M.Tech (Bioinformatics)

SHREYA BHARDWAJ

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T-CELL AND B-CELL EPITOPE PREDICTION FOR MULTI-EPITOPE PEPTIDE VACCINE AGAINST KYASANUR FOREST DISEASE VIRUS

A DISSERTATION

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MASTER OF TECHNOLOGY

In

BIOINFORMATICS

Submitted by

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CANDIDATE'S DECLARATION

I, **SHREYA BHARDWAJ** (2k21/BIO/04) of M.Tech (Bioinformatics), hereby declare that the project Dissertation titled **"T-cell and B-cell epitope prediction for multi-epitope peptide vaccine against Kyasanur Forest Disease Virus"** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in the partial fulfillment of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

Shreya

SHREYA BHARDWAJ

Place: Delhi

Date: 13-06-2023

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CERTIFICATE

I hereby certify that the project Dissertation titled **"T-cell and B-cell epitope prediction for multi-epitope peptide vaccine against Kyasanur Forest Disease Virus"** which is submitted by **SHREYA BHARDWAJ, 2K21/BIO/04** to the Department of Biotechnology, Delhi Technological University, Delhi in the partial fulfillment of the requirement for the award of the degree of Master of Technology. is record of the project work carried out by the students under my supervision. To the best of my knowledge this work has not been submitted in part for any Degree or Diploma to this University or elsewhere.

Place: Delhi

Date: 13-06-2023

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Shreya

SHREYA BHARDWAJ

(2K21/BIO/04)

ABSTRACT

Kyasanur Forest Disease Virus (KFDV) is a tick-borne flavivirus that is transmitted to humans through the bite of infected ticks, it endemics primarily in south-western regions of the Indian subcontinent, mainly Karnataka, Kerala, Tamil Nadu, Goa, and Maharashtra. The disease poses a significant public health challenge, with no licensed vaccines currently available for its prevention. KFDV is a seriously underreached virus, despite its high morbidity and mortality rates. This study aims to address this critical gap by employing an in-silico approach to design a peptide-based vaccine against KFDV. Through a systematic in-silico approach involving structural and antigenic analyses, B-cell and T-cell epitope prediction, as well as molecular docking studies, we identified four B-cell epitopes and 11 T-cell epitopes with desirable characteristics such as high affinity, low allergenicity, strong antigenicity, minimal toxicity, stability, and the ability to induce IFN-gamma. Furthermore, molecular docking analyses confirmed the robust binding of the epitopes with their respective MHC alleles. These findings provide valuable insights into potential targets for vaccine development against KFDV. However, further experimental validation and preclinical studies are warranted to evaluate the immunogenicity and protective efficacy of these epitopes. The development of an effective vaccine against KFDV would be crucial in controlling the spread of this disease and safeguarding vulnerable populations at risk of infection.

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LIST OF ABBREVIATION

KFDV	Kyasanur Forest Disease Virus
KFD	Kyasanur Forest Disease
WHO	World Health Organisatio
TBE	Tick-Borne Encephalitis
NS	Non-Structural
NCBI	National Center for Biotechnology Information

CHAPTER 1

INTRODUCTION

Since Dr. Edward Jenner's discovery of vaccines in the late 18th century, the world of disease prevention has been revolutionized. Vaccines have emerged as an important component of global health efforts, effectively reducing disease transmission and impact worldwide. According to the WHO data, proper vaccination has prevented millions of deaths due to infectious diseases every year. [1]Community protection is another main advantage of vaccines, it not only protects individuals from getting infected, but it also has a role in limiting the transmission of disease in commumity. Research has shown that vaccines are a highly cost-effective investment, with exceptional returns on investment in terms of both direct healthcare expenditures and broader economic advantages. [2]

Vaccinations have proved highly efficient in controlling a wide range of infectious diseases, yet, there are still a number of diseases for which effective vaccinations have yet to be discovered. To fill this void, novel approaches to vaccinology, such as non-viral vaccine technologies and viral vector platforms, have evolved. Non-pathogenic viral backbones are used in viral vector vaccines to deliver antigens unrelated to the vector itself. [3]Another interesting option is the use of immunoinformatic technologies to generate Multi-Epitope Vaccines (MEVs), this includes predicting epitopes using dependable servers, linking epitopes with appropriate linkers, and assessing their physicochemical, immunological, and structural features using bioinformatics techniques. Bioinformatics approaches and applications have been used to support many stages of vaccine development, from the preclinical to the post-clinical trial stage.[4]

Vaccines are particularly relevant when dealing with diseases that currently lack a cure or viable and effective treatment options. For such diseases, prevention is the primary focus in mitigating the repercussions that may arise if the disease progresses unchecked.

KFD is a viral haemorrhagic fever transmitted by ticks and commonly found in the southern-western parts of India.[5] KFDV is spread by infected hard ticks, specifically *Haemaphysalis spinigera*, which act as reservoirs for the virus. KFD, can have lethal consequences for both humans and primates. It belongs to the Flavivirus genus and is part of the TBE complex. While H. spinigera is considered the primary vector, a variety of tick species, small rodents, primates, and birds are thought to contribute to the KFDV transmission cycle. Cattle, on the other hand, are key hosts for adult H. spinigera ticks but have immunological responses that prevent virus proliferation.[6] Although 80% of individuals recover without incident, still 20% may experience a biphasic presentation of symptoms, with a few developing serious haemorrhagic or neurological indications. [7] In recent years, the burden of KFD has spread beyond Karnataka's Western Ghats, reaching neighbouring states along the Western Ghats' route. The rising number of cases highlights the need for increased efforts to combat this disease.[8]

Even though KFD is common in endemic areas, little is know about it , and unfortunately, there is no cure for KFD at this time. Therefore, the primary and the present vaccination has low efficacy and limited coverage. Currently, there are very few studies on KFDV vaccine development, so through this project, an attempt is made to construct an epitope-based vaccine against KFDV. Epitopes are short amino acid sequences capable of generating specific immunological responses. This trait will also address the drawbacks of currently available live attenuated vaccines against KFDV.

CHAPTER-2

LITERATURE REVIEW

2.1 Kyasanur Forest Disease

Kyasanur Forest disease (KFD) is attributed to the KFDV, is classified within the Flaviviridae family and Flavivirus genus. Serological investigations and phylogenetic sequencing suggest that KFDV belongs to a cluster TBV associated with hemorrhagic fever in mammals, sharing close similarity with the Alkhurma virus [9][10] where it resulted in a sizable number of fatalities. It is believed that a number of variables contributed to the genesis of this novel viral illness.

Common clinical symptoms start to manifest in 3 to 8 days [11] after the exposure to the virus, clinical symptoms include fever, headache, body aches, vomiting, and prostration.[12] Typically, 14 days after the initial infection, the majority of people start to feel better. However, occasionally there is a 1-2 week remission period, which is followed by a second phase with neurological symptoms. Intense headaches, confusion, trembling, stiff muscles, sensitivity to light, discomfort in the eyes, and blurred vision are some of these symptoms that may be present. [5]

2.2 Epidemiology and the Geographical Presence.

In recent studies, scientists concentrated on figuring out the vector that transmits KFDV. Investigations identified the most likely vector as the tick species Hemaphysalis spinigera. Ticks belonging to the Hemaphysalis spp. family were found on dead or ill monkeys, and viral isolation from these ticks' larvae and nymphs proved the tick species' involvement in KFDV transmission. Due to its association with forest environments and the involvement of various vertebrate species in its transmission cycle, KFDV is classified as a risk group 4 pathogen, indicating its potential danger. It also causes an endemic disease with unpredictable ecology and epidemiology.

The spread of KFDV started in Forest of the Shimoga, Karnataka, in March 1957. [10]Although there is no record of the illness prior to the epidemic in 1956–1957, it is conceivable that the virus had been circulating in the region and occasionally infecting people. In addition, local non-human primate mortality might not have been recognised as the result of a novel disease or might have been attributed to other factors. [13]

A considerable expansion of population occurred in the during the 1950s, which resulted in significant environmental changes, including deforestation and modifications to land-use practises. These changes might have made it easier for people to invade the primitive forest, perhaps exposing them to novel infections like KFDV. [13]A more recent study on the spread of KFD beyond its usual geographical boundary has also clearly indicate that deforestation plays role in determining the suitability of landscapes for the expansion of this severe

infection. Furthermore, the study reveals a strong correlation between the rise in KFD cases, the loss of forest cover, and the presence of diverse wildlife species.[14]

Notably, KFDV infections have extended beyond the initial outbreak in Shimoga district. Recent studies have also detected the KFDV presence in neighbouring states, suggesting its prevalence and potential spread to nearby geographical locations. [15]Outbreaks have occurred in different regions, including Thirthahalli and Shimoga District in Karnataka, Goa, and Sindhudurg district in Maharashtra. The new areas in which the disease has highlights the dynamic nature of KFDV and its ability to affect regions beyond its traditional hotspots.[16][17][18]

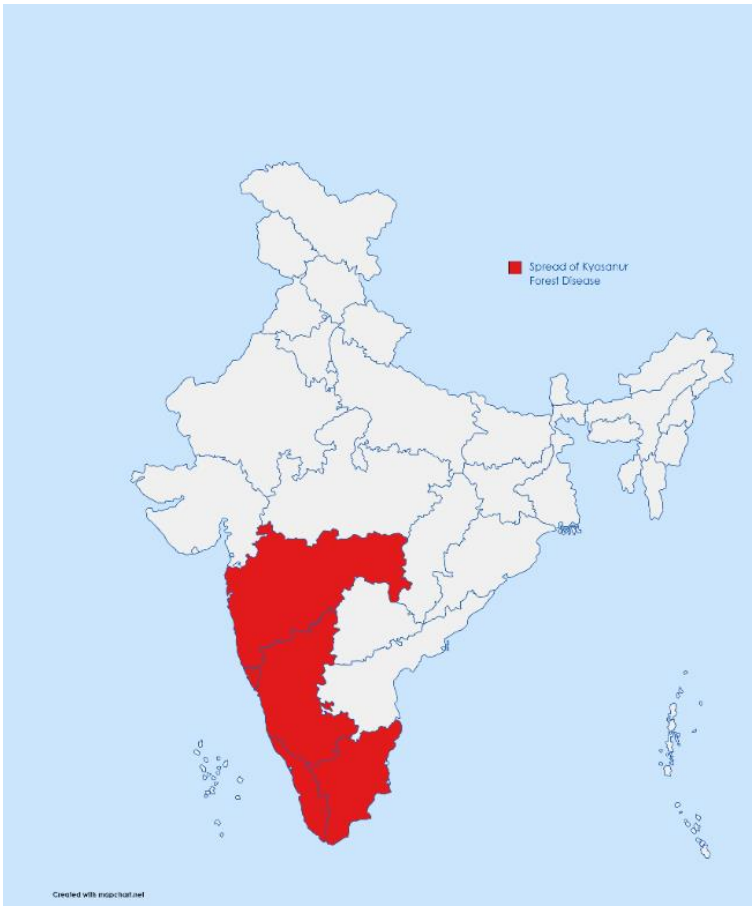

Figure-2.2.1 : Epidemiological presence of KFDV

The estimated number cases of KFD from year 1957 to 2017 were 9594 within 16 districts in India .The largest outbreak in recent times was observed in 2017, with a considerable number of cases reported. These evolving patterns indicate the changing epidemiology of KFDV, with an increasing number of cases occurring outside the traditional Shimoga region.[19], [20]

2.3 Structure of KFDV

KFDV has a single-stranded, + RNA genome. The genome is roughly 11 kb in size and seven non-structural in addition to3 proteins, C, E, M. The icosahedral that surrounds the virus is spherical in shape and ranges in size from 40 to 65 nm. A single-stranded, positively polarised RNA molecule with a length of 10,774 nucleotides makes up its genome. [21]

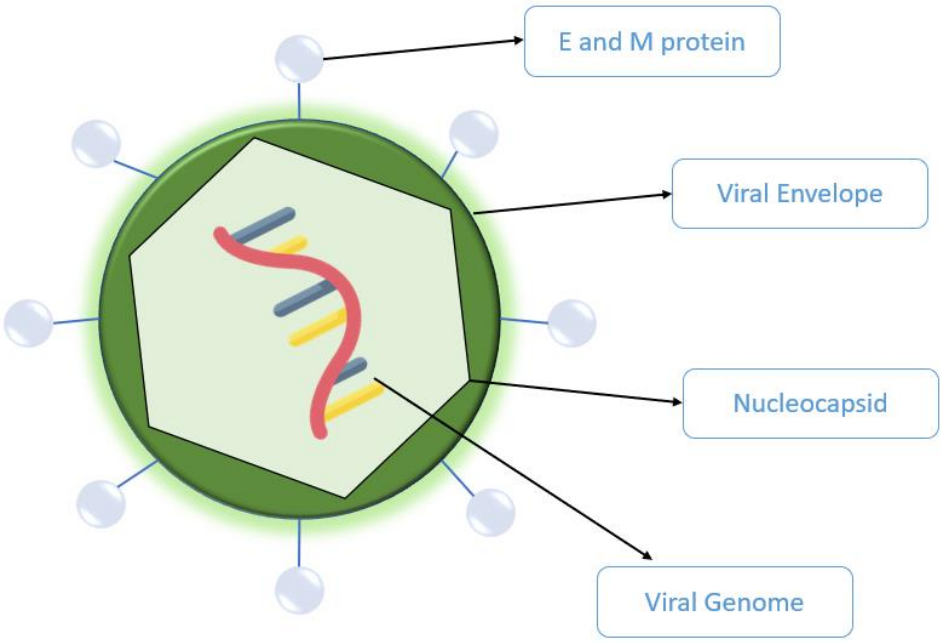

Figure-2.3.1 : Structure of KFDV

Individual viral proteins, such as C, prM, Eand NS5, are produced by proteolytic polyprotein that the RNA codes for.

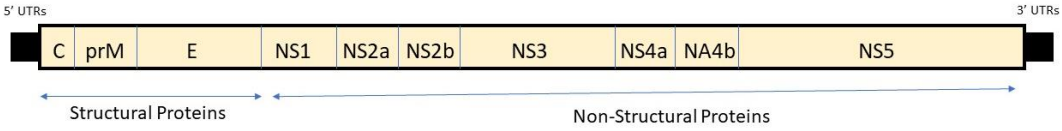

Figure-2.3.2 : KFDV Polyprotein Construct

Several proteins that the KFDV virus contains are crucial to its pathogenesis, immunological evasion, and replication. Capsid protein C interferes with the host Dicer enzyme to prevent RNA silencing. To ensure normal virion maturation and immune evasion, prM acts as a shield for the fusion peptide of envelope protein E. The tiny envelope protein M, via triggering a mitochondrial apoptotic pathway, may contribute to virus budding and have cytotoxic effects.[22] [23]

Non-structural protein 1 (NS1) participates in replication complex and is involved in immune evasion, viral replication. The non-structural protein 2A (NS2A) inhibits the host immune response. The serine protease activity of NS3, which carries out autocleavage and cleaves the viral polyprotein, requires the serine protease subunit NS2B as a cofactor. NS3 also functions as an NTPase and an RNA helicase. The ATPase activity of the NS3 helicase is regulated by non-structural protein 4A (NS4A). The interferon antagonistic activity of NS4B is aided by peptide 2k, which serves as a signal peptide for NS4B. NS4B suppresses the host interferon-alpha/beta pathway while inducing the creation of membrane vesicles derived from ER for viral replication. Finally, NS5 blocks the interferon signalling pathway while replicating the viral RNA genome and performing capping to avoid the induction of a cellular antiviral state. [23] [22][21]

2.4 Life-cycle of *Haemaphysalis spinigera* and transmission of KFDV

Small mammals, especially rodents, have long been known to act as reservoirs for flaviviruses carried by ticks, including KFD. These animals are crucial in the transmission cycle and can harbour the virus without displaying symptoms. [24] Recent studies, however, indicate that ticks themselves may be able to maintain the diseases without primarily relying on mammalian reservoirs through transstadial and trans-ovarian transmission[25]. Also thought to be a more effective method of virus transmission among ticks is co-feeding on a mammalian host.

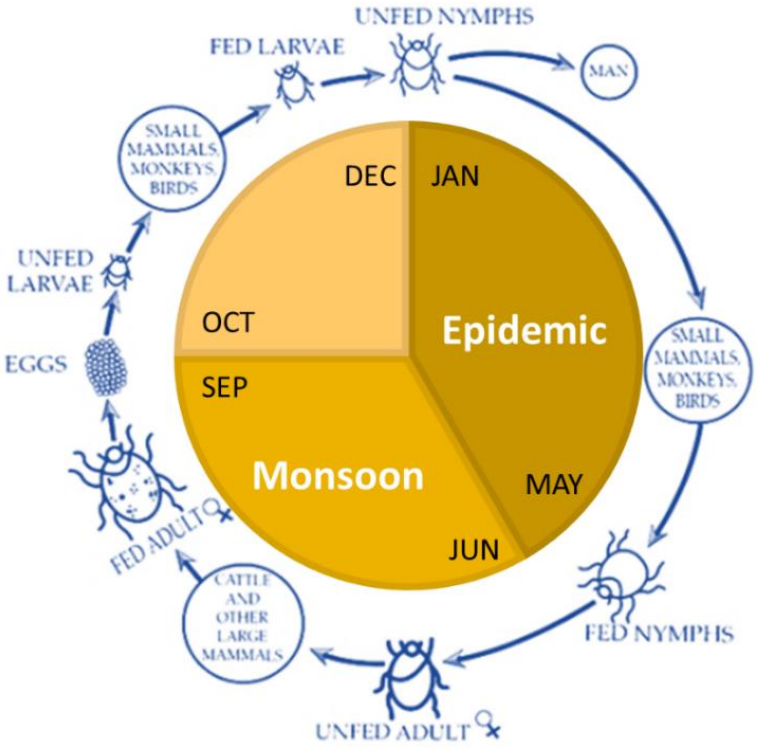

Figure-2.4.1 : Life cycle of *Haemaphysalis spinigera*

In the endemic region of Karnataka, India, H. spinigera ticks are the main vector species and KFD is largely transmitted through the bites of infected Haemophysalis ticks . According to Ajesh et al. , these ticks can pick up and spread the virus at any point in their life cycle, including through transstadial transmission and vertical transmission to their offspring. [26]In order to facilitate virus transmission between ticks without infecting the host.[27]

In strictly controlled laboratory settings, the life cycle of H. spinigera lasts between 118 and 160 days and. [28] Male and female adult ticks, after remaining unfed for a period of 8-13 days. They separate from the host once they are fully engorged, and the females deposit eggs in 2 to 5 days. [28]The eggs hatch into larvae after a 25–30-day incubation period. After 5-7 days, these larvae typically eat small animals, monkeys, and birds, then in the next few days the larvae moult and change into nymphs. For a period of 25 to 30 days after moulting, H. spinigera tick nymphs can feed on animals, including humans, primates, small mammals, & birds. [29]

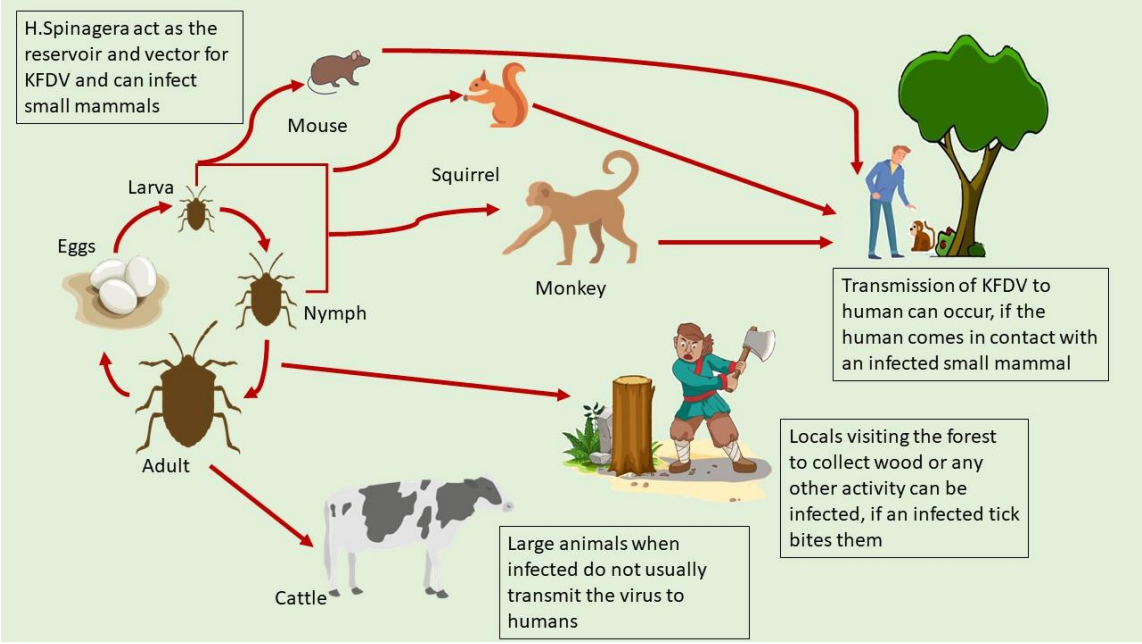

Figure-4.4.2 : Transmission cycle of KFDV

In the course of the KFD life cycle, the virus is spread from infected nymphs to various species, such as monkeys and small mammals, which unintentionally infect humans while dining on ticks. The transmission cycle is maintained by the ticks as they develop through a variety of life stages, from eggs to larvae, nymphs, and adults.

Although mosquitoes can transmit some viruses vertically, the long-term persistence of a virus within mosquito populations is constrained by its short lifespan. Ticks, on the other hand, live for several years, which is a far longer lifespan. According to Pattnaik's study, these investigations involved surveys of animals taken from the wild or experimental infections carried out in lab settings.[29] However, no small animals

has emerged as a significant reservoir for KFDV, demonstrating once again that ticks are the virus' primary host.

In the past 60 years there have been no documented instances of nosocomial KFD infections or cases reported by hospitals, indicating the absence of transmission within healthcare settings or close contacts. Furthermore, no patterns of clustered cases suggestive of person transmission have been observed. [30]

2.5 Mechanism of KFDV Pathogenesis

Despite having a long history, our knowledge about the dynamics of KFDV, its distribution in tissues and bodily fluids, and the immune response following infection remains limited. Gaining insights into viral RNA in different biological fluids is crucial for comprehending the pathogenesis and transmission patterns. Research indicates that KFDV RNA copies per milliliter are more abundant in blood compared to other body fluids, and the presence of viremia can persist for up to post-infection. Recent studies conducted on Macaca radiata monkeys suggest that infected monkeys can excrete the virus in various bodily fluids. [31] Furthermore, degenerative changes have been observed in tissues and organs. Despite having a long history, our knowledge about the dynamics KFDV, its distribution in tissues and bodily fluids, and the immune response following infection remains limited. Gaining insights into the presence of viral RNA in different biological fluids is crucial for comprehending the pathogenesis and transmission patterns of KFDV in humans. Research indicates that KFDV RNA copies per milliliter are more abundant in blood compared to other body fluids, and the presence of viremia can persist for up to two weeks. Recent studies conducted on Macaca radiata monkeys suggest that infected monkeys can excrete the virus in various bodily fluids during the early stages of illness. Furthermore, degenerative changes have been observed in tissues and organs. [32][33]

2.6 Management and Prevention of KFDV

Since KFDV in humans is currently untreatable with no proven antiviral drug, prompt hospitalisation and supportive therapy are crucial when it comes to dealing with KFDV infections to avoid escalation of the disease due to negligence. With documented fatality rates ranging from 0.9% to 33%, KFDV is a serious condition that can be fatal. [18][34] [35]While supportive care is primary course of action for the management of KFD, more intensive care may be needed in some circumstances to prevent mortality.

Supportive care to treat KFDV includes preserving normal blood cell counts [36] Anticonvulsants and corticosteroids are available for nerve problems, whereas antipyretics, painkillers, antibiotic therapy, and blood transfusions may be required for secondary infections [34]. IFN-α2a, a routinely used therapy, was found to be ineffective in lowering KFDV viral titres in studies examining its efficacy. Other IFN-/ subtype concentrations were also evaluated, however even though they appeared to cause less cellular damage, KFDV replication remained resistant to some subtypes. It has been determined that the KFDV protein NS5 has a role in how well the virus resists the antiviral effects of IFN. Therefore, it is necessary to investigate alternate therapeutic alternatives for those who have KFD ([37]).

Since there are no licenced antivirals to treat KFDV, vaccination and avoiding tick bites are crucial for infection control. Villagers should avoid cattle grazing in deep forests, refraining from sleeping on outdoor surfaces, and regularly inspecting and removing ticks from cattle through weekly washing. While entering forest measures should be taken to avoid being bitten by an infected tick, these measures include generous use of insect repellents, wearing clothes that covers the entire body, it is also advisable to treat the clotting with substances like permethrin to repel ticks. By following these practices, individuals can minimize the risk of tick bites and potential transmission of diseases. [38]

Despite India's efforts to spread preventive vaccines, which have had only modest success [39] KFD continues to spread. The effects of this illness are particularly severe for a number of vulnerable populations, which includes farmers, fire-wood collectors, and tribal populations who depend on forests for their livelihood. It is

necessary to comprehend KFD and its transmission better because of things like poor healthcare access, low disease awareness, and the virus's high pathogenicity.

2.7 History of Development of Vaccine

The WRAIR and the ICMR worked together to develop the first KFD vaccine, which was derived from a formalin-inactivated, mouse-brain preparation of RSSEV [40] Although the RSSEV vaccine showed protection against KFDV in mice, it did not induce a strong immune response in humans and failed to offer a substantial level of protection in vaccinated people during subsequent epidemiologic studies [41]

Then efforts were made to create a vaccine using KFDV as the basis. The Haffkine Institute in Bombay generated a formalin-inactivated vaccination utilising chick embryo fibroblasts that had a mediocre level of efficacy. After receiving the two-dose vaccine, 50% of previously non-immune participants in a study at the Virus Research Centre in Poona produced neutralising antibodies against KFDV [42]

India currently has a licenced KFDV-based formalin-inactivated vaccine that was created in the early 1990s. It was initially field-tested in a sizable population, with over 61,000 people receiving both doses, administered on a two-dose regimen along with regular booster shots. [43] Studies conducted revealed a 59% efficacy of the vaccine In the ongoing battle to control KFD in impacted areas, this vaccine serves as a critical preventive tool.

Given the current limitations of vaccine efficacy and the absence of FDA-approved vaccines for KFDV, the exploration of alternative vaccine options such as synthetic peptide vaccines holds promise for further development. KFDV is categorized as a priority pathogen by the NIAID due to its highly pathogenic nature and the lack of approved vaccines and therapeutics by the US FDA. Additionally, the infectious dose required for KFDV infection remains unknown [44]

S.no	Year	Type of Vaccine	Developed by	Efficacy	Reference
1	1962	Russian Spring Summer Encephalitis vaccine	ICMR and Walter Reed Institute	Zero efficacy	[40][19] [41]
2	1967	tissue culture vaccine	Experimental Vaccine	The mice were effectively protected by these vaccines.	[45], [46]
3	1991	formalin-inactivated vaccination utilising chick embryo fibroblasts	The Haffkine Institute in Bombay	59% efficacy post administration of two doses	[42][43] [47]

Table-2.7.1 : Evolution of Vaccines against KFDV

2.8 New-generation Peptide Vaccines

The development of peptide vaccines employing a rational design approach has been greatly aided by developments in our understanding of how antigens are recognised at the molecular level. The idea behind peptide vaccines is to create certain immunodominant B and T-cell epitopes that can trigger focused immune reactions. The resultant peptide vaccine becomes immunogenic and can trigger a particular immune response by coupling the B-cell epitope of a target molecule with a T-cell epitope.[48]) Due to the simplicity of manufacturing, chemical stability, and lack of infectious potential of peptides, they have become appealing candidates for the

development of vaccines. They have made progress through clinical studies with positive clinical outcomes, showing encouraging results in the creation of vaccinations against many forms of cancer. For both possible therapeutic and preventive immunotherapy uses, peptide vaccination is currently the subject of intensive research. There are still some issues, though, including the need for more potent adjuvants and carriers as well as the problem of poor immunogenicity. Nevertheless, continuing studies are significantly advancing the removal of these obstacles and raising the effectiveness of peptide vaccines.[49]

Figure-2.8.1 : Advantages of using Peptide vaccines

CHAPTER 3

METHODOLOGY

3.1 Retrieval of polyprotein sequence

In order to predict peptide vaccine epitopes for KFDV, The NCBI database was explored to retrieve FASTA sequence KFDV's structural and non-structural proteins, accession number of the KFDV genome polyprotein was MG720120. There are 3 structural protein components in the polyprotein –Capsid Protein, Protein M, protein E-protein and there are 7 non-structural proteins -. The FASTA sequences for all ten proteins were retrieved from NCBI, providing the necessary information for further analysis and prediction of potential of a multi-epitope peptide vaccine against KFDV.[50], [51]

3.2 Antigenicity and physiochemical properties of protein sequence

In the second step of the methodology for predicting peptide vaccine epitopes for KFDV, the antigenicity and physicochemical characteristics of the target protein sequences were assessed. To begin, the VaxiJen v2.0 server (available at was employed with a threshold value of 0.4 to determine the antigenicity of the protein sequences. VaxiJen is a bioinformatics tool specifically designed for predicting potent antigens within pathogens. [52]

In the provided space, the protein sequence was entered, and the target organism (virus) was selected. The threshold value was set to 0.4, and then the submission button was clicked.

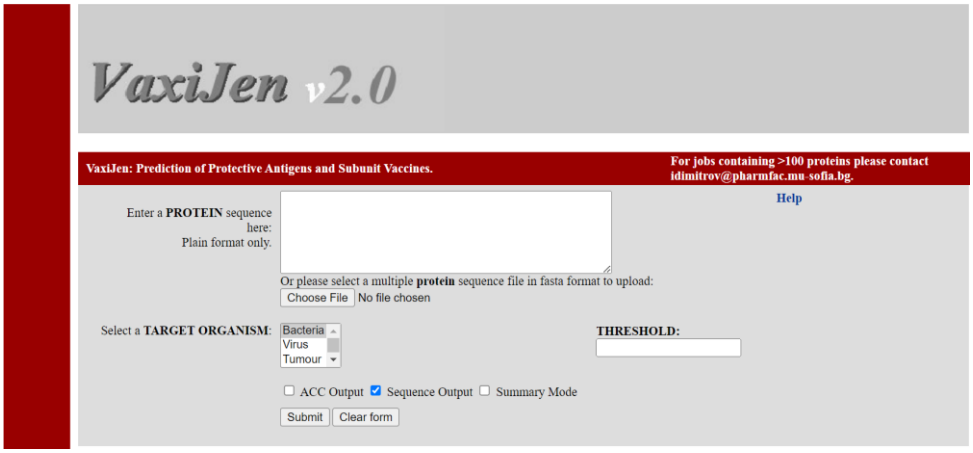

Figure-3.2.1: VaxiJen interface

The next tool used is Expasy Protparam tool was utilized to study the physicochemical properties of the target proteins. This tool computes various parameters, including molecular weight, theoretical pI, amino acid composition, GRAVY, and instability index. The ProtParam tool is capable of calculating these properties for a given protein sequence without requiring additional information about the protein[52]

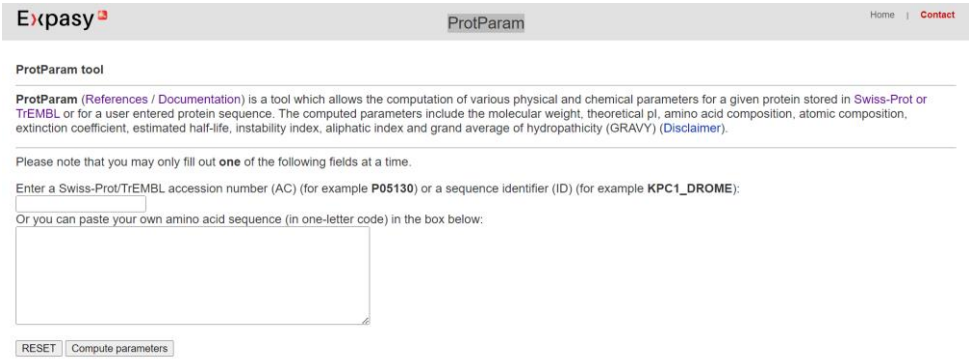

Figure-8: Expasy Protparam Server interface

In the space provided protein sequence was pasted and parameters were computed

By performing these analyses, the antigenicity and various physicochemical properties of the target protein sequences from KFDV can be evaluated, providing

valuable information for the subsequent prediction of potential epitopes for the development of a multi-epitope vaccine for KFDV.

3.3 B-cell epitope prediction

To accomplish this, the B cell epitope prediction was done by IEDB B cell epitope prediction server available at Numerous databases are accessible for B-cell epitopes; however, the IEDB database is widely recognized for its comprehensiveness and extensive collection. It encompasses a vast number of confirmed epitopes and non-epitopes, making it an optimal choice for this study. This process enables the identification of specific regions within proteins that are likely to elicit an immune response, potentially serving as targets for the development of KFDV-specific peptide vaccines in the future. In the space provided, submit the sequence.

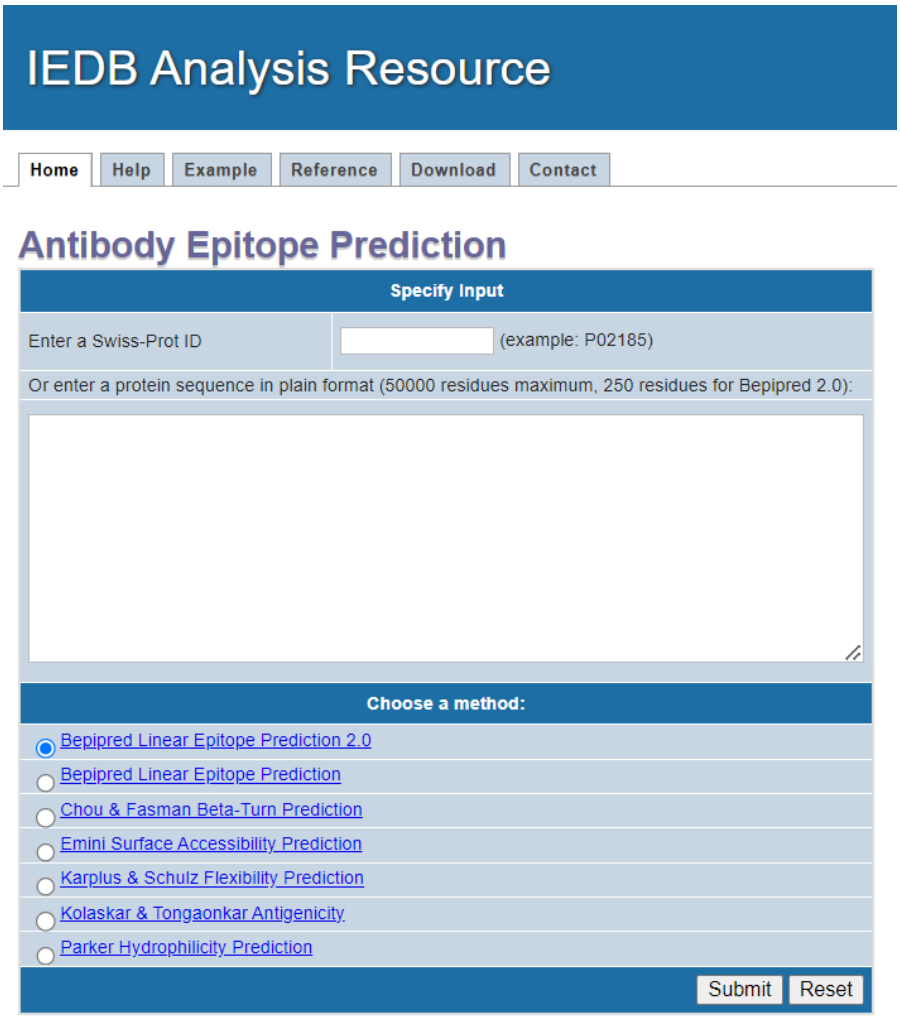

Figure-3.3.1 : IEDB B-cell epitope interface

3.4 T-cell – MHC-I and MHC-II epitope prediction

T-cells play a crucial role in adaptive, hence are important when designing epitopes for peptides CTLs are involved in directly killing infected cells, while HTLs recognize antigens and activate B-cells and CTLs to eliminate infected target cells. To predict the T-cell epitopes, IEDB was utilized[53]

For MHC-I, the ANN 4.0 prediction method was employed. This method uses artificial neural networks to predict the binding affinity of peptide sequences to MHC-I molecules.

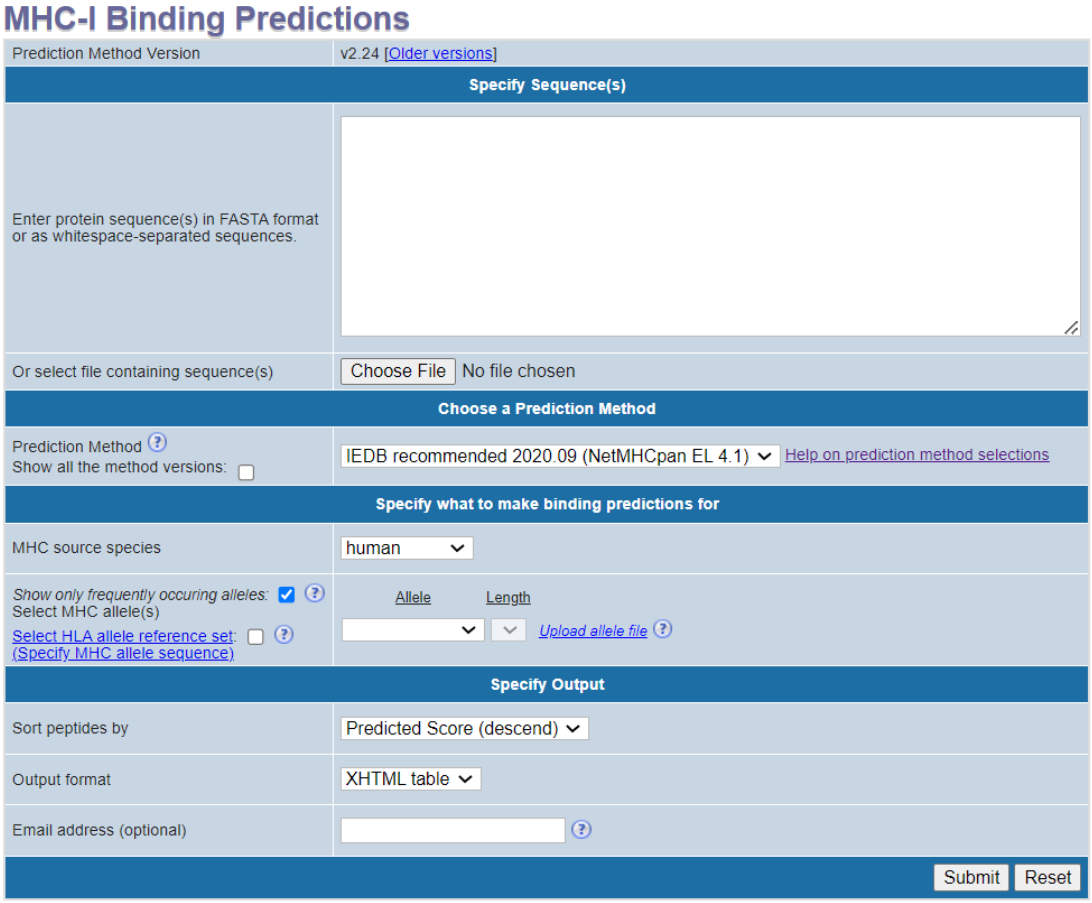

Figure- 3.4.1 : IEDB T-cell MHC -I epitope interface

MHC-II restricted CD4+ epitopes were predicted using the NN-align 3.0 prediction method.[53]This method utilizes a neural network-based algorithm to predict the binding affinity between peptide sequences and MHC-II molecules.

The parameters used to screen the epitopes were low IC50 value pI values. The ic50 value threshold for this study was select as less than or equal to 100 nm.

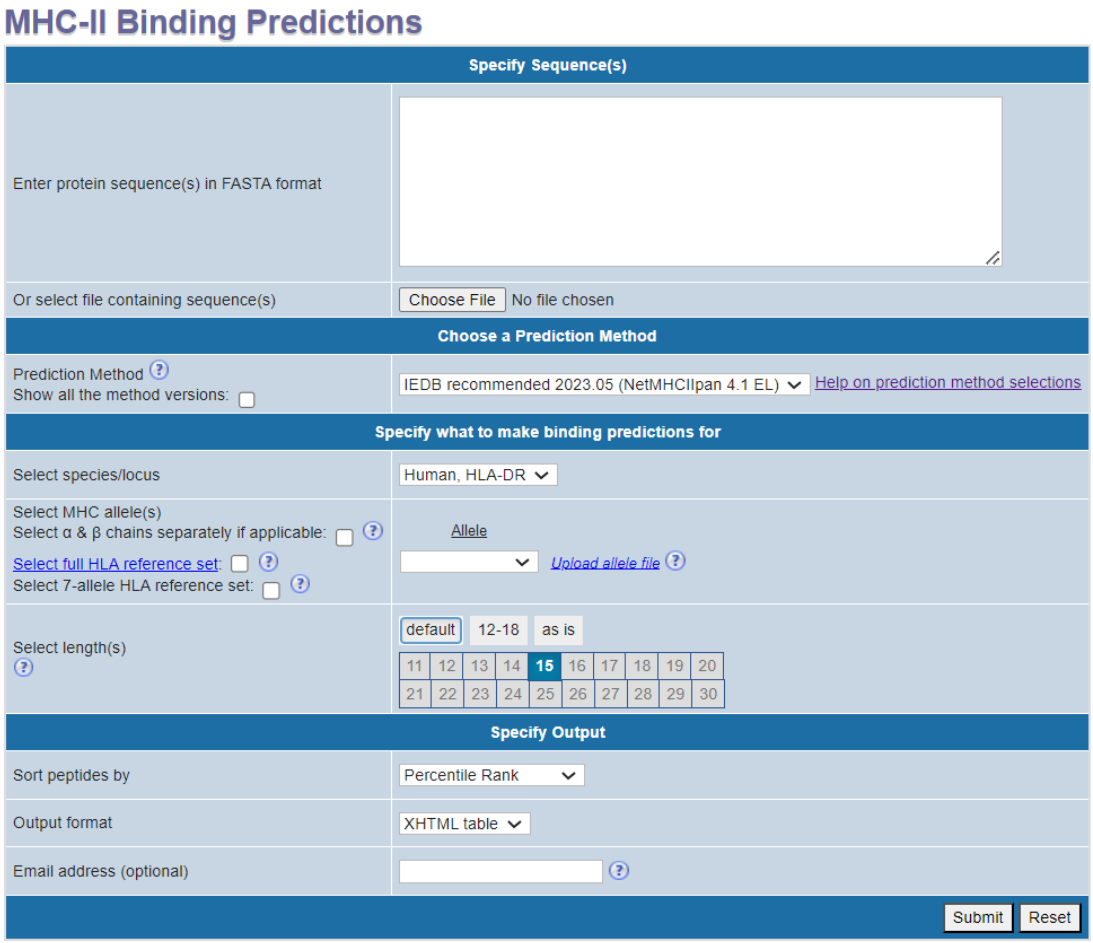

Figure- 3.4.2: IEDB T-cell MHC -II epitope interface

3.5 Antigenicity, allergenicity, and toxicity analysis of the selected epitopes

Anitigen of the selected epitopes was checked using VaxiJen and Physical properties were studies by Expasy Protparam same as done in 3.2

To assess the allergenicity, the tool AllerTOP v.2.0 was used. AllerTOP is an. [54]With the help of this tool, the potential allergenic properties of the selected epitopes were evaluated, this is done to ensure the safety of the vaccine.

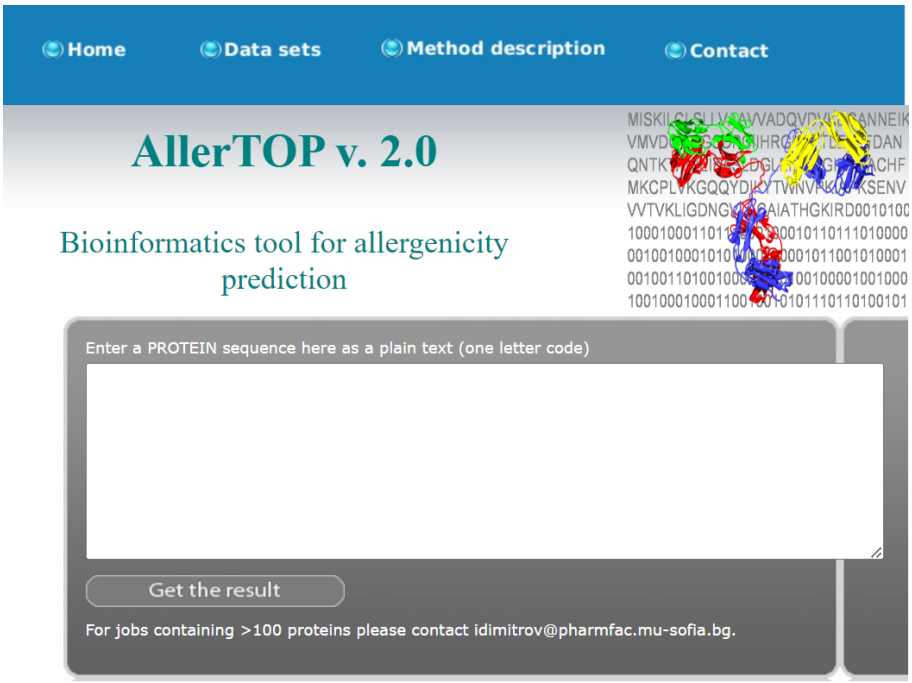

Figure-3.5.1 : AllerTOP v.2.0 interface

The toxicity prediction was performed using ToxinPred [55]. Which uses SVM based method to predict the toxicity of protein sequences by analysing the epitopes. This is crucial for the evaluation and selection of safe and effective epitope for the vaccine.

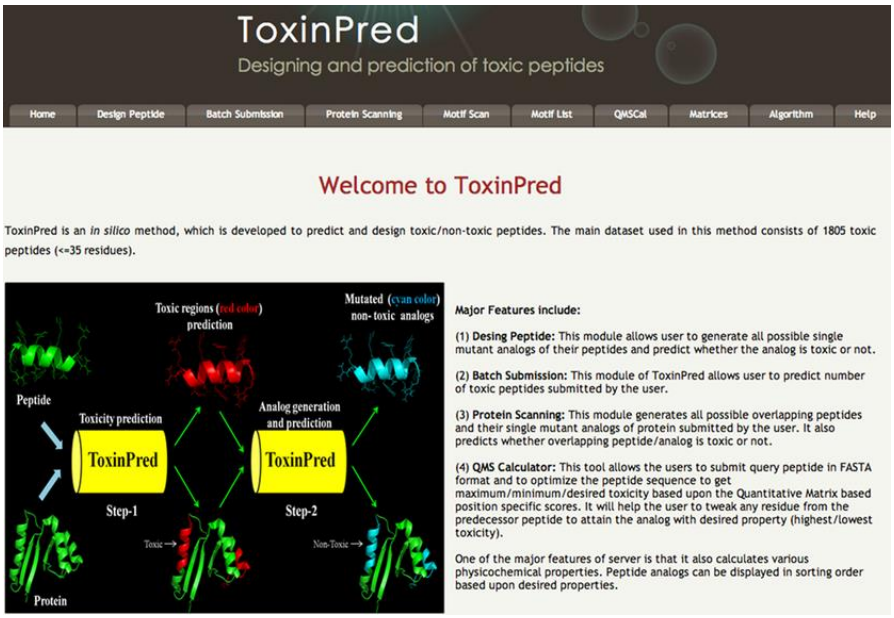

Figure-3.5.2 : ToxinPred interface

IFN-gamma using an online tool IFNepitope. This is done as IFN-gamma plays a key role in antigen presentation, enhances immune responses, regulates inflammation, and supports immune surveillance[56]

3.6 Human Homology Studies

Homology studies are done to select antigens that do not share sequence similarity with host proteins, particularly human proteins and other related viruses, this is done mainly to eliminate the risk of cross-reactivity and adverse immune reactions and . To confirm the lack of sequence similarity, a homology prediction analysis was done using the BLASTp Epitopes with E-values score of .05 were taken.

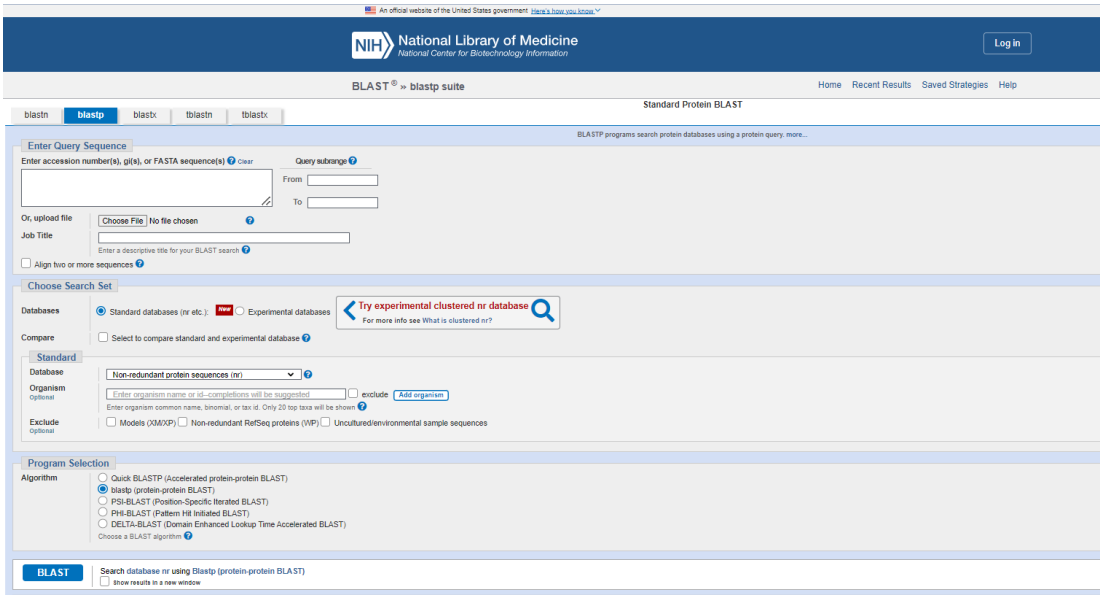

Figure -3.6.1 BLASTp interface

3.7 Peptide modelling

After all the testings the best suitable epitopes are taken for epitope prediction, The peptide modelling plays a role in understanding basic structural attributes of the selected epitopes, which is essential for evaluating their potential interaction with HLAs and subsequent immune responses. The 3D structures of the selected epitopes were generated using the PEP-FOLD3, which is a freely available tool. PEP-FOLD3 is a tool that utilizes a Hidden-Markov Model[57]

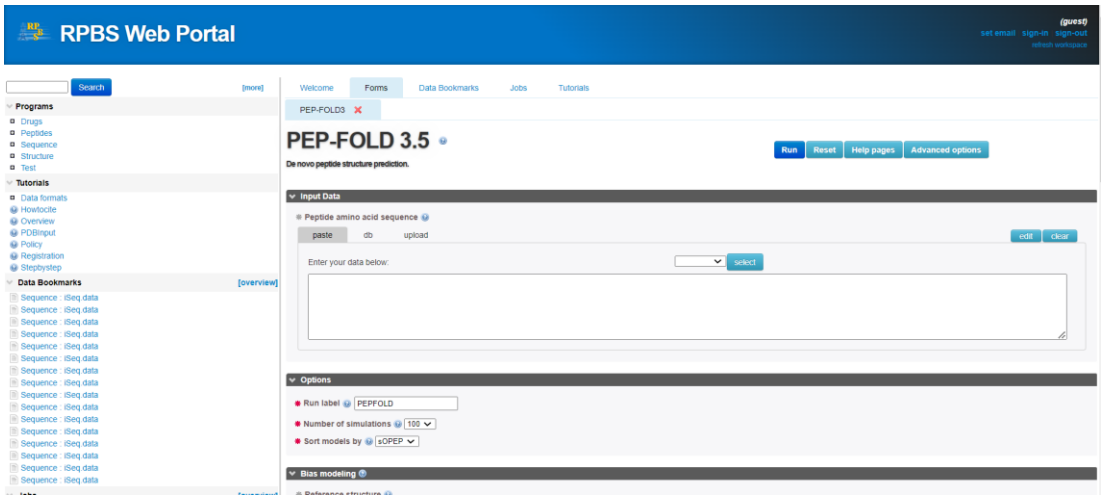

Figure -3.7.1 PEP-FOLD interface

3.8 Molecular Docking

In the final step the best binding model of the epitopes was determined using the ClusPro2.0 software. ClusPro2.0(https://cluspro.bu.edu/dimer_predict/submit.php) is a tool [58], [59] specifically designed for molecular docking, where the ligand (epitope) and receptor (MHC molecule) are inputted. The output of ClusPro2.0 provides a list of suitable complexes arranged in the order of best binding based and lowest score. The molecular docking analysis provides insights into the binding interactions, helping to understand the potential antigenic properties and the likelihood of immune recognition by T cells

For the analysis of HLA binding alleles specific to each epitope, HLA-B*57:01 was considered for MHC-I epitopes, while HLA-DRB1*07:01 was taken for MHC-II epitopes. The crystal structures of these two binding HLA alleles were retrieved from the PDB .The results of the docking were visualized usingPyMOL.

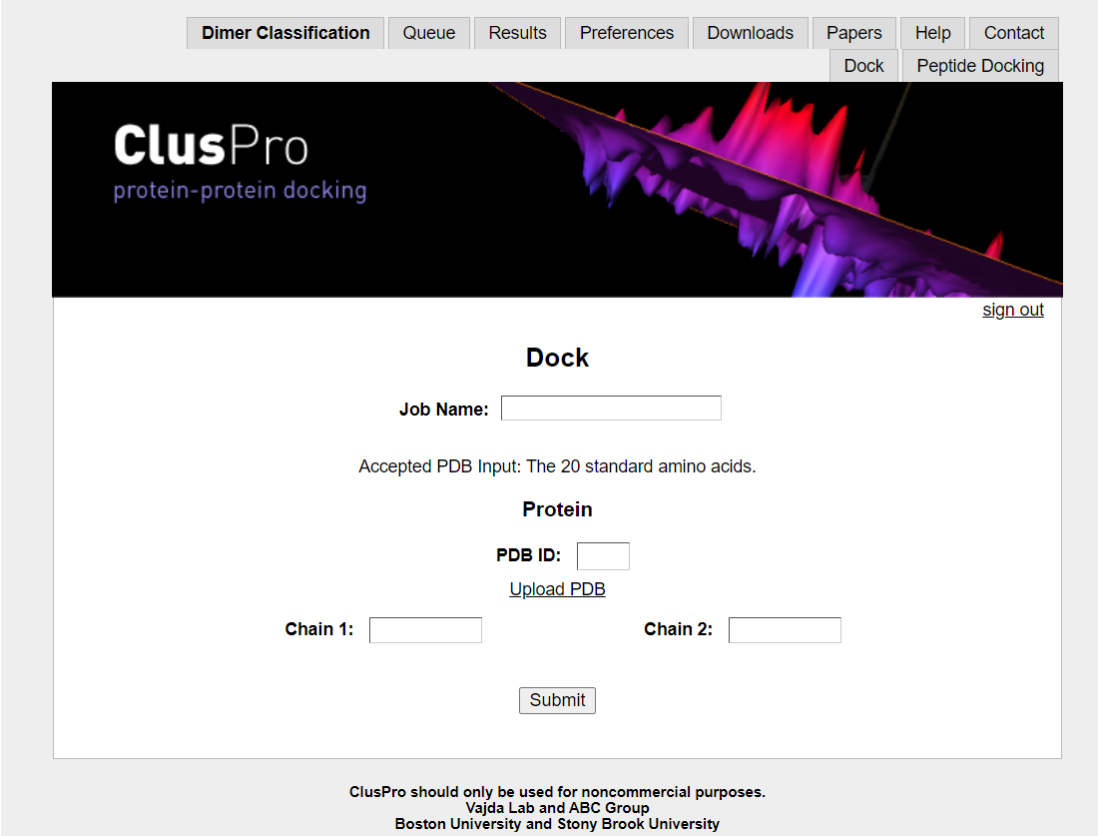

Figure-3.8.1 : ClusPro 2.0 interface

CHAPTER- 4

RESULTS

4.1 Selection of Stable Antigenic proteins

When the all the 3 structural protein 7 non-structural were subjected to antigenic and physiological analysis. It was found that structural protein C is non-antigenic and unstable Protein and non-structural Protein NS1 was also found unfit for epitope prediction because it is also an unstable protein. Therefore protein-C and NS1 were eliminated for further epitope prediction process.

Protein	Antigen	M.W.	Pi	Estimated half-life	Stable/Unstable	GRAVY
1	NON-ANTIGEN	12731.55	12.4	30 hours	unstable	0.022
2	ANTIGEN	17990.68	7.75	4.4 hours	stable	-0.054
3	ANTIGEN	53704.16	7.26	7.2 hours	stable	-0.172
4	ANTIGEN	39144.6	6.78	1.1 hours	unstable	-0.298
5	ANTIGEN	24620.45	9.71	1.4 hours	stable	0.713
6	ANTIGEN	14340.87	5.1	1.9 hours	stable	0.492
7	ANTIGEN	69288.24	7.64	1.9 hours	stable	-0.523
8	ANTIGEN	15888.66	6.04	1.9 hours	stable	0.73
9	ANTIGEN	27096.59	8.04	1.4 hours	stable	0.256
10	ANTIGEN	102897	7.84	30 hours	stable	-0.493

Table-4.1.1 : Structural and Non-structural Proteins and their physiological properties

4.2 B-cell epitope prediction

B-cell epitopes for individual protein was predicted using IEDB's B-cell epitope prediction tool. A selection of 32 epitopes were determined as the most acceptable options based on their optimal length out of the 97 epitopes. Additional testing was done on these 25 epitopes to determine their allergenicity, antigenicity, toxicity, and stability, and upon testing only 4 epitopes were deemed suitable for vaccine development.

PEPTIDE	ANTIGENICITY	ALLERGENICITY	STABLILITY	TOXICITY
prM Protein				
IDSGEEPVDV	ANTIGENIC	NON-ALLERGENIC	UNSTABLE	NONTOXIC
E Protein				
FAWKRPPTDSGH	NONANTIGENIC	NON-ALLERGENIC	UNSTABLE	NONTOXIC
TAEHLPKAW	NONANTIGENIC	NON-ALLERGENIC	STABLE	NONTOXIC
GDYLAANESHSNRK	NONANTIGENIC	NON-ALLERGENIC	STABLE	NONTOXIC
IHQENPAKT	NONANTIGENIC	NON-ALLERGENIC	UNSTABLE	NONTOXIC
NS2a Protein				
FAWKRPPTDSGH	NONANTIGENIC	NON-ALLERGENIC	UNSTABLE	NONTOXIC
TAEHLPKAW	NONANTIGENIC	NON-ALLERGENIC	STABLE	NONTOXIC
GDYLAANESHSNRK	NONANTIGENIC	NON-ALLERGENIC	STABLE	NONTOXIC

IHQENPAKT	NONANTIGENIC	NON-ALLERGENIC	UNSTABLE	NONTOXIC
NS2b Protein				
MIKGQRDQKGL	ANTIGENIC	NON-ALLERGENIC	STABLE	NONTOXIC
RRPTTGTSV	NONANTIGENIC	ALLERGENIC	UNSTABLE	NONTOXIC
NS3 Protein				
TFEKDYLRVR	NONANTIGENIC	NON-ALLERGENIC	UNSTABLE	NONTOXIC
AQRRGRVGRTSG	ANTIGENIC	ALLERGENIC	UNSTABLE	NONTOXIC
NIKPEEVDGKVELT	ANTIGENIC	ALLERGENIC	UNSTABLE	NONTOXIC
CDDDDTSLVQWKEA	ANTIGENIC	NONALLERGENIC	STABLE	NONTOXIC
NS4a Protein				
ENPGSRAMRMA	ANTIGENIC	NONALLERGENIC	STABLE	NONTOXIC
EPGKQRSSDDN	NON-ANTIGENIC	NONALLERGENIC	UNSTABLE	NONTOXIC
NS5 Protein				
DVHSLEAHR	NONANTIGENIC	NONALLERGENIC	STABLE	NONTOXIC
LSWPWNARE	ANTIGENIC	ALLERGENIC	STABLE	NONTOXIC
RDQRGSGQV	NONANTIGENIC	NONALLERGENIC	STABLE	NONTOXIC
TEATGSAASLI	NONANTIGENIC	NONALLERGENIC	STABLE	NONTOXIC
SSPDPLVEGERSR	ANTIGENIC	NONALLERGENIC	UNSTABLE	NONTOXIC

ARVSPGCGWSVRE	ANTIGENIC	ALLERGENIC	UNSTABLE	NON-TOXIC
TQYGDSWHVDKEHPY	NONANTIGENIC	NONALLERGENIC	STABLE	NON-TOXIC
HWASRDLSGAGVEGT	ANTIGENIC	NONALLERGENIC	STABLE	NON-TOXIC

Table 4.2.1 – List B-cell 25 epitopes

MIKGQRDQKGL, CDDDDTSLVQWKEA, ENPGSRAMRMA, HWASRDLSGAGVEGT are the final 4 B-cell epitopes that should be considered for multi-epitope vaccine construction against KFDV

4.3 T-cell epitope prediction

In the initial analysis of all suitable proteins using IEDB, a total of 17,272 epitopes were confirmend as MHC-I binders and 5,674 epitopes were predicted as MHC-II binders. Further, all epitope molecules were screened based on their IC50 values. A lower IC50 value signifies a higher affinity, therefore epitopes with good affinity were selected for further analysis. For this study, an IC50 threshold of 100mM was used. Following the screening on the basis of IC50 values, a total of 46 MHC-I molecules and 80 MHC-II molecules were deemed fit for further analysis. Linke in B-cell epitope selection, T-cell epitopes also underwent thorough evaluation for allergenicity, antigenicity, toxicity, stability, and IFN-gamma induction in the case of MHC-II epitopes. After comprehensive testing, only 16 epitopes out of the initial 126 were deemed suitable for further consideration.[60]

To ensure that the selected epitopes are safe for human use homology studies were performed. As a result, five epitopes were found to have homology with other sequences and were consequently eliminated from the study.

In the end, a total of 11 epitopes were identified as promising candidates for further examination. Out of these, two epitopes were found to bind to MHC-I, while the remaining nine epitopes exhibited binding to MHC-II. The specific epitopes can be found in the table provided below:

EPITOPE	**ALLELE**	**MHC-I/II**
RGSRAIWYMW	HLA-B*57:01	I
GSRAIWYMW	HLA-B*57:01	I
LTVVGVMLTVASGMV	HLADRB1*07:01	II
PLTVVGVMLTVASGM	HLADRB1*07:01	II
TVVGVMLTVASGMVR	HLADRB1*07:01	II
VGVMLTVASGMVRHT	HLADRB1*07:01	II
GVMLTVASGMVRHTS	HLADRB1*07:01	II
TMWHVTRGAALVVDE	HLADRB1*07:01	II
MWHVTRGAALVVDEA	HLADRB1*07:01	II
RMKTLVLAPTRVVLR	HLADRB1*07:01	II
VEVAVLGVATLGILW	HLADRB1*07:01	II

Table 4.3.1 – List T-cell epitopes

The above-mentioned epitopes exhibit desirable characteristics such as high affinity, low allergenicity, strong antigenicity, minimal toxicity, stability, and the ability to induce IFN-gamma.

4.4 Structural and Docking Analysis

The structural analysis of the final 11 final epitopes was done with the help of structural prediction tool PEP-FOLD-3, and the resulting 3D structures. The visualised structure through RasMol are shown below in the figure 4.4.1

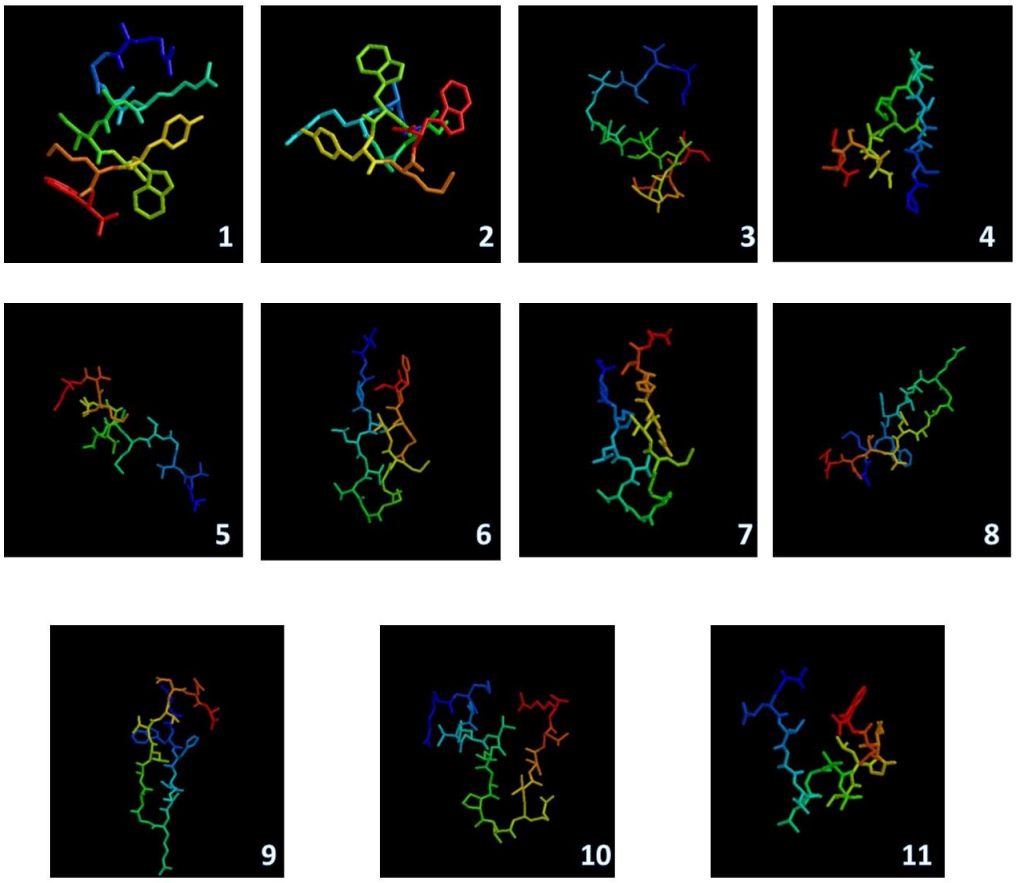

Figure-4.4.1 : Predicted structure of the 11 T-cell epitopes -RGSRAIWYMW, GSRAIWYMW, LTVVGVMLTVASGMV, PLTVVGVMLTVASGM, TVVGVMLTVASGMVR, VGVMLTVASGMVRHT, GVMLTVASGMVRHTS, TMWHVTRGAALVVDE, MWHVTRGAALVVDEA, RMKTLVLAPTRVVLR, VEVAVLGVATLGILW respectively

Molecular docking studies via ClusPro 2.0 results from the docking show optimal interaction between the HLA alleles and the peptide epitope. Hence all 11 moles are good candidates for vaccine synthesis. MHC class-II epitopes, the interaction

between the epitope "MWHVTRGAALVVDEA" and the HLA-DRB1*07:01 allele displayed the lowest and most favourable score, indicating a robust binding, similarly MHC class-I epitopes, the interaction between the epitope " RGSRAIWYMW " and the HLA-B*57:01allele displayed the lowest and most favourable score, indicating a robust binding. The visualised docking results for the best binging peptides are shown below.

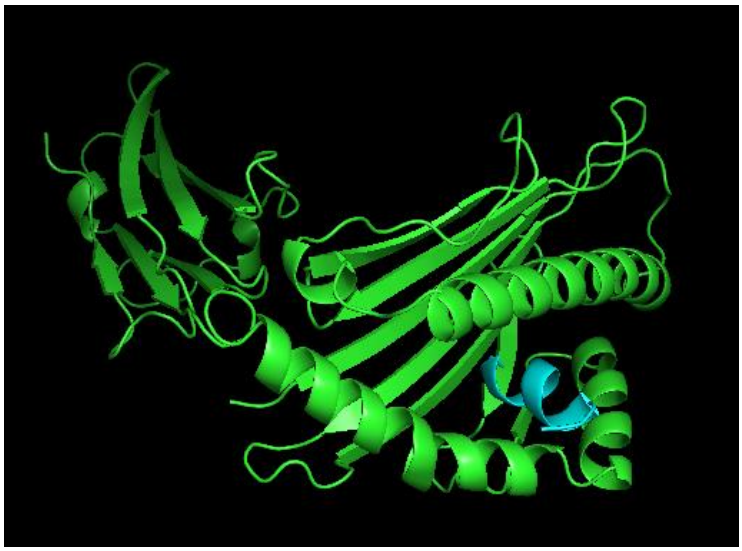

Figure- 4.4.2 : The interaction between the epitope " RGSRAIWYMW " and the HLA-B*57:01allele

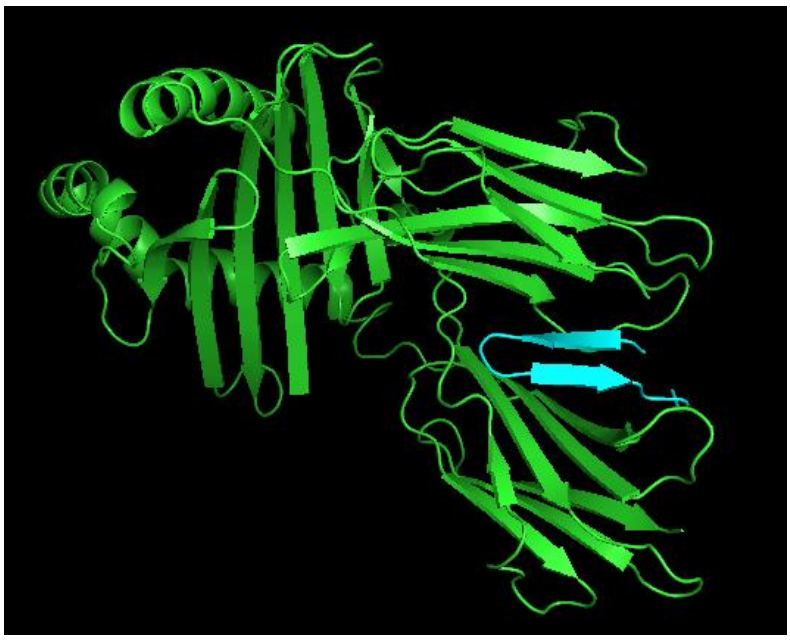

Figure-19: The interaction between the epitope " MWHVTRGAALVVDEA " and the HLA-DRB1*07:01 allele

These findings suggest that the epitopes "RGSRAIWYMW" and "MWHVTRGAALVVDEA" exhibit strong binding affinities with their respective MHC-I and MHC-II molecules, as indicated by their favourable global docking scores. These results support the potential of these epitopes to serve as good and effective candidates for further investigation in the manufacturing of a peptide-based vaccine against KFDV.

Chapter- 5

KFDV, is a severe public health concern in South Indian regions. The virus can infect humans when they are bitten by ticks carrying the virus, particularly the Haemaphysalis spinigera species, or through contact with other infected small mammals. Despite the recurrent occurrence of KFD outbreaks, no authorized vaccines or targeted accessible for this disease. This study exploits the research of this gap and aims to address the need for an effective vaccine against KFDV

The study aimed to identify potential epitopes vaccine against KFDV. Through a series of antigenic and physiological analyses, it was determined that the structural protein C and non-structural protein NS1 were non-antigenic and unstable, leading to their exclusion from further epitope prediction. The remaining proteins, exhibited antigenicity and stability, making them suitable candidates for epitope prediction. B-cell epitope prediction resulted in the selection of four epitopes, MIKGQRDQKGL, CDDDDTSLVQWKEA, ENPGSRAMRMA, and HWASRDLSGAGVEGT, which displayed favourable characteristics such as the peptides are non-allergen, stable, and non-toxic. These epitopes hold promise for vaccine development against KFDV.

T-cell prediction yielded a pool of MHC-I and MHC-II binders, which were screened based on IC50 values to identify epitopes with high affinity. Further evaluation for allergenicity, antigenicity, toxicity, stability, and IFN-gamma induction led to the selection of 16 epitopes. Homology studies eliminated five epitopes with significant sequence similarity to other proteins. Finally, 11 epitopes remained as potential candidates for vaccine development, with two MHC-I binding epitopes (RGSRAIWYMW and GSRAIWYMW) and nine MHC-II binding epitopes.

Structural analysis of the epitopes done by PEP-FOLD-3 provided their 3D structures, which were then subjected to docking analysis to check binding affinity with MHC class I and MHC class II molecules using ClusPro 2.0. The docking results confirmed strong affinity between the epitopes and their MHC HLA-alleles against which they were docked. Specifically, the epitope "RGSRAIWYMW" demonstrated robust binding with the HLA-B*57:01 allele, while "MWHVTRGAALVVDEA" displayed strong binding with the HLA-DRB1*07:01 allele.

In conclusion, our findings suggest that the identified epitopes possess desirable characteristics, including high affinity, low allergenicity, strong antigenicity, minimal toxicity, stability, and the ability to induce IFN-gamma. These epitopes hold promise for further investigation in the manufacturing of a vaccine for KFDV. However, it is crucial to emphasize that experimental validation is necessary to confirm their binding affinities and immunogenic properties. Future studies should focus on in vitro and in vivo experiments in order of assessing the efficacy and safety of the selected epitopes and their potential as vaccine candidates against KFDV.

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