codons used more frequently than other amino acids like UGC (Cys), CAG (Gln), and AAC (Asn) are also highly preferred codons of Nipah virus strains of India, Malaysia, and Bangladesh, used 60%. And AAC used 40%. Methionine (AUG) and Tryptophan (UGG) are universally used at the full frequency of 1.0 in all nine strains from different countries. The least frequently used codons were GC-rich, such as **CGC (Arg; as low as 0.02), UCG (Ser; as low as 0.03),** and CCG **(Pro; as low as 0.08),** indicating strong codon bias against these variants. (Table 4). The overall codon usage frequency range spanned from **0.02** (CGC in MK673562) to **1.00** (AUG and UGG across all strains). This pattern was consistent across geographic origins, suggesting that mutational pressure and base composition, rather than host-specific selection, are the dominant forces shaping codon preference in Nipah virus. Such bias toward A/U-ending codons may enhance compatibility with host translational machinery while maintaining genome stability, a feature that should be considered in reverse genetics systems and codon optimization strategies for vaccine development.

Table 4. Most Preferred and Least Preferred Codons per Amino Acid Across Three Groups Based on Fractional Usage

		Malaysia	India	India	India	India	Bangladesh	Bangladesh	Bangladesh	Bangladesh
Codon	Amino acid	MK673562	MH396625	MH523640	MH523641	FJ513078	JN808857	JN808863	MK673592	MK673571
Ala	**GCG**	**0.05**	**0.08**	**0.06**	**0.07**	**0.09**	**0.08**	**0.08**	**0.09**	**0.06**
	GCA	**0.46**	**0.48**	**0.53**	**0.48**	**0.45**	**0.46**	**0.46**	**0.45**	**0.35**
	GCU	0.29	0.27	0.26	0.27	0.28	0.27	0.27	0.28	0.36
	GCC	0.2	0.18	0.16	0.19	0.18	0.18	0.18	0.18	0.22
Cys	UGU	0.53	0.56	0.59	0.52	0.56	0.55	0.55	0.55	0.6
	UGC	**0.47**	**0.44**	**0.41**	**0.48**	**0.44**	**0.45**	**0.45**	**0.45**	**0.4**
Asp	GAU	0.68	0.71	0.64	0.67	0.67	0.67	0.67	0.66	0.64
	GAC	0.32	0.29	0.36	0.33	0.33	0.33	0.33	0.34	0.36
Glu	GAG	0.52	0.53	0.46	0.46	0.54	0.53	0.53	0.54	0.36
	GAA	0.48	0.47	0.54	0.54	0.46	0.47	0.47	0.46	0.64
Phe	UUU	0.53	0.56	0.59	0.63	0.55	0.56	0.56	0.56	0.61
	UUC	0.47	0.44	0.41	0.37	0.45	0.44	0.44	0.44	0.39
Gly	GGG	0.23	0.23	0.23	0.17	0.25	0.25	0.25	0.25	0.18

	GGA	0.42	0.42	0.41	0.44	0.39	0.39	0.39	0.4	0.45
	GGU	0.18	0.2	0.25	0.19	0.21	0.21	0.21	0.21	0.19
	GGC	0.17	0.15	0.11	0.2	0.15	0.15	0.15	0.14	0.18
His	CAU	0.66	0.66	0.61	0.68	0.65	0.65	0.65	0.65	0.65
	CAC	0.34	0.34	0.39	0.32	0.35	0.36	0.35	0.35	0.35
IIe	AUA	0.37	0.37	0.37	0.28	0.37	0.38	0.38	0.37	0.22
	AUU	0.31	0.33	0.34	0.38	0.32	0.32	0.32	0.32	0.4
	AUC	0.32	0.31	0.3	0.34	0.31	0.31	0.31	0.31	0.37
Lys	AAG	0.46	0.46	0.46	0.43	0.46	0.46	0.46	0.46	0.39
	AAA	0.54	0.54	0.54	0.57	0.54	0.54	0.54	0.54	0.61
Leu	UUG	0.24	0.24	0.23	0.22	0.24	0.25	0.25	0.24	0.16
	UUA	0.2	0.21	0.2	0.21	0.21	0.21	0.21	0.2	0.23
	CUG	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.1
	CUA	0.19	0.18	0.19	0.15	0.18	0.18	0.18	0.18	0.14
	CUU	0.11	0.12	0.13	0.16	0.12	0.2	0.12	0.12	0.21
	CUC	0.11	0.1	0.1	0.11	0.09	0.09	0.09	0.09	0.16
Met	AUG	1	1	1	1	1	1	1	1	1
Asn	AAU	0.59	0.62	0.62	0.65	0.64	0.63	0.63	0.63	0.63
	AAC	**0.41**	**0.38**	**0.38**	**0.35**	**0.36**	**0.37**	**0.3**	**0.37**	**0.37**
Pro	CCG	0.15	0.18	0.18	0.13	0.16	0.16	0.16	0.16	0.08
	CCA	0.37	0.37	0.36	0.33	0.36	0.36	0.36	0.36	0.34
	CCU	0.28	0.25	0.27	0.32	0.26	0.26	0.26	0.26	0.34
	CCC	0.2	0.19	0.19	0.22	0.22	0.21	0.21	0.21	0.24
Gln	**CAG**	**0.49**	**0.48**	**0.48**	**0.4**	**0.47**	**0.47**	**0.47**	**0.47**	**0.29**
	CAA	0.51	0.52	0.52	0.6	0.53	0.53	0.53	0.53	0.71
Arg	AGG	0.28	0.3	0.26	0.25	0.29	0.29	0.29	0.29	0.24
	AGA	0.43	0.43	0.48	0.47	0.45	0.44	0.44	0.44	0.48
	CGG	0.09	0.06	0.06	0.08	0.06	0.06	0.06	0.06	0.09
	CGA	0.11	0.11	0.11	0.1	0.1	0.03	0.11	0.11	0.1
	CGU	0.06	0.07	0.05	0.06	0.07	0.06	0.06	0.06	0.05
	CGC	0.02	0.03	0.05	0.04	0.03	0.03	0.03	0.03	0.05
Ser	AGU	0.16	0.15	0.13	0.18	0.14	0.15	0.15	0.15	0.24

	AGC	0.13	0.13	0.12	0.15	0.14	0.14	0.14	0.14	0.14
	UCG	0.08	0.07	0.08	0.05	0.06	0.06	0.06	0.06	0.03
	UCA	0.34	0.33	0.35	0.28	0.32	0.33	0.33	0.33	0.23
	UCU	0.17	0.2	0.2	0.21	0.19	0.19	0.19	0.19	0.23
	UCC	0.11	0.13	0.11	0.13	0.14	0.14	0.14	0.14	0.13
Thr	ACG	0.13	0.14	0.11	0.12	0.14	0.14	0.14	0.14	0.06
	ACA	0.47	0.46	0.48	0.41	0.46	0.46	0.46	0.46	0.33
	ACU	0.23	0.21	0.23	0.27	0.22	0.22	0.22	0.21	0.38
	ACC	0.17	0.19	0.18	0.2	0.19	0.19	0.19	0.19	0.23
Val	GUG	0.29	0.31	0.29	0.23	0.3	0.3	0.3	0.3	0.17
	GUA	0.29	0.27	0.21	0.23	0.27	0.27	0.27	0.27	0.21
	GUU	0.22	0.22	0.3	0.27	0.22	0.22	0.22	0.22	0.34
	GUC	0.2	0.21	0.19	0.27	0.21	0.21	0.21	0.21	0.29
Trp	UGG	1	1	1	1	1	1	1	1	1
Tyr	UAU	0.63	0.61	0.59	0.66	0.61	0.61	0.61	0.62	0.7
	UAC	0.37	0.39	0.41	0.34	0.39	0.39	0.39	0.38	0.3
End	UGA	0.37	0.37	0.32	0.46	0.37	0.37	0.37	0.37	0.49
	UAG	0.26	0.25	0.27	0.17	0.25	0.25	0.25	0.25	0.14
	UAA	0.37	0.37	0.41	0.34	0.38	0.38	0.38	0.38	0.37

5.Comparative analysis of Relative Synonymous Codon Usage (RSCU): - The analysis to assess synonymous codon usage patterns in the Nipah virus (NiV), Relative Synonymous Codon Usage (RSCU) values were computed. This analysis of RSCU values. Codons exhibiting a higher-than-expected frequency of usage, termed overrepresented codons, were identified and are specifically highlighted within their respective genes (Table 5). We have observed the Nipah Virus strains from three different countries, which are highly preferred and non–preferred codons (Table 5). We observed that the strong bias is shown by some amino acids. In India, the ACA (Thr) and AGA(Arg), in Malaysia, UCA (Ser)and GCA (Ala), and in Bangladesh, also lean into UUA (Leu) and GGA (Gly). Codons such as GCA and GCU for alanine, GGA for glycine, CCU and CCA for proline, and ACA for threonine are consistently preferred in all three strains, suggesting evolutionary stability in codon selection among regional variants of the virus.

Table 5: Comparative Analysis of RSCU of Nipah virus Across India, Malaysia, and Bangladesh. The most preferred codon is highlighted.

Malaysia Nipah virus isolates RSCU	**Indian Nipah virus isolates RSCU.**				**Bangladesh Nipah virus isolates RSCU**			
MK673562	MH396625	MH523640	MH523641	FJ513078	JN808857	JN808863	MK673592	MK673571
UUU	UUU	UUU	UUU	UUU	UUU	UUU	UUU	UUU

(Phe)1.06	(Phe)1.12	(Phe)1.18	(Phe)1.27	(Phe)1.11	(Phe)1.12	(Phe)1.12	(Phe)1.11	(Phe)1.22
UUC (Phe)0.94	UUC (Phe)0.88	UUC (Phe)0.82	UUC (Phe)0.73	UUC (Phe)0.89	UUC (Phe)0.88	UUC (Phe)0.88	UUC (Phe)0.89	UUC (Phe)0.78
UUA (Leu)1.19	UUA (Leu)1.24	UUA (Leu)1.18	UUA (Leu)1.27	UUA (Leu)1.24	UUA (Leu)1.23	UUA (Leu)1.23	UUA (Leu)1.22	UUA (Leu)1.38
UUG(Leu)1.44	UUG (Leu)1.43	UUG(Leu)1.39	UUG(Leu)1.33	UUG(Leu)1.46	UUG(Leu)1.47	UUG(Leu)1.47	UUG(Leu)1.46	UUG(Leu)0.93
CUU (Leu)0.66	CUU (Leu)0.74	CUU (Leu)0.77	CUU (Leu)0.97	CUU (Leu)0.71	CUU (Leu)0.7	CUU (Leu)0.7	CUU (Leu)0.71	CUU (Leu)1.29
CUC (Leu)0.68	CUC (Leu)0.6	CUC (Leu)0.59	CUC (Leu)0.68	CUC (Leu)0.56	CUC (Leu)0.56	CUC (Leu)0.56	CUC (Leu)0.56	CUC (Leu)0.95
CUA (Leu)1.13	CUA (Leu)1.07	CUA (Leu)1.15	CUA (Leu)0.87	CUA (Leu)1.09	CUA (Leu)1.08	CUA (Leu)1.08	CUA (Leu)1.08	CUA (Leu)0.86
CUG (Leu)0.91	CUG (Leu)0.92	CUG (Leu)0.92	CUG (Leu)0.87	CUG (Leu)0.95	CUG (Leu)0.95	CUG (Leu)0.95	CUG (Leu)0.95	CUG (Leu)0.59
AUU (Ile)0.93	AUU (Ile)0.98	AUU (Ile)1.01	AUU (Ile)1.14	AUU (Ile)0.95	AUU (Ile)0.96	AUU (Ile)0.96	AUU (Ile)0.95	AUU (Ile)1.2
AUC(Ile)0.96	AUC(Ile)0.92	AUC(Ile)0.89	AUC(Ile)1.03	AUC(Ile)0.93	AUC(Ile)0.92	AUC(Ile)0.92	AUC(Ile)0.93	AUC(Ile)1.12
AUA(Ile)1.11	AUA(Ile)1.11	AUA(Ile)1.1	AUA(Ile)0.84	AUA(Ile)1.12	AUA(Ile)1.13	AUA(Ile)1.13	AUA(Ile)1.12	AUA(Ile)0.67
GUU (Val)0.88	GUU (Val)0.87	GUU (Val)1.21	GUU (Val)1.1	GUU (Val)0.86	GUU (Val)0.87	GUU (Val)0.87	GUU (Val)0.89	GUU (Val)1.36
GUC(Val)0.78	GUC(Val)0.83	GUC(Val)0.78	GUC(Val)1.08	GUC(Val)0.85	GUC(Val)0.85	GUC(Val)0.85	GUC(Val)0.84	GUC(Val)1.14
GUA(Val)1.18	GUA(Val)1.07	GUA(Val)0.85	GUA(Val)0.91	GUA(Val)1.08	GUA(Val)1.09	GUA(Val)1.09	GUA(Val)1.09	GUA(Val)0.83
GUG(Val)1.16	GUG(Val)1.23	GUG(Val)1.17	GUG(Val)0.91	GUG(Val)1.2	GUG(Val)1.19	GUG(Val)1.19	GUG(Val)1.19	GUG(Val)0.67
UCU(Ser)1	UCU(Ser)1.17	UCU(Ser)1.22	UCU(Ser)1.26	UCU(Ser)1.13	UCU(Ser)1.11	UCU(Ser)1.11	UCU(Ser)1.11	UCU(Ser)1.37
UCC(Ser)0.66	UCC(Ser)0.78	UCC(Ser)0.68	UCC(Ser)0.78	UCC(Ser)0.82	UCC(Ser)0.83	UCC(Ser)0.83	UCC(Ser)0.82	UCC(Ser)0.8
UCA(Ser)2.05	UCA(Ser)1.97	UCA(Ser)2.12	UCA(Ser)1.71	UCA(Ser)1.94	UCA(Ser)1.97	UCA(Ser)1.97	UCA(Ser)1.97	UCA(Ser)1.38

UCG(Ser)0.51	UCG(Ser)0.4	UCG(Ser)0.5	UCG(Ser)0.29	UCG(Ser)0.39	UCG(Ser)0.36	UCG(Ser)0.36	UCG(Ser)0.37	UCG(Ser)0.16
AGU(Ser)0.98	AGU(Ser)0.88	AGU(Ser)0.77	AGU(Ser)1.07	AGU(Ser)0.87	AGU(Ser)0.89	AGU(Ser)0.89	AGU(Ser)0.87	AGU(Ser)1.42
AGC(Ser)0.79	AGC(Ser)0.79	AGC(Ser)0.71	AGC(Ser)0.89	AGC(Ser)0.85	AGC(Ser)0.85	AGC(Ser)0.85	AGC(Ser)0.85	AGC(Ser)0.86
CCU (Pro)1.11	CCU (Pro)1.01	CCU (Pro)1.07	CCU (Pro)1.29	CCU (Pro)1.03	CCU (Pro)1.04	CCU (Pro)1.04	CCU (Pro)1.05	CCU (Pro)1.35
CCC (Pro)0.8	CCC (Pro)0.77	CCC (Pro)0.78	CCC (Pro)0.89	CCC (Pro)0.87	CCC (Pro)0.86	CCC (Pro)0.86	CCC (Pro)0.84	CCC (Pro)0.98
CCA(Pro)1.5	CCA(Pro)1.49	CCA(Pro)1.44	CCA(Pro)1.31	CCA(Pro)1.44	CCA(Pro)1.45	CCA(Pro)1.45	CCA(Pro)1.46	CCA(Pro)1.37
CCG(Pro)0.59	CCG(Pro)0.73	CCG(Pro)0.72	CCG(Pro)0.51	CCG(Pro)0.66	CCG(Pro)0.65	CCG(Pro)0.65	CCG(Pro)0.66	CCG(Pro)0.3
ACU(Thr)0.9	ACU(Thr)0.83	ACU(Thr)0.92	ACU(Thr)1.06	ACU(Thr)0.87	ACU(Thr)0.87	ACU(Thr)0.87	ACU(Thr)0.85	ACU(Thr)1.51
ACC(Thr)0.69	ACC(Thr)0.77	ACC(Thr)0.72	ACC(Thr)0.8	ACC(Thr)0.76	ACC(Thr)0.76	ACC(Thr)0.76	ACC(Thr)0.75	ACC(Thr)0.93
ACA(Thr)1.87	ACA(Thr)1.84	ACA(Thr)1.91	ACA(Thr)1.65	ACA(Thr)1.82	ACA(Thr)1.82	ACA(Thr)1.82	ACA(Thr)1.85	ACA(Thr)1.32
ACG (Thr)0.54	ACG (Thr)0.56	ACG (Thr)0.45	ACG (Thr)0.48	ACG (Thr)0.54	ACG (Thr)0.54	ACG (Thr)0.54	ACG (Thr)0.54	ACG (Thr)0.24
GCU (Ala)1.16	GCU (Ala)1.06	GCU (Ala)1.04	GCU (Ala)1.07	GCU (Ala)1.13	GCU (Ala)1.1	GCU (Ala)1.1	GCU (Ala)1.14	GCU (Ala)1.43
GCC (Ala)0.81	GCC (Ala)0.7	GCC (Ala)0.62	GCC (Ala)0.76	GCC (Ala)0.71	GCC (Ala)0.71	GCC (Ala)0.71	GCC (Ala)0.72	GCC (Ala)0.9
GCA (Ala)1.82	GCA (Ala)1.92	GCA (Ala)2.1	GCA (Ala)1.9	GCA (Ala)1.82	GCA (Ala)1.86	GCA (Ala)1.86	GCA (Ala)1.78	GCA (Ala)1.41
GCG (Ala)0.21	GCG (Ala)0.31	GCG (Ala)0.23	GCG (Ala)0.27	GCG (Ala)0.34	GCG (Ala)0.34	GCG (Ala)0.34	GCG (Ala)0.36	GCG (Ala)0.26
UAU (Tyr)1.25	UAU (Tyr)1.22	UAU (Tyr)1.18	UAU (Tyr)1.33	UAU (Tyr)1.23	UAU (Tyr)1.23	UAU (Tyr)1.23	UAU (Tyr)1.24	UAU (Tyr)1.4
UAC (Tyr)0.75	UAC (Tyr)0.78	UAC (Tyr)0.82	UAC (Tyr)0.67	UAC (Tyr)0.77	UAC (Tyr)0.77	UAC (Tyr)0.77	UAC (Tyr)0.76	UAC (Tyr)0.6
CAU(His)1.33	CAU(His)1.33	CAU(His)1.21	CAU(His)1.37	CAU(His)1.3	CAU(His)1.3	CAU(His)1.3	CAU(His)1.3	CAU(His)1.3
CAC (His)0.67	CAC (His)0.67	CAC (His)0.79	CAC (His)0.63	CAC (His)0.7	CAC (His)0.7	CAC (His)0.7	CAC (His)0.7	CAC (His)0.7
CAA (Gln)1.02	CAA (Gln)1.05	CAA (Gln)1.04	CAA (Gln)1.19	CAA (Gln)1.05	CAA (Gln)1.05	CAA (Gln)1.05	CAA (Gln)1.06	CAA (Gln)1.41

CAG (Gln)0.98	CAG (Gln)0.95	CAG (Gln)0.96	CAG (Gln)0.81	CAG (Gln)0.95	CAG (Gln)0.95	CAG (Gln)0.95	CAG (Gln)0.94	CAG (Gln)0.59
AAU (Asn)1.18	AAU (Asn)1.25	AAU (Asn)1.24	AAU (Asn)1.29	AAU (Asn)1.27	AAU (Asn)1.27	AAU (Asn)1.27	AAU (Asn)1.26	AAU (Asn)1.26
AAC (Asn)0.82	AAC (Asn)0.75	AAC (Asn)0.76	AAC (Asn)0.71	AAC (Asn)0.73	AAC (Asn)0.73	AAC (Asn)0.73	AAC (Asn)0.74	AAC (Asn)0.74
AAA(Lys)1.08	AAA(Lys)1.08	AAA(Lys)1.09	AAA(Lys)1.14	AAA(Lys)1.08	AAA(Lys)1.08	AAA(Lys)1.08	AAA(Lys)1.07	AAA(Lys)1.22
AAG (Lys)0.92	AAG (Lys)0.92	AAG (Lys)0.91	AAG (Lys)0.86	AAG (Lys)0.92	AAG (Lys)0.92	AAG (Lys)0.92	AAG (Lys)0.93	AAG (Lys)0.78
GAU (Asp)1.36	GAU (Asp)1.41	GAU (Asp)1.28	GAU (Asp)1.35	GAU (Asp)1.34	GAU (Asp)1.33	GAU (Asp)1.33	GAU (Asp)1.33	GAU (Asp)1.29
GAC (Asp)0.64	GAC (Asp)0.59	GAC (Asp)0.72	GAC (Asp)0.65	GAC (Asp)0.66	GAC (Asp)0.67	GAC (Asp)0.67	GAC (Asp)0.67	GAC (Asp)0.71
GAA(Glu)0.97	GAA(Glu)0.93	GAA(Glu)1.08	GAA(Glu)1.09	GAA(Glu)0.93	GAA(Glu)0.95	GAA(Glu)0.95	GAA(Glu)0.92	GAA(Glu)1.28
GAG(Glu)1.03	GAG(Glu)1.07	GAG(Glu)0.92	GAG(Glu)0.91	GAG(Glu)1.07	GAG(Glu)1.05	GAG(Glu)1.05	GAG(Glu)1.08	GAG(Glu)0.72
UGU (Cys)1.05	UGU (Cys)1.12	UGU (Cys)1.18	UGU (Cys)1.05	UGU (Cys)1.12	UGU (Cys)1.11	UGU (Cys)1.11	UGU (Cys)1.1	UGU (Cys)1.19
UGC (Cys)0.95	UGC (Cys)0.88	UGC (Cys)0.82	UGC (Cys)0.95	UGC (Cys)0.88	UGC (Cys)0.89	UGC (Cys)0.89	UGC (Cys)0.9	UGC (Cys)0.81
CGU (Arg)0.36	CGU (Arg)0.42	CGU (Arg)0.31	CGU (Arg)0.35	CGU (Arg)0.39	CGU (Arg)0.39	CGU (Arg)0.39	CGU (Arg)0.39	CGU (Arg)0.32
CGC(Arg)0.12	CGC(Arg)0.19	CGC(Arg)0.28	CGC(Arg)0.26	CGC(Arg)0.19	CGC(Arg)0.19	CGC(Arg)0.19	CGC(Arg)0.19	CGC(Arg)0.27
CGA (Arg)0.69	CGA (Arg)0.67	CGA (Arg)0.66	CGA (Arg)0.61	CGA (Arg)0.63	CGA (Arg)0.66	CGA (Arg)0.66	CGA (Arg)0.65	CGA (Arg)0.58
CGG (Arg)0.55	CGG (Arg)0.38	CGG (Arg)0.33	CGG (Arg)0.5	CGG (Arg)0.38	CGG (Arg)0.37	CGG (Arg)0.37	CGG (Arg)0.39	CGG (Arg)0.52
AGA(Arg)2.61	AGA(Arg)2.56	AGA(Arg)2.85	AGA(Arg)2.79	AGA(Arg)2.67	AGA(Arg)2.65	AGA(Arg)2.65	AGA(Arg)2.67	AGA(Arg)2.9

AGG(Arg)1.67	AGG(Arg)1.78	AGG(Arg)1.56	AGG(Arg)1.49	AGG(Arg)1.74	AGG(Arg)1.74	AGG(Arg)1.74	AGG(Arg)1.73	AGG(Arg)1.42
GGU (Gly)0.71	GGU (Gly)0.81	GGU (Gly)0.99	GGU (Gly)0.77	GGU (Gly)0.85	GGU (Gly)0.85	GGU (Gly)0.85	GGU (Gly)0.82	GGU (Gly)0.78
GGC (Gly)0.68	GGC (Gly)0.62	GGC (Gly)0.43	GGC (Gly)0.8	GGC (Gly)0.62	GGC (Gly)0.58	GGC (Gly)0.58	GGC (Gly)0.58	GGC (Gly)0.72
GGA (Gly)1.69	GGA (Gly)1.66	GGA (Gly)1.66	GGA (Gly)1.75	GGA (Gly)1.55	GGA (Gly)1.56	GGA (Gly)1.56	GGA (Gly)1.6	GGA (Gly)1.79
GGG (Gly)0.92	GGG (Gly)0.9	GGG (Gly)0.92	GGG (Gly)0.67	GGG (Gly)0.99	GGG (Gly)1.01	GGG (Gly)1.01	GGG (Gly)1	GGG (Gly)0.71

7. Estimation of the CAI (Codon Adaptation Index) Values. The CAI Cal web server was used to estimate the CAI indices of the Nipah Virus isolates by taking humans as a reference set and choosing a standard genetic code. The observed CAI values for the Nipah virus in Malaysia, India and Bangladesh were observed as range 0.718-0.741 respectively. As the index range, Nipah virus from different origin isolates has a higher efficiency of the virus in adapting to the host environment. We conclude that the Nipah Virus isolates are efficient in adapting to the human host environment, as observed by CAI values.

Table 6: Codon Adaptation Index (CAI) values of Nipah virus isolates from Malaysia, India and Bangladesh

Strains and origin	MK673562 Malaysia	MH396625 India	MH523640 India	MH523641 India	FJ513078 India	JN808857 Bangladesh	JN808863 Bangladesh	MK673592 Bangladesh	MK673571 Bangladesh
Length	18231	18210	18132	18027	18252	18252	18252	18234	18240
CAI	0.719	0.718	0.71	0.735	0.719	0.719	0.719	0.718	0.741

8. Comparative Assessment of Nipah Virus Envelope Protein Translation Efficiency in the Human Host: The translational efficiency of the Nipah virus (NiV) envelope protein in humans was assessed by comparing its preferred codons with the abundance of corresponding human tRNA isoacceptors. Data from Indian, Malaysian, and Bangladeshi NiV isolates (Table 7) revealed that 13 of the 18 preferred codons in the envelope gene match the most abundant human tRNAs. While some codons correspond to tRNAs with low or no gene copies in the human genome, several—including Ala (GCA/GCU), Gly (GGA), Lys (AAA), and Asp (GAU)—show strong compatibility, suggesting efficient translation in human cells. Codons for Pro, Thr, Ser, Arg, Leu, and Glu exhibited moderate compatibility, likely reflecting nucleotide composition constraints. Overall, the envelope gene displays a codon usage pattern indicative of evolutionary adaptation to the human translational system, balancing protein expression efficiency with genomic composition limitations.

Table 7: Comparative Assessment of Most Preferred Codons in Nipah Virus Strains from India, Malaysia, and Bangladesh concerning Human tRNA Isotype Availability

Amino Acids	Indian Nipah virus: Most Preferred codon	Malaysia Nipah virus Most Preferred codon	Bangladesh Nipah virus: Most Preferred codon	Human tRNA isotype
Ala	**GCA**, GCU	**GCA**, GCU	**GCA**, GCU	AGC(22) GGC(0) CGC(4) UGC(8)

Gly	GGA	GGA	**GGA**, GGG	ACC(0) GCC(14) CCC(5) UCC(9)
Pro	CCU, **CCA**	CCU, **CCA**	CCU, **CCA**	AGG(9) GGG(0) CGC(4) UGG(7)
Thr	ACA, ACU	ACA	ACA,ACU	AGU(9) GGU(0) CGU(5) UGU(6)
Val	GUA, **GUG,GUU,**GUC	**GUA,** GUG	GUA, **GUG**,**GUU, GUC**	AAC(9) GCA(0) CAC(11) UAC(5)
Ser	**UCA,** UCU, AGU	UCU,**UCA**	UCA, UCU,**AGU**	AGA(9) GGA(0) CGA(4) UGA(4) ACU(0) GCU(8)
Arg	**AGA,** AGG	**AGA**, AGG	**AGA**, AGG	ACG(7) GCG(0) CCG(4) UCG(6) CCU(5) UCU(6)
Leu	UUA, **UUG,** CUA	UUA, **UUG,** CUA	UUA, **UUG,** CUA,CUU	AAG(9) GAG(0) CAG(9) UAG(3)CAA(6) UAA(4)
Ile	AUA,AUU,AUC	AUA	AUA,AUU,AUC	AAU(14) GAU(3) CAU(0) UAU(5)
Glu	GAG,GAA	GAG	GAG,GAA	CUC(8) UUC(7)
Phe	UUU	UUU	UUU	AAA(0) GAA(10)
Asn	AAU	AAU	AAU	AUU(0) GUU(20)
Lys	AAA	AAA	AAA	CUU(15) UUU(12)
Asp	GAU	GAU	GAU	AUC(0) GUC(13)
His	CAU	CAU	CAU	AUG(0) GUG(10)
Gln	CCA	CCA	CCA	CUG(13) UUG(6)
Tyr	UAU	UAU	UAU	AUA(0) GUA(13)
Cys	UGU	UGU	UGU	ACA(0) GCA(29)

DISCUSSION

The comparative analysis of codon usage in Nipah virus strains from different geographical origins underscores the virus's evolutionary plasticity and varying degrees of adaptation to the human host. The interplay between nucleotide composition, codon preference, and host tRNA availability reveals a complex optimization landscape that shapes viral fitness and translational efficiency. These insights not only deepen our understanding of NiV-host interactions but also inform strategies for the rational design of vaccines and therapeutic constructs. In summary, the codon usage analysis of Nipah virus strains from India, Malaysia, and Bangladesh underscores the intricate balance between evolutionary pressures and functional adaptation. The observed codon biases, shaped by mutational patterns and host translation machinery, have important implications for viral pathogenesis, epidemiology, and biomedical intervention strategies. As genomic surveillance expands and more host-specific data becomes available, codon usage studies will remain a vital tool in understanding emerging viral threats and developing precision-based countermeasures. Overall, the analysis highlights the significance of codon-tRNA matching in viral adaptation and underscores its potential application in rational vaccine development. Specifically, understanding codon preferences can aid in the design of codon-deoptimized or attenuated viral constructs that reduce viral fitness while preserving antigenicity—an approach valuable for live-attenuated or synthetic vaccine strategies**.**

CONCLUSIONS

The codon usage analysis of Nipah virus strains from India, Malaysia, and Bangladesh demonstrates that Nipah virus genomes are characterized by low codon bias, largely influenced by mutational pressure with additional contributions from translational selection. The preferential use of AT-rich codons and their correspondence with highly abundant human tRNAs suggest a degree of host adaptation that may enhance viral replication efficiency and fitness. These findings emphasize the role of codon bias as both an evolutionary signature and a functional determinant in NiV–host interactions. Furthermore, the insights gained hold translational value, particularly for the rational design of codon-optimized vaccine candidates and codon-deoptimized constructs for live-attenuated vaccine strategies. Thus, codon usage studies represent a powerful approach to understanding viral evolution, host adaptation, and informing precision-based biomedical interventions against emerging pathogens**.**

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