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In-silico Screening of T-cell Epitopes as Vaccine Candidate from Proteome of Menangle Virus

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ABSTRACT

Menangle virus, a zoonotic paramyxovirus of pteropid bats, isolated in 1997 from still born piglets in an outbreak of reproductive disease in New South Wales, Australia. Symptoms of disease include fetal abnormalities, fetal death and stillborn piglets. These viruses are capable of causing disease in pigs and humans & hence there is an urgent need for vaccine designing against these viruses. In this study, screening of T-Cell epitopes from Menangle virus proteome followed by highest binding affinity of selected T-cell epitopes with their corresponding HLA alleles has been done. The server ProPred facilitates the binding prediction of HLA class II allele with specific epitopes from the antigenic protein sequences of Menangle virus. We docked the selected T-cell epitopes with their corresponding HLA allele structures using the HPEPDOCK Server. The screened T-cell epitopes are anticipated to be valuable in designing comprehensive epitope-based vaccines against Menangle virus after further *in-vivo* studies.

Keywords: Menangle virus , T-cell epitope, HLA alleles, vaccine designing.

INTRODUCTION

Menangle virus, a zoonotic paramyxovirus of pteropid bats was isolated in 1997 from still born piglets in the study of a serious outbreak of reproductive disease at a huge commercial piggery in New South Wales, Australia (Philbey et al., 1998). Macroscopic pathology in affected stillborn piglets was differentiate by serious degeneration of the brain and spinal cord as well as arthrogryposis, brachygnathia and kyphosis (Love et al.,2001). These viruses are capable of causing disease in pigs and humans (Barr et al., 2012; Chant et al., 1998). Tioman virus which was closely related to Menangle virus was isolated from urine of pteropid bat from Malaysia (Chua et al., 2001). Important implication of this study is to screen promiscuous T-cell epitopes from Menangle virus proteins viz phosphoprotein, attachment protein, non-structural protein, nucleocapsid, matrix protein, RNA-directed RNA polymerase, fusion glycoprotein. The screened T-cell epitopes may be the promising targets for epitope-based vaccine design for Menangle virus.

MATERIALS AND METHODS

Complete proteome of Menangle virus was retrieved from protein sequence database from NCBI (http://www.ncbi.nlm.nih.gov/protein) and their accession number were shown in Table 4.

Primary and secondary structure prediction: A proteomics server, ExPASy ProtParam (www.expasy.org) was used to analyze the primary structure of the target protein. Several parameters given by ProtParam tool for example amino acid composition, estimated half-life, theoretical pI, molecular weight, extinction coefficient, atomic composition, aliphatic index, grand average of hydropathicity and instability index were examined. SOPMA (Geourjon and Deleage, 1995) server used to check the secondary structure (alpha helix, beta plated sheets, turns and coils) of the proteins,

its aim to predict solvent accessibility, coiled-coil regions, transmembrane helices, globular regions and ultimately determines the stability and function of proteins.

Protein antigenicity determination: The sequence of proteins were analyzed by VaxiJen server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) (Doytchinova and Flower, 2007) with default parameters to find out the antigenicity. All the antigenic proteins with their respective predicted score were computed.

Prediction of HLA class II binding nanomer T-cell epitopes: Properd is used to predict the potential HLA class II binding nanomer epitopes (Singh and Raghava, 2001). Threshold percentage of highest scoring peptides is taken at 3%. Top four binders for different HLA allele are taken into consideration.

Toxicity prediction: ToxinPred (http://crdd.osdd.net/raghava/toxinpred/) was used to predict toxicity of selected T- cell epitopes (Gupta et al., 2013). ToxinPred is an in-silico tool to predict the selected epitope as toxic or non-toxic. Only non-toxic T-cell epitopes were selected for further study.

Structure-based Modeling of T-cell epitopes & HLA allele: The PEPstrMOD (Singh et al., 2015) method performed to find out the tertiary structure of selected nanomer epitopes. The PEPstrMOD tool prediction strategy utilizes the secondary structure data & β-turns data anticipated by PSIPRED and BetaTurns respectively. The amino acid sequences of HLA alleles were retrieved from IMGT/HLA database (http://www.ebi.ac.uk/ipd/imgt/hla/intro.html) (Robinson et al., 2012) and

homology model of alleles was constructed using program HPEPDOCK (http://huanglab.phys.hust.edu.cn/hpepdock/).

Molecular docking: HPEPDOCK Server has been used to perform docking of selected epitopes with highest scoring alleles models (Zhou et al., 2018). Docking studies was performed to study the interaction of epitopes with alleles. For such interaction studies, the most important requirement was the proper orientation and conformation of epitope, which fit to the binding site of the allele appropriately and form the epitope-allele complex. The obtained docking scores was tabulated and analysed.

RESULTS AND DISCUSSION

Primary and secondary structure analysis: Primary structure analysis viz molecular weight, theoretical isoelectric point (PI), total number of positively charged residues (Arg+Lys) and negatively charged residues (Asp+Glu), estimated half-life (in vitro) in mammalian reticulocytes and instability index are shown in table 1 while secondary structure analysis viz alpha helix, extended strand, beta turn & random coil are shown in table 2.

Table 1: Primary structure analysis using ProtParam

Name of Protein	No.of Amino acids	Molecular weight	Theoretical PI	Total No. of Negativety charged residues (ASP-GLU)	Total No. of Positivety charged residues (ASP-LYS)	Extinction coefficient	half-life	Instability Index	Aliphatic Index	Grand average of hydropathicity
Phosphoprotein	388	41823.59	8.67	49	52	18450	30	48.01	82.50	-0.511
Attachment Protein	595	65887.50	8.14	44	48	78030	30	39.81	85.13	-0.027
Non-structural Protein v	227	25077.35	7.59	33	34	25355	30	45.34	63.57	-0.759
Nucleocapsid	519	58913.58	4.94	74	54	39350	30	51.25	73.78	-0.545
Matrix protein	373	41963.84	9.36	32	48	52995	30	47.57	86.25	-0.174
RNA-directed RNA polymerase L	2269	257803.32	6.13	245	218	274920	30	43.58	101.96	-0.070
Fusion glycoprotein F0	554	60395.08	8.53	32	37	32610	30	34.53	112.62	0.300

Table 2: The secondary structure analysis using Sopma

Protein	Alpha helix	Extended strand	Beta turn	Random coil
Phosphoprotein	159 (40.98%)	14 (3.61%)	13 (3.35%)	202 (52.06%)
Attachment Protein	120 (20.17%)	152 (25.55%)	23 (3.87%)	300 (50.42%)
Non-structural Protein v	46 (20.26%)	18 (7.93%)	10 (4.41%)	153 (67.40%)
Nucleocapsid	271 (52.22%)	54 (10.40%)	20 (3.85%)	174 (33.53%)
Matrix protein	87 (23.32%)	96 (25.74%)	19 (5.09%)	171 (45.84%)
RNA-directed RNA polymerase L	1116 (49.18%)	250 (11.02%)	75 (3.31%)	828 (36.49%)
Fusion glycoprotein F0	223 (40.25%)	133 (24.01%)	28 (5.05%)	170 (30.69%)

Protein antigenicity determination: Antigenicity of all the proteins were screened by VaxiJen. All the proteins were found antigenic except, matrix protein & RNA-directed RNA polymerase which were non-antigenic at threshold value of 0.4 (default threshold for viral proteins) (Table 3). Antigenic proteins selected for further analysis.

Table 3: VaxiJen result of antigenicity

S.No.	Protein	Overall Antigen Prediction
1	Phosphoprotein	0.5193 (Probable ANTIGEN)
2	Attachment Protein	0.4486 (Probable ANTIGEN).
3	Non-structural Protein v	0.5256 (Probable ANTIGEN).
4	Nucleocapsid	0.5741 (Probable ANTIGEN).
5	Matrix protein	0.3939 (Probable NON-ANTIGEN).
6	RNA-directed RNA polymerase L	0.3979 (Probable NON-ANTIGEN).
7	Fusion glycoprotein F0	0.5115 (Probable ANTIGEN).

Prediction and analysis of HLA Class II binding peptides: Menangle virus proteins were subjected to Propred for selection of HLA Class II specific T- cell epitopes binders. Epitopes showing % of highest score with the maximum number of HLA alleles binders were selected (Table 4).

Table 4: ProPred predicted T-cell epitopes for HLA Class II with binding scores

Protein name	Amino acid length	Accession no.	Position	Epitopes	HLA class alleles	Propred (% of highest score)
Phosphoprotein	388	Q91MK1	126	IMSMMPLQS	DRB1_1311	60.24
Phosphoprotein	388	Q91MK1	87	VRPIDVEPS	DRB1_0308	57.95
Phosphoprotein	388	Q91MK1	217	IHYLQTLET	DRB1_0410	47.87
Attachment Protein	595	Q91MJ8	426	IVYMYIQSA	DRB1_0404	50.00
Attachment Protein	595	Q91MJ8	447	LQLKQNRLQ	DRB1_0402	60.42
Non-structural Protein v	227	Q91MK2	83	VRGKVRPID	DRB1_0817	55.94
Nucleocapsid	519	Q91MK3	222	IRSSLTIRQ	DRB1_0410	52.42
Nucleocapsid	519	Q91MK3	198	YQQQGRLDQ	DRB1_1321	51.69
Matrix protein	373	Q91MK0	212	LRIDCAADS	DRB1_0306	76.14
Matrix protein	373	Q91MK0	133	VRRLPPIFN	DRB1_1321	55.06
RNA-directed RNA polymerase L	2269	Q2Z1L2	479	IFMKDKAIS	DRB1_0804	81.25
RNA-directed RNA polymerase L	2269	Q2Z1L2	2043	LQSQQKRVS	DRB1_1327	63.64
Fusion glycoprotein F0	554	Q91MJ9	499	IVLCIVIII	DRB1_0701	68.97
Fusion glycoprotein F0	554	Q91MJ9	409	LRVCQKLTL	DRB1_1104	54.22
Fusion glycoprotein F0	554	Q91MJ9	8	IVLYLTHSQ	DRB1_0806	75.58
Fusion glycoprotein F0	554	Q91MJ9	504	VIIIYINVQ	DRB1_0410	59.57

Toxicity prediction: ToxinPred (Gupta et al., 2013) used for toxicity prediction of selected T- cell epitopes. ToxinPred tool is a unique in-silico method based on Support Vector Machine (SVM) in predicting toxicity of peptides along with important physico-chemical properties viz Hydrophobicity, Hydropathicity, Hydrophilicity, Charge and Molecular weight. The selected epitopes were subjected to ToxinPred and only non-toxic T-cell epitopes were selected for further studies (Table 5).

Table 5: Toxicity prediction of the peptides by ToxinPred

PEPTIDE SEQUENCE	SVM SCORE	PREDICTION	HYDRO PHOBICITY	HYDRO PATHICITY	HYDRO PHILICITY	CHARGE	MOL .WT.
IMSMMPLQS	-1.00	Non-Toxin	0.08	0.81	-0.74	0.00	1037.45
VRPIDVEPS	-0.81	Non-Toxin	-0.19	-0.29	0.50	-1.00	1011.26
IHYLQTLET	-1.09	Non-Toxin	-0.03	-0.09	-0.64	-0.50	1117.41
IVYMYIQSA	-0.96	Non-Toxin	-0.18	-1.11	-1.22	0.00	1087.43
LQLKQNRLQ	-0.88	Non-Toxin	-0.44	-1.22	0.16	2.00	1140.50
VRGKVRPID	-0.90	Non-Toxin	-0.38	-061	-0.80	2.00	1039.37
IRSSLTIRQ	-0.46	Non-Toxin	-0.32	-0.22	0.11	2.00	1073.39
YQQQGRLDQ	-1.00	Non-Toxin	-0.50	-2.21	0.30	0.00	1135.34
LRIDCAADS	-0.78	Non-Toxin	-0.18	0.23	0.41	-1.00	963.18
VRRLPPIFN	-1.13	Non-Toxin	-0.21	-0.04	-0.16	2.00	1111.48
IFMKDKAIS	-1.15	Non-Toxin	-0.07	0.38	0.16	1.00	1052.42
LQSQQKRVS	-0.89	Non-Toxin	-0.49	-1.39	0.43	2.00	1073.35
IVLCIVIII	-0.39	Non-Toxin	0.59	4.13	-1.64	0.00	998.53
LRVCQKLTL	-0.88	Non-Toxin	-017	0.61	-0.23	2.00	1073.50
IVLYLTHSQ	-1.02	Non-Toxin	0.09	0.76	-1.07	0.50	1073.40
VIIIYINVQ	-0.78	Non-Toxin	0.30	2.01	-1.34	0.00	1074.48

Molecular Docking: 3D structures of selected epitopes were predicted by PEPstrMOD while HPEPDOCK Server was employed to generate homology model of alleles. Template PDB ID

(protein data bank) formed by server was used for alleles model (table 6). HPEPDOCK Server has been utilized to perform docking study of selected epitopes with alleles models (Figure 1-4). The best conformation of docked complex was chosen on the basis of minimum docking score (table 7).

Table 6: Template PDB ID for modeling of selected HLA alleles

S.No.	Allele	Template of model	Sequence Identity
1	DRB1_0308	1A6A B	99.5%
2	DRB1_0817	6CPL B	96.8%
3	DRB1_0410	4MD5 B	99.0%
4	DRB1_0804	6CPL B	96.3%,
5	DRB1_0410	4MD5 B	99.0%
6	DRB1*13:21	6CPL B	98.9%

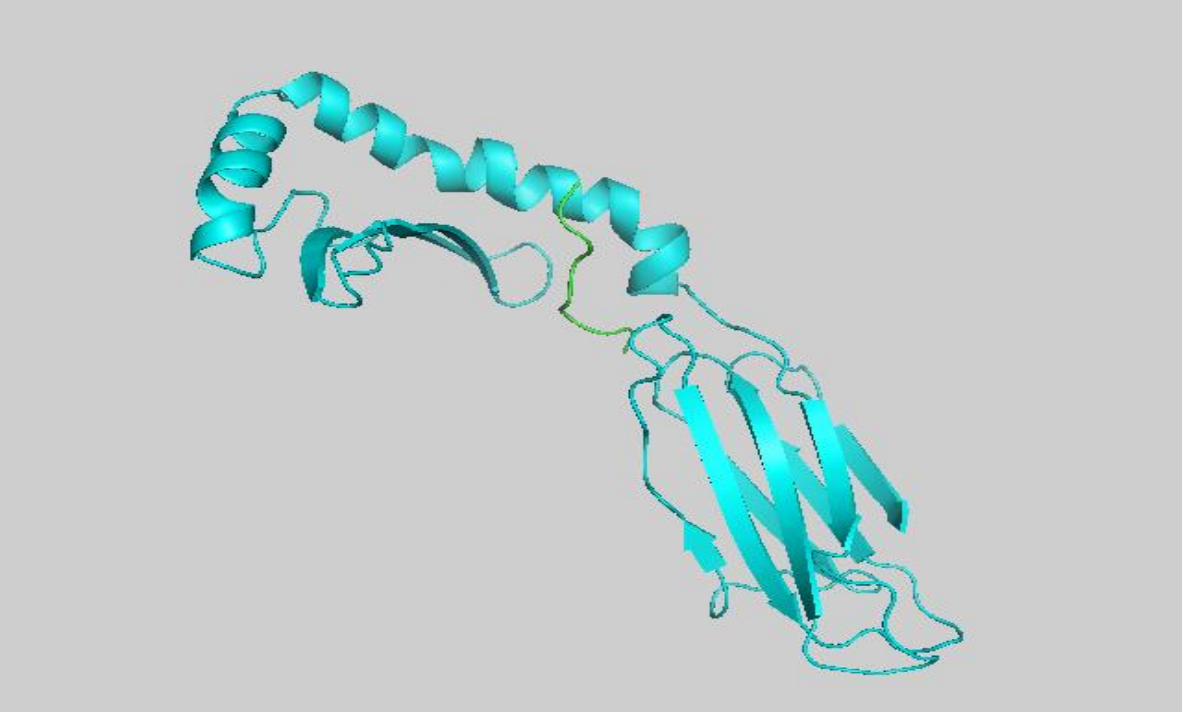

Figure 1. Docked complex of Phosphoprotein epitope VRPIDVEPS & DRB1_0308 allele

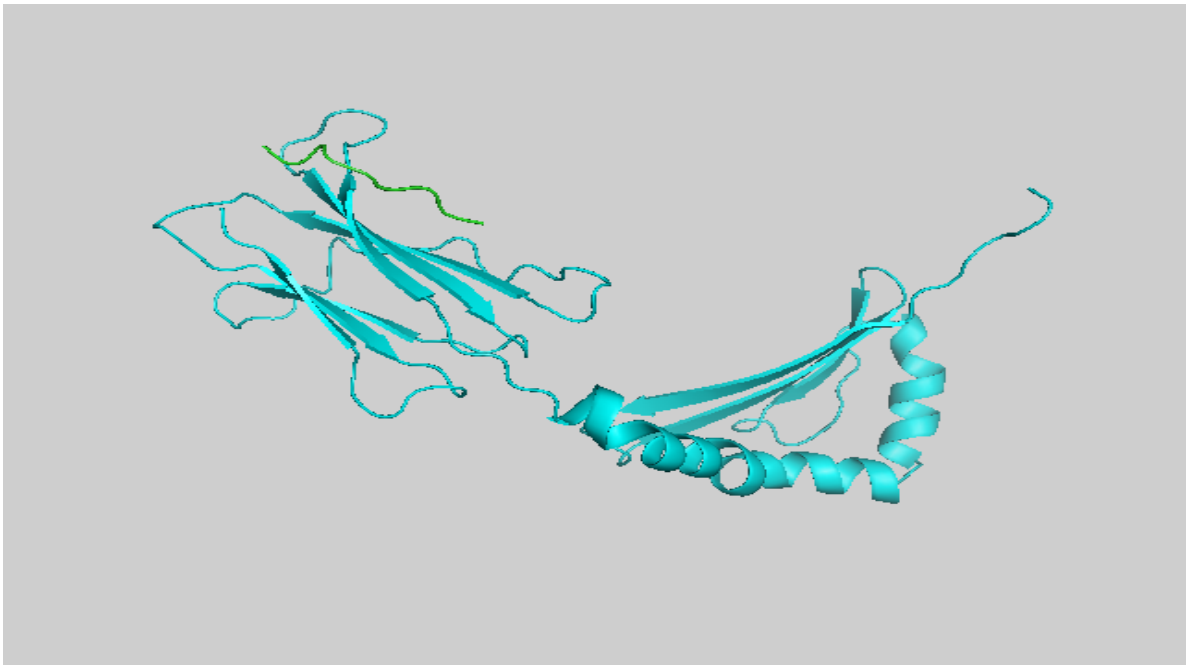

Figure 2. Docked complex of Non-structural Protein epitope VRGKVRPID & DRB1_0817 allele

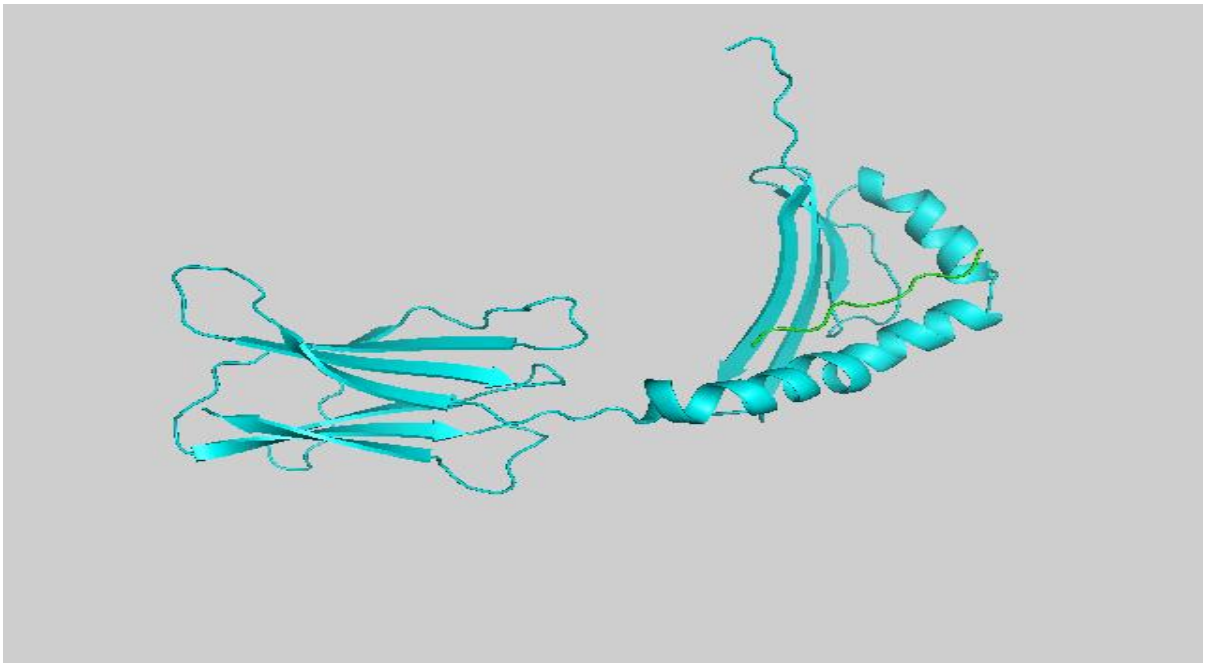

Figure 3. Docked complex of Nucleocapsid protein epitope IRSSLTIRQ & DRB1_0410 allele

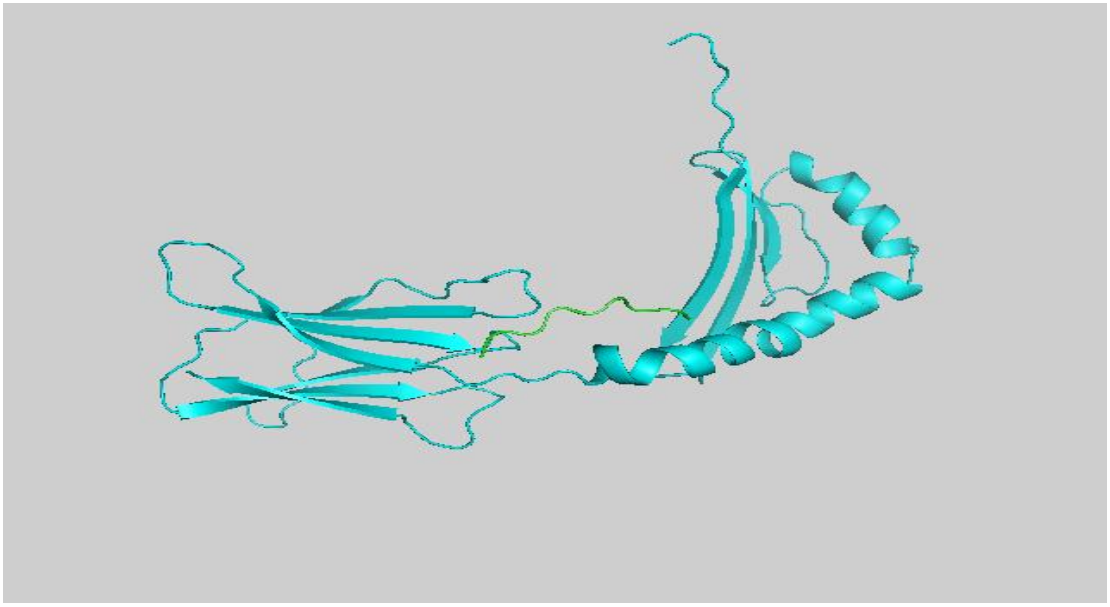

Figure 4. Docked complex of Fusion glycoprotein epitope VIIIYINVQ & DRB1_0410 allele

Table 7: Docking result of selected T-cell epitopes with allele structures.

S. No.	Protein Name	Epitopes	HLA class Alleles	Docking Score
1	Phosphoprotein	VRPIDVEPS	DRB1_0308	-159.479
2	Non-structural Protein v	VRGKVRPID	DRB1_0817	-162.380
3	Nucleocapsid	IRSSLTIRQ	DRB1_0410	-143.287
4	Fusion glycoprotein F0	VIIIYINVQ	DRB1_0410	-226.124

Epitope antigenicity determination: VaxiJen is used with default parameters to predict the antigenicity of selected epitopes as vaccines candidate. All the antigenic epitopes with their respective predicted score were selected (table 8).

Table 8: Vaxijen for T-cell epitopes

S. No.	SEQUENCE	VAXIJEN RESULT
1	VRPIDVEPS	2.0104 (Probable ANTIGEN).
2	VRGKVRPID	1.9895 (Probable ANTIGEN).
3	IRSSLTIRQ	1.1561 (Probable ANTIGEN).
4	VIIIYINVQ	0.9121 (Probable ANTIGEN).

DISCUSSION

Menangle viruses are emerging zoonotic infectious viruses that cause fatal diseases in pigs and humans (Barr et al., 2012). New efficient vaccines against Menangle virus infection are urgently needed to control the disease and its proliferation. In the present study, prediction and modeling of T cell epitopes of Menangle virus antigenic proteins followed by docking studies of predicted highest binding scores with their corresponding HLA class II alleles have been performed.

Menangle virus proteins were subjected to Propred for selection of HLA Class II specific T- cell epitopes binders (Singh and Raghava, 2001). Epitopes showing highest score with the maximum number of HLA Class II alleles binders were selected at a threshold value of 3% (Table 4). Docking of selected nanomer T-cell epitopes VRPIDVEPS, VRGKVRPID, IRSSLTIRQ, VIIIYINVQ with their corresponding allele DRB1_0308, DRB1_0817, DRB1_0410, DRB1_0410 respectively showed stable HLA–peptide complexes with docking Score -159.479, -162.380, -143.287, -226.124 respectively (Table 7). These epitopes also show positive values of antigenicity as shown in table 8. We have previously published similar work for HLA class II alleles for Hendra viruses (Kamthania et al., 2019) & H9N2 virus (Renu et al., 2020).

CONCLUSION

In this study, we screened the potential nanomer T-Cell epitopes as vaccine candidate against Menangle virus. The result confirming high binding affinity of selected T-cell epitopes with HLA alleles, stable complex formation tendency with HLA allele and tendency to induce high antigenicity makes the selected T-Cell epitopes to be a potential candidate for epitope based vaccine development

against Menangle virus infection. Hence reported nanomer T-cell epitopes may undergo further in-vivo trials to develop vaccine against Menangle virus infection.

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