ISSN: 2475-6296

Research Article

Journal of Clinical & Experimental Immunology

Computational Prediction of Multi-Epitopes Vaccine from Envelope E Protein against Louping Ill Virus via Reverse Vaccinology

Khadiga Osman Yousif[1], Yassir A Almofti[2*], Khoubieb Ali Abd-elrahman[3], Mashair AA Nouri[2] and Elsideeq EM Eltilib[2]

[1]*AlneelainMedical Research institute, Faculty of Medicine, Alneelain University, Khartoum- Sudan*

[2]*Department of Molecular Biology and Bioinformatics, College of Veterinary Medicine, University of Bahri, Khartoum- Sudan*

[3]*Department of pharmaceutical technology, College of Pharmacy, University of Medical Science and Technology (MUST) Khartoum-Sudan*

[*]Corresponding author:
Yassir A Almofti, University of Bahri, College of Veterinary Medicine, Department of Molecular Biology and Bioinformatics- Khartoum/ Sudan

Submitted: 26 Feb 2020; **Accepted**: 04 Mar 2020; **Published**: 18 Mar 2020

Abstract

Louping ill disease is a zoonotic viral disease caused by louping ill virus in the genus Flavivirus. It belongs to the tick-borne flavivirus that is a part of the tick-borne encephalitis virus complex.The envelope E protein of louping ill virus is the major structural protein that plays an important role in membrane binding and inducing a protective immune response.The aim of the present study was to design multi epitopes vaccine from the envelope E glycoprotein against louping ill virus using immunoinformatic tools that elicited humoral and cellular immunity. Eighteen envelope E protein sequences were retrieved from NCBI and subjected to various immunoinformatics tools from IEDB to assess their conservancy, surface accessibility and antigenicity as promising epitopes against B cells. The binding affinity of the conserved predicted epitopes was analyzed against MHC-I and MHC-II alleles of the T cells. The predicted epitopes were further assessed for their population coverage. For B-cell 25, 18 and 12 epitopes were predicted as linear conserved epitopes, surface accessibility and antigenic respectively. However, nine epitopes overlapped all the B cell prediction tools. Among them three epitopes (205-TAEHLP-210,336-KPCR-339 and 349-SPDV-352) were proposed as B cell epitopes. For T cell, 75 epitopes were found to interact with MHC-I alleles. The epitopes 130-YVYDANKV-138and356-MLITPNPTI-364 were proposed as a peptide vaccine since they interacted with the highest number of MHC-1 alleles.Moreover a total of 195core epitopes were found to interact with MHC-II alleles. The core epitopes 130-YVYDANKV-138, 219-WFNDLALPW-227, 415-VIGEHAWDF-423 and 462-VALAWLGLN-470 interacted with higher number of MHC-II alleles and proposed as vaccine since they demonstrated high affinity to MHC-II alleles.The population coverage epitopes set for MHC-I and MHC-II alleles was 74.69% and 99.98%, respectively. While the epitopes set for all T cell, proposed epitopes was 100%. Nine epitopes were predicted eliciting B and T cells and proposed as vaccine candidates against louping ill virus. However, these proposed epitopes require clinical trials studies to ensure their efficacy as vaccine candidates.

Keywords: Louping ill virus, Envelope E glycoprotein, IEDB, NCBI, B-cell, T-cell

Introduction

Louping ill disease is a zoonotic viral disease caused by louping ill virus (LIV) [1]. The virus is a member of the genus Flavivirus in the family Flaviviridae [2]. It belongs to the tick-borne flavivirus that is a part of the tick-borne encephalitis virus complex (TBEV) [3, 4]. Louping ill virus differs from other members of the TBEV serocomplex by not being associated with a forest environment and has economic and welfare importance by causing illness and death in livestock [5]. In endemic areas, the mortality rate is usually 60%, and most cases occur in animals [6]. The disease was reported throughout upland areas of Scotland, Ireland, northern England, and Wales [7]. Human infection was first reported in 1934 [8].

Louping ill disease transmitted mainly by tick's vector, Ixodes ricinus, which is called the sheep tick [9, 10]. Humans can be infected via tick bites or by contact with the virus in tissues or laboratory cultures. The virus may also be transmitted through skin wounds, aerosol exposure in laboratories, drinking unpasteurized milk; particularly high viral titers occur in goat milk [1]. The disease affects the central nervous system CNS caused encephalomyelitis and death in livestock and occasionally affects other domestic animals and humans [11].

Flaviviruses share a similar genomic organization and replication strategy [4, 12, 13]. Flaviviruses are enveloped; positive-sense single stranded RNA viruses with a genome of approximately 9.4–13 kb in length. The virions are spherical in shape and have diameter about 40-50 nm [13, 14]. The genome contains only one open reading frame (ORF) flanked by 5' and 3'untranslated regions (UTRs) that encodes a polyprotein. The translated polyprotein is directed into host endoplasmic reticulum (ER) membrane by signal sequences and

cleaved by host protease into three structural proteins, a nucleocapsid protein (C protein), a precursor membrane glycoprotein (prM protein) and a glycosylated envelope protein (E protein), as well as seven non-structural (NS) proteins [15-19]. The C protein is responsible for encapsulation of the virus to protect the genetic material. The PrM contributed in forming the viral envelope and played an important role in maintaining the E protein locative structure. Both prM and E form the surface structure of virion [20-22]. The envelope E protein (53-60 KD) is the major structural protein and plays an important role in membrane binding and inducing a protective immune response [23]. The E envelope glycoprotein comprised of three domains—I, II, and III—and the stem anchor region [24]. Domain I contain an N-linked glycosylation site, domain II is involved in membrane fusion and dimerization, and domain III, an immunoglobulin-like module, is recognized as the receptor-binding domain [25]. This rearrangement facilitates fusion with the endosomal membrane and release of the viral genome into the cytosol [16, 25]. Multiple studies indicated that envelope E protein in flaviviruses is the main protein responsible for pathogenicity [24, 26, 27]. Moreover some studies denoted that the nonstructural protein, 5 NS5, can participate with the envelope E protein in virus pathogenicity because it carried some genes that made virus. In addition to that, the envelope E protein is a major determinant of cell distribution and virulence due to its role in viral entry and immune activation [26, 27]. Also, it is important for cell passaging, neutralizing antibody escape mutants, and reverse genetics have been used extensively to study the role of E protein in louping ill pathogenicity [16, 28-30].

Despite the presence of vaccines for LIV like inactivated vaccines and recombinant vaccines, which preserve them from morbidity and mortality, LIV infection is not obstructed due to persistence in ticks with the involvement of wildlife hosts. Strategies that both protect livestock and reduce the persistence of virus through tick control will be of benefit to the farming industry and minimize the impact of disease [31-35]. Epitope based Vaccines are mostly based on specific epitopes capable for inducing B cell immunity. However recently vaccines based on T cell epitope have been encouraged as the host can generate a strong immune response by CD8+ T cell against the infected cells. Moreover, in silico approach has become handy in vaccine designing as it provides clue to select target protein sequence [36, 37]. In this study, we aimed to design a peptide vaccine for LIV particularly from the envelope protein (E) using computational methods. The method can predict epitopes inducing positive, desirable T cell and B cell mediated immune response. This criteria can be used later to create a new peptide vaccine that could replace conventional vaccines depending on an in silicon approaches and information in databases [38-40].

Materials and methods

Protein sequences retrieval and alignment tool

The protein sequences of eighteenenvelope E protein were retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/protein/?term=louping+ill+envelope+protein). The protein strains were retrieved according to their accession numbers, country and date of collectionand shown in (Table 1). The protein sequences were further aligned to obtain the conserved regions using multiple sequence alignment (MSA) tools, Clustal W in the BioEdit program, version 7.0.9.0 [41].

Molecular Evolution Analysis

The retrieved sequences were subjected to evolutionary divergence analysis and a phylogenetic tree was constructed to determine the common ancestor of each strain using the following website (http://www.phylogeny.fr/).

Determination of B Cells Epitopes

The conserved regions of the candidate epitopes were analyzed by different prediction software tools obtained by Immune Epitope Database (IEDB) analysis (https://www.iedb.org/). The reference sequence was used as an input for the IEDB software analysis.Tools from IEDB at (http://tools.iedb.org/bcell/) was used to identify the B cell epitopes, including Bepipred for linear epitope analysis, Emini for surface accessibility and Kolaskar and Tongaonkar for antigenicity scale

Table 1: Retrieved strains of LIV with their date of collection, accession number, and geographical region

Accession	Year of Collection	Country
AAA46282.1*	1993	JAPAN
sp|P35766.1	2018	SCOTLAND
sp|Q02478.1	2018	JAPAN
sp|P35765.1	2018	SCOTLAND
sp|P35764.1	2018	SCOTLAND
sp|Q02012.1	2018	JAPAN
NP_740271.1	2018	SIBERIA, UNITED KINGDOM
BAA02312.1	2016	GREAT BRITIAN
BAA02311.1	2016	NORWAY
BAA02313.1	1992	NORWAY
CAA60480.1	2016	BRITISH ISLES
CAA60481.1	2016	IRELAND
CAA60484.1	2016	SCOTLAND
CAA60482.1	2016	WALES
CAA60483.1	2016	ENGLAND
CAA49595.1	2016	GREAT BRITIAN, NORWAY, IRLAND
AAA46678.1	1993	N/A
A43383	2000	N/A

*refseq of louping ill envelope E protein
N/A: not available

T-cell epitopes prediction

The IEDB tools were used for the identification of the T cell epitopes prediction. The prediction method includes the major histocompatibility complex class I and II (MHC-I, MHC-II).

MHC-I Binding Predictions

Analysis of epitopes binding to MHC-I molecules was assessed by the software of IEDB MHC-I prediction tools (http://tools.iedb.org/mhci/). The prediction method was obtained by Artificial Neural Network (ANN), Stabilized Matrix Method (SMM) or Scoring Matrices derived from combinatorial peptide libraries. Before the prediction step, epitopes lengths were set as 9mers. The conserved epitopes that bind to alleles at score equal to or less than 300 (half-maximal inhibitory concentrations, IC50) was considered as B cell epitopes and were selected for further analysis

MHC-II Binding Predictions

Analysis of epitopes binding to MHC-II molecules wasperformed by the IEDB MHC-II prediction tools (http://tools.iedb.org/mhcii/). The neural networks align (NN-align) that allow for simultaneous identification of the MHC-II binding core epitopes and binding affinity was used. All the predicted conserved epitopes that bind to many alleles at score equal to or less than 3000 half-maximal inhibitory concentration (IC50≤3000) were selected for further analysis.

Population Coverage

For the calculation of the population, coverage for all potential MHC-I and II epitopes, the IEDB tools was used (http://tools.iedb.org/population/). The envelope E protein was assessed for population coverage against the whole world with selected MHC-I and MHC-II interacted alleles.

Homology Modeling

Phyre 2 protein3D structure prediction server was used for creation the 3D structure of the reference envelope E protein [42]. The reference sequence was used as an input and Chimera 1.8 was used as a tool to visualize the selected epitopes belonging to B cell and T cell (MHC-I and MHC-II) [43]. Homology modeling was used for visualization of the surface accessibility of the B-lymphocytes predicted candidate epitopes as well as for visualization of all predicted T cell epitopes in the structural level.

Results

Epitopes Conservancy

Sequence alignment of all retrieved strains of envelope E protein was performed using ClustalW that presented by Bio edit software. Sequence alignment was performed to obtain 100%-conserved epitopes from the retrieved strains. As shown in figure (1) the retrieved sequences of the envelope E protein demonstrated conservancy when they were aligned. The conserved regions were recognized by the identity of amino acid sequences among the retrieved sequences.

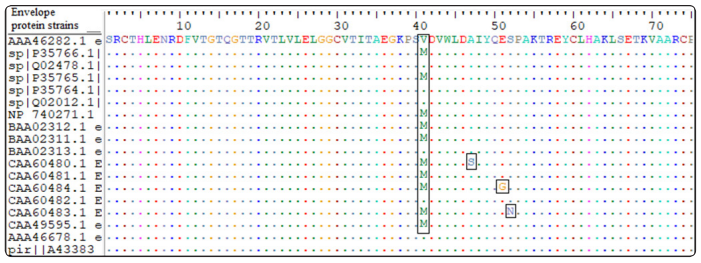

Figure 1: Multiple sequence alignment showed the conservancy between sequences of the retrieved strains of envelope E protein. The alignment was performed using BioEdit software tool. Dots showed the conserved regions while rectangular within the sequences showed the mutated or the unconserved region between strains

Phylogenetic Analysis

Figure (2) provided the phylogenetic relationship of the 18 retrieved strains of the envelope E protein of louping ill viruses. The phylogeny demonstrated evolutionary divergence among the retrieved strains of louping ill envelope protein.

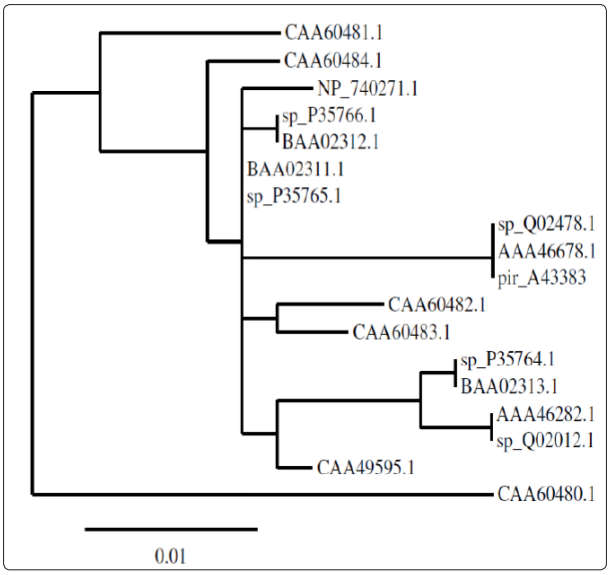

Figure 2: phylogenetic tree of relationship analysis of envelope E protein of different strains of LIV

B Cell Epitopes Prediction

The envelope E protein was subjected to Bepipred linear epitope prediction, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity prediction methods from IEDB. The thresholds of Bepipred linear epitope, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity were shown in Figure (3). In Bepipred linear epitope prediction method; the average score of envelope E protein to B-lymphocytes was 0.039 (minimum: -0.004 and maximum: 2.041). Values equal to or greater than the default threshold 0.039 were predicted as conserved linear epitope. Table (2) showed that 25 epitopes were predicted by Bepipred method as a linear epitopes. In Emini surface accessibility prediction, the average score of louping ill envelope E protein was 1.000 (minimum: 0.075 and maximum: 6.764). Values equal to or greater than the default threshold 1.000 were regarded potentially on the surface. Emini surface accessibility method predicted 18epitopes on the surface that have potential binding to B-lymphocytes cells (Table 2). In Kolaskar and Tongaonkar antigenicity prediction method, the average score of louping ill envelope E protein was 1.032 (minimum: 0.876 and maximum: 1.223). Values equal to or greater than the default threshold 1.032 were considered as antigenic epitopes. This method predicted 12 antigenic epitopes with potential binding to B-lymphocytes cells (Table 2). However, nine epitopes overlapped the three prediction tools of B cell. Among them three conserved epitopes were successfully predicted to elicit the B cell lymphocytes since they were conserved among all retrieved strains, got higher score values in Emini surface accessibility and Kolaskar and Tongaonkar antigenicity prediction methods. These three epitopes were 205-TAEHLP-210, 336-KPCR-339 and 349-SPDV-352. The three dimension structural (3D) level of these epitopes in the E glycoprotein was shown in Figure (4).

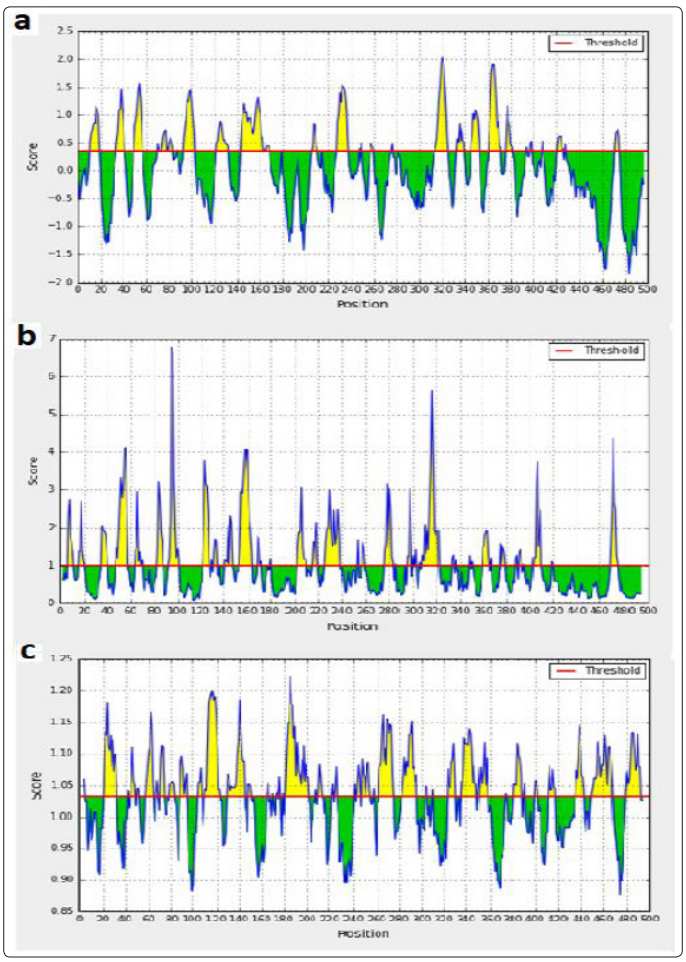

Figure 3: Threshold values of B-cell epitopes using (a) Bepipred linear epitope, threshold value 0.039 (b) Emini surface accessibility, threshold value 1.000and (c) Kolaskar&Tongaonkar antigenicity methods, threshold value 1.032. Yellow areas above the threshold (red line) are suggested to be a part of B cell epitope, while green areas are not

Table 2: B cell predicted epitopes. The table demonstrated the predicted conserved epitopes with their surface accessibility score and antigenicity score

Peptide	Start	End	Length	Emini[a] 1.000	Kolskar1.032[b]
RNPT	472	475	4	2.926	0.905
PAKT	53	56	4	1.877	0.992
GYVY	129	132	4	0.751	1.145
TFTVS	164	168	5	0.594	1.061
WDFGS	421	425	5	0.668	0.947
TAEHLP**	205	210	6	1.169	1.04
LPPGDN	376	381	6	1.398	0.982
EAKKKA	122	127	6	3.772	0.962
SGSKPCR	333	339	7	1.235	1.025
KPCR	336	339	4	1.07	1.351
TAEGKPS	34	40	7	2.215	0.958
CPTMGPA	74	80	7	0.392	1.03
PTDSGHD	318	324	7	2.403	0.957
VAHGSPDV	345	352	8	0.446	1.094
SPDV	349	352	4	1.069	1.081

FVTGTQGTT	11	19	9	0.654	0.986
RDQSDRGWG	94	102	9	3.539	0.905
NPTIENDGGGF	361	371	11	0.906	0.919
VEPHTG-DYVAANETH	143	157	15	1.71	1.024
EPHTGDYVAA	144	153	10	1.169	1.034
PHTGDYVA	145	152	8	1.015	1.053
PHTGDYV	145	151	7	1.249	1.052
HTGDYV**	146	151	6	1.007	1.05
VEPHT	143	147	5	1.292	1.062
VEPH	143	146	4	1.126	1.101

a= threshold for Emini b=threshold for kolskar
** Proposed peptide that has high score in both Emini and kolskar

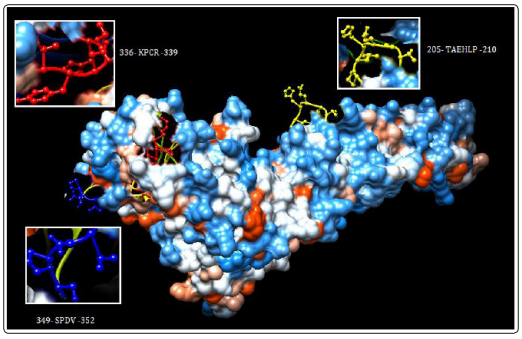

Figure 4: Position of proposed conserved B cell epitopes in structural level of envelope E protein of LIV. Three epitopes ($_{205}$-TAEHLP$_{210}$ and $_{336}$-KPCR$_{339}$ and $_{349}$-SPDV$_{352}$) were predicted to interact with B cell. The epitopes showed conservancy, surface accessibility and antigenicity using IEDB software

T Lymphocytes Epitopes Binding Prediction

MHC-I Binding Predictions

Envelope E protein was analyzed using IEDB MHC-1 binding prediction tool to predict T lymphocytes epitopes that have binding affinity with MHC-I alleles based on Artificial Neural Network (ANN) with half-maximal inhibitory concentration (IC50) ≤ 300. As shown in Table (3) a total of75 epitopes were found to interact with MHC-I alleles. The epitopes 130-YVYDANKV-138and356-MLITPNPTI-364 interacted with the highest number of alleles and the best binding affinity to MHC-1 alleles. The three dimensional structural level (3D) of these epitopes within envelope E protein of louping ill was shown in Figure (5).

Table 3: Position of epitopes in the envelope E protein of LIV that bind with the human MHC class 1 alleles

Peptide	Start	End	Allele	ic50
TQGTTRVTL	15	23	HLA-B*39:01	37.17
GTTRVTLVL	17	25	HLA-B*58:01	130.02
TRVTLVLEL	19	27	HLA-B*27:05	96.41
			HLA-B*39:01	56.78
LVLELGGCV	23	31	HLA-A*02:06	30.56
LELGGCVTI	25	33	HLA-B*18:01	165.65
			HLA-B*40:01	14.52
			HLA-B*40:02	59.58
CVTITAEGK	30	38	HLA-A*68:01	92.8
KTREYCLHA	55	63	HLA-A*30:01	3.43
REYCLHAKL	57	65	HLA-B*18:01	52.95
			HLA-B*40:01	5.9
			HLA-B*40:02	21.84
			HLA-B*44:03	163.7
			HLA-B*48:01	246.99
KLSETKVAA	64	72	HLA-A*02:01	282.49
			HLA-A*02:06	195.79

LSETKVAAR	65	73	HLA-A*31:01	76.52
KVAARCPTM	69	77	HLA-A*30:01	260.03
			HLA-C*14:02	197.17
AARCPTMGP	71	79	HLA-A*30:01	38.97
GLFGKGSIV	106	114	HLA-A*02:01	205.64
CVKAACEAK	116	124	HLA-A*68:01	217.31
YVYDANKIV	130	138	HLA-A*02:01	145.37
			HLA-A*02:06	31.17
			HLA-A*68:02	289.93
			HLA-C*03:03	81.19
			HLA-C*06:02	124.52
			HLA-C*07:01	130
			HLA-C*12:03	9.02
			HLA-C*14:02	41.19
			HLA-C*15:02	96.56
DANKIVYTV	133	141	HLA-A*68:02	25.77
			HLA-C*12:03	256.59
ANKIVYTVK	134	142	HLA-A*30:01	200.5
KVEPHTGDY	142	150	HLA-A*30:02	64.65
EPHTGDYVA	144	152	HLA-B*35:01	150.15
FTVSSEKTI	165	173	HLA-C*03:03	297.65
KTAEHLPTA	204	212	HLA-A*02:01	172.18
			HLA-A*02:06	19.39
			HLA-A*30:01	43.35
TAEHLPTAW	205	213	HLA-B*53:01	46.98
RDWFNDLAL	217	225	HLA-B*40:02	250.1
WFNDLALPW	219	227	HLA-A*29:02	300.69
			HLA-B*53:01	291.34
LGDQTGVLL	257	265	HLA-C*05:01	39.74
GSKPCRIPV	334	342	HLA-A*30:01	35.87
			HLA-A*02:06	124.84
SPDVNVAML	349	357	HLA-B*07:02	170.96
			HLA-B*39:01	275.95
MLITPNPTI	356	364	HLA-A*02:01	27.13
			HLA-A*02:06	57.23
			HLA-A*32:01	272.66
IENDGGGFI	364	372	HLA-B*40:01	25.34
MQLPPGDNI	374	382	HLA-A*02:06	33.47
LPPGDNIIY	376	384	HLA-B*35:01	13.08
LTVIGEHAW	413	421	HLA-B*57:01	23.96
			HLA-B*58:01	6
AVHTVLGGA	436	444	HLA-A*30:01	134.52
FLPKLLMGV	454	462	HLA-A*02:01	4.63
			HLA-A*02:06	4.98
			HLA-A*68:02	171.04
LPKLLMGVA	455	463	HLA-B*07:02	297.12

KLLMGVALA	457	465	HLA-A*02:01	30.23
			HLA-A*02:06	45.55
LLMGVALAW	458	466	HLA-A*32:01	71.29
			HLA-B*15:01	253.7
			HLA-B*53:01	112.2
			HLA-B*58:01	11.74
LMGVALAWL	459	467	HLA-A*02:01	162.63
GVALAWLGL	461	469	HLA-A*02:06	163.3
LVLAMTLGV	486	494	HLA-A*02:01	27.07
			HLA-A*02:06	7.43
			HLA-A*68:02	228.68
LAMTLGVGA	488	496	HLA-A*02:06	236.29

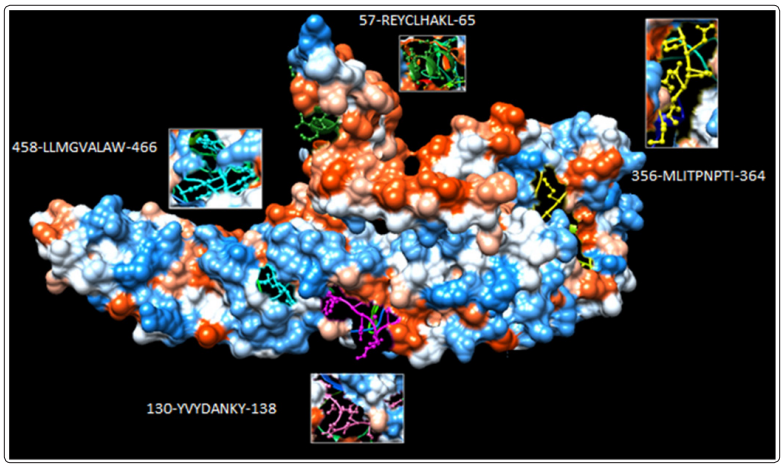

Figure 5: T cell proposed epitopes that interact with MHC-I shown in the 3D structural level of envelope E protein of LIV, the epitope 1) 130-YVYDANKV-138,2) 356-MLITPNPTI-364,3)57-REYCLHAKL-65,4)458-LLMGVALAW-466

MHC-II binding predictions

Envelope E protein was analyzed using IEDB MHC-II binding prediction tool to predict T lymphocytes epitopes that have binding affinity with MHC-II alleles based on NN-align with half-maximal inhibitory concentration (IC50) ≤ 3000. Total of 195 core epitopes were found to interact with MHC-II alleles. Table (4) demonstrated the best four core epitopes that interacted with MHC-II alleles. The core epitopes $_{130}$-YVYDANKV-$_{138}$, $_{219}$-WFNDLALPW-$_{227}$, $_{415}$-VIGEHAWDF-$_{423}$ and $_{462}$-VALAWLGLN-$_{470}$ interacted with higher number of MHC-II alleles. The three dimensional structural level (3D) of these epitopes within envelope E protein was shown in Figure (6).

Table 4: Position of the best four epitopes in the envelope E protein of LIV binding affinity with the human MHC class II alleles

Core Sequence	Peptide Sequence	Start	End	Allele	IC50	Rank
YVYDANKIV	KKKATGYVYDANKIV	124	138	HLA-DPA1*01:03/ DPB1*02:01	1935.2	41.46
				HLA-DPA1*02:01/ DPB1*01:01	2145.8	56.72
				HLA-DQA1*05:01/ DQB1*03:01	363.2	30.81
				HLA-DRB1*01:01	149.1	36.08
				HLA-DRB1*03:01	2132.6	30.24
	KKATGYVYDANKIVY	125	139	HLA-DPA1*01:03/ DPB1*02:01	1761.3	39.76
				HLA-DPA1*02:01/ DPB1*01:01	1478.8	49.72
				HLA-DQA1*05:01/ DQB1*03:01	261	26.04
				HLA-DRB1*01:01	57	22.85
				HLA-DRB1*03:01	599.8	15.58
				HLA-DRB1*04:01	193.2	14.59
	KATGYVYDANKIVYT	126	140	HLA-DPA1*01:03/ DPB1*02:01	1956.9	41.67
				HLA-DPA1*02:01/ DPB1*01:01	1115.2	44.48
				HLA-DPA1*03:01/ DPB1*04:02	2207.6	43.25
				HLA-DRB1*01:01	29	15.11
				HLA-DRB1*03:01	343.7	11.23
				HLA-DRB1*04:01	137.2	10.9
	ATGYVYDANKIVYTV	127	141	HLA-DPA1*01:03/ DPB1*02:01	1908.7	41.21
				HLA-DPA1*02:01/ DPB1*01:01	729.7	37.03
				HLA-DPA1*03:01/ DPB1*04:02	1356.1	36
				HLA-DRB1*01:01	14.8	8.48
				HLA-DRB1*03:01	189.6	7.77
				HLA-DRB1*04:01	109.6	8.86
	TGYVYDANKIVYTVK	128	142	HLA-DPA1*01:03/ DPB1*02:01	2368.8	45.33
				HLA-DPA1*02:01/ DPB1*01:01	633.5	34.63
				HLA-DPA1*03:01/ DPB1*04:02	1152.8	33.76
				HLA-DQA1*05:01/ DQB1*03:01	142.3	18.53
				HLA-DRB1*01:01	18.7	10.64
				HLA-DRB1*03:01	249.8	9.21
				HLA-DRB1*04:01	155.5	12.17
	GYVYDANKIVYTVKV	129	143	HLA-DPA1*01:03/ DPB1*02:01	2595.8	47.16
				HLA-DPA1*02:01/ DPB1*01:01	604	33.84

				HLA-DPA1*03:01/ DPB1*04:02	1231.9	34.65
				HLA-DQA1*05:01/ DQB1*03:01	162.7	20.06
				HLA-DRB1*01:01	21.8	12.15
				HLA-DRB1*03:01	486.8	13.87
				HLA-DRB1*04:01	234.1	16.95
	YVYDANKIVYTVKVE	130	144	HLA-DQA1*05:01/ DQB1*03:01	228.2	24.26
				HLA-DRB1*01:01	39.4	18.46
				HLA-DRB1*03:01	1092.4	21.62
	WQVHRDWFNDLALPW	213	227	HLA-DPA1*01:03/ DPB1*02:01	279	15.63
				HLA-DPA1*02:01/ DPB1*01:01	296	22.87
				HLA-DQA1*03:01/ DQB1*03:02	709.2	12.63
				HLA-DQA1*05:01/ DQB1*02:01	250.1	5.55
				HLA-DQA1*05:01/ DQB1*03:01	2675.9	67.97
				HLA-DRB1*03:01	245	9.1
				HLA-DRB1*04:01	130.5	10.43
				HLA-DRB1*04:05	1066	42.61
				HLA-DRB1*09:01	624.8	29.45
				HLA-DRB1*11:01	778.4	37.1
				HLA-DRB1*13:02	1530.8	32.75
				HLA-DRB1*15:01	665.6	33.48
				HLA-DRB3*01:01	30.6	1.86
				HLA-DRB5*01:01	2566.2	59.4
	QVHRDWFNDLALPWK	214	228	HLA-DPA1*01:03/ DPB1*02:01	259.8	15
				HLA-DQA1*03:01/ DQB1*03:02	775.5	13.83
				HLA-DQA1*05:01/ DQB1*02:01	335.1	7.57
				HLA-DQA1*05:01/ DQB1*03:01	2771.6	68.72
				HLA-DRB1*01:01	1840.7	76.23
				HLA-DRB1*03:01	212.2	8.31
				HLA-DRB1*04:01	99.5	8.07
				HLA-DRB1*04:05	999.5	41.37
				HLA-DRB1*07:01	1040.1	44.87
				HLA-DRB1*09:01	539.3	26.81
				HLA-DRB1*11:01	372.5	27.35
				HLA-DRB1*13:02	1353.9	30.64
				HLA-DRB3*01:01	31	1.88
	VHRDWFNDLALPWKH	215	229	HLA-DPA1*01:03/ DPB1*02:01	271.8	15.39
				HLA-DPA1*02:01/ DPB1*01:01	241.8	20.13

				HLA-DQA1*03:01/ DQB1*03:02	735.6	13.11
				HLA-DQA1*05:01/ DQB1*02:01	432.3	9.74
				HLA-DQA1*05:01/ DQB1*03:01	2103.1	62.88
				HLA-DRB1*01:01	945.4	66.45
				HLA-DRB1*03:01	194.9	7.91
				HLA-DRB1*04:01	75.5	6.1
				HLA-DRB1*04:05	1020.9	41.78
				HLA-DRB1*07:01	936.4	43.15
				HLA-DRB1*09:01	433.2	23.18
				HLA-DRB1*11:01	183	19.38
				HLA-DRB1*13:02	1133.9	27.78
				HLA-DRB1*15:01	292.3	21.58
				HLA-DRB3*01:01	33	2
WFNDLALPW	HRDWFNDLALPWKHD	216	230	HLA-DPA1*01/ DPB1*04:01	1200.8	24.02
				HLA-DPA1*01:03/ DPB1*02:01	367.6	18.23
				HLA-DPA1*02:01/ DPB1*01:01	241.5	20.12
				HLA-DQA1*03:01/ DQB1*03:02	752.8	13.41
				HLA-DQA1*05:01/ DQB1*02:01	552.1	12.25
				HLA-DQA1*05:01/ DQB1*03:01	2002.8	61.86
				HLA-DRB1*01:01	928.4	66.18
				HLA-DRB1*03:01	201.8	8.08
				HLA-DRB1*04:01	65.5	5.23
				HLA-DRB1*04:05	1242.8	45.62
				HLA-DRB1*07:01	1282.4	48.5
				HLA-DRB1*09:01	389.7	21.52
				HLA-DRB1*11:01	125.3	15.72
				HLA-DRB1*13:02	1077.5	27.01
				HLA-DRB1*15:01	340.2	23.6
				HLA-DRB3*01:01	32.4	1.97
	RDWFNDLALPWKHDG	217	231	HLA-DPA1*01:03/ DPB1*02:01	428.8	19.81
				HLA-DPA1*02:01/ DPB1*01:01	346.1	25.08
				HLA-DQA1*03:01/ DQB1*03:02	842.1	15.02
				HLA-DQA1*05:01/ DQB1*02:01	694.5	15.08
				HLA-DQA1*05:01/ DQB1*03:01	2247.5	64.27
				HLA-DRB1*01:01	1414.2	72.33
				HLA-DRB1*03:01	514.2	14.29
				HLA-DRB1*04:01	100.8	8.17

				HLA-DRB1*04:05	1583.8	50.49
				HLA-DRB1*07:01	1878	55.36
				HLA-DRB1*09:01	498.8	25.48
				HLA-DRB1*11:01	173.5	18.83
				HLA-DRB1*13:02	1340.3	30.45
				HLA-DRB1*15:01	418	26.45
				HLA-DRB3*01:01	54.5	2.97
	DWFNDLALPWKHDGN	218	232	HLA-DPA1*01:03/ DPB1*02:01	779.3	26.92
				HLA-DPA1*02:01/ DPB1*01:01	1197.7	45.81
				HLA-DQA1*01:01/ DQB1*05:01	1204.5	16.8
				HLA-DQA1*03:01/ DQB1*03:02	950.6	16.89
				HLA-DQA1*05:01/ DQB1*02:01	859.2	18.08
				HLA-DQA1*05:01/ DQB1*03:01	2173.4	63.57
				HLA-DRB1*03:01	1332.2	24.01
				HLA-DRB1*04:01	171.6	13.23
				HLA-DRB1*04:05	2335.6	58.56
				HLA-DRB1*07:01	2737.9	62.42
				HLA-DRB1*09:01	861.9	35.75
				HLA-DRB1*11:01	269.8	23.54
				HLA-DRB1*13:02	1617.4	33.71
				HLA-DRB1*15:01	707.1	34.44
				HLA-DRB3*01:01	103.7	4.58
	WFNDLALPWKHDGNP	219	233	HLA-DPA1*01:03/ DPB1*02:01	951.2	29.73
				HLA-DPA1*02:01/ DPB1*01:01	1583.8	50.98
				HLA-DQA1*01:01/ DQB1*05:01	1681.1	20.77
				HLA-DQA1*03:01/ DQB1*03:02	1982.4	31.7
				HLA-DQA1*05:01/ DQB1*02:01	1110.4	22.18
				HLA-DQA1*05:01/ DQB1*03:01	2934.8	69.95
				HLA-DRB1*01:01	1941.4	77.05
				HLA-DRB1*03:01	2777.4	34.3
				HLA-DRB1*04:01	249.2	17.76
				HLA-DRB1*09:01	2040.6	56.14
				HLA-DRB1*11:01	342.5	26.31
				HLA-DRB1*13:02	2678.4	43.88
				HLA-DRB1*15:01	863.4	37.66
				HLA-DRB3*01:01	179.8	6.44
	GIERLTVIGEHAWDF	409	423	HLA-DPA1*01:03/ DPB1*02:01	331.7	17.21

				HLA-DPA1*02:01/ DPB1*01:01	1566.9	50.79
				HLA-DQA1*05:01/ DQB1*02:01	258.2	5.74
				HLA-DRB1*03:01	2709.8	33.89
				HLA-DRB1*07:01	189.5	20.79
				HLA-DRB3*01:01	274	8.26
				HLA-DRB5*01:01	119.1	16.83
	IERLTVIGEHAWDFG	410	424	HLA-DPA1*01:03/ DPB1*02:01	314	16.69
				HLA-DPA1*02:01/ DPB1*01:01	1523.6	50.28
				HLA-DQA1*05:01/ DQB1*02:01	133.2	2.6
				HLA-DQA1*05:01/ DQB1*03:01	2174.9	63.59
				HLA-DRB1*03:01	2253.3	31.01
				HLA-DRB1*07:01	295.6	26.05
				HLA-DRB3*01:01	251.9	7.86
				HLA-DRB5*01:01	116.2	16.61
	ERLTVIGEHAWDFGS	411	425	HLA-DPA1*01:03/ DPB1*02:01	311.1	16.6
				HLA-DPA1*02:01/ DPB1*01:01	1840.4	53.8
				HLA-DPA1*03:01/ DPB1*04:02	2662.6	46.22
				HLA-DQA1*05:01/ DQB1*02:01	214.8	4.68
				HLA-DQA1*05:01/ DQB1*03:01	1373.7	54.14
				HLA-DRB1*01:01	644.7	60.72
				HLA-DRB1*03:01	1452.5	25.08
				HLA-DRB1*04:01	1449.5	51.76
				HLA-DRB1*07:01	377.6	29.32
				HLA-DRB1*09:01	2902.8	65.29
				HLA-DRB3*01:01	216.2	7.18
				HLA-DRB5*01:01	96.4	14.98
VIGEHAWDF	RLTVIGEHAWDFGSA	412	426	HLA-DPA1*01:03/ DPB1*02:01	309.7	16.57
				HLA-DPA1*02:01/ DPB1*01:01	1843.6	53.83
				HLA-DPA1*03:01/ DPB1*04:02	2674.2	46.29
				HLA-DQA1*05:01/ DQB1*02:01	323.2	7.28
				HLA-DQA1*05:01/ DQB1*03:01	1008.2	48.14
				HLA-DRB1*01:01	323.4	49.44
				HLA-DRB1*03:01	956.6	20.17
				HLA-DRB1*04:01	1512.4	52.77
				HLA-DRB1*07:01	482.1	32.76

				HLA-DRB1*09:01	2900.1	65.27
				HLA-DRB1*13:02	1569.3	33.17
				HLA-DRB3*01:01	191.7	6.69
				HLA-DRB5*01:01	79.3	13.38
	LTVIGEHAWDFGSAG	413	427	HLA-DPA1*01:03/ DPB1*02:01	421.2	19.62
				HLA-DQA1*05:01/ DQB1*02:01	473.3	10.62
				HLA-DRB1*01:01	989.2	67.11
				HLA-DRB1*03:01	1892.3	28.52
				HLA-DRB1*04:01	2644.1	66.66
				HLA-DRB1*07:01	931.3	43.07
				HLA-DRB1*15:01	2214.3	54.27
				HLA-DRB3*01:01	322.4	9.07
				HLA-DRB4*01:01	1174.6	43.95
				HLA-DRB4*01:01	1174.6	43.95
				HLA-DRB5*01:01	160	19.67
	TVIGEHAWDFGSAGG	414	428	HLA-DPA1*01:03/ DPB1*02:01	831.3	27.81
				HLA-DQA1*05:01/ DQB1*02:01	719.4	15.55
				HLA-DRB1*07:01	1549.4	51.86
				HLA-DRB3*01:01	564	12.61
				HLA-DRB4*01:01	2238.5	58.37
				HLA-DRB4*01:01	2238.5	58.37
				HLA-DRB5*01:01	286.3	26.05
	VIGEHAWDFGSAGGF	415	429	HLA-DPA1*01:03/ DPB1*02:01	1079.9	31.6
				HLA-DQA1*05:01/ DQB1*02:01	814.8	17.29
				HLA-DRB3*01:01	820.1	15.8
				HLA-DRB5*01:01	480.8	32.54
	KLLMGVALAWLGLNT	457	471	HLA-DQA1*03:01/ DQB1*03:02	2180.1	34
	LLMGVALAWLGLNTR	458	472	HLA-DQA1*03:01/ DQB1*03:02	2512.8	37.65
	LMGVALAWLGLNTRN	459	473	HLA-DQA1*03:01/ DQB1*03:02	2873.2	41.27
VALAWLGLN	MGVALAWLGLNTRNP	460	474	HLA-DPA1*01:03/ DPB1*02:01	1372.5	35.45
				HLA-DQA1*01:01/ DQB1*05:01	2624.7	27.2
				HLA-DQA1*01:02/ DQB1*06:02	752	34.65
				HLA-DQA1*04:01/ DQB1*04:02	2704.4	35.87
				HLA-DRB1*08:02	2644.1	42.78
	GVALAWLGLNTRNPT	461	475	HLA-DPA1*01:03/ DPB1*02:01	2398.2	45.59
				HLA-DQA1*01:02/ DQB1*06:02	1377.9	46.77

				HLA-DQA1*05:01/ DQB1*03:01	337.5	29.74
				HLA-DRB1*08:02	2530.3	41.71
	VALAWLGLNTRNPTM	462	476	HLA-DQA1*01:02/ DQB1*06:02	2099.4	55.84
				HLA-DQA1*05:01/ DQB1*03:01	355.8	30.51

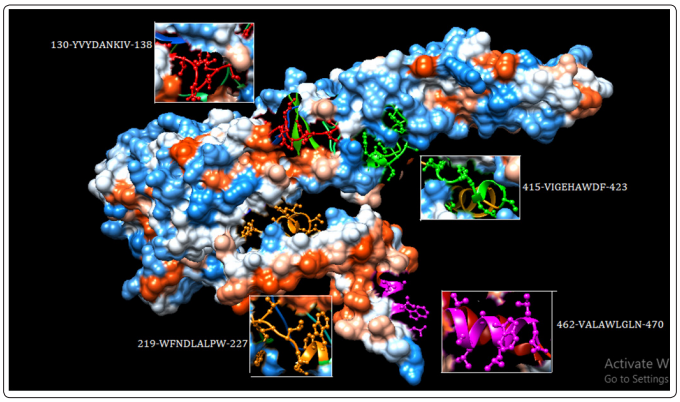

Figure 6: T cell proposed epitopes (130-YVYDANKV-138, 219-WFNDLALPW-227, 415-VIGEHAWDF-423 and 462-VALAWLGLN-470) that interact with MHC-II shown in the 3D structural level of envelope E protein of LIV

Population Coverage

The suggested epitopes that demonstrated higher affinity to interact with MHC-I and that bound to different sets of alleles were selected for population coverage analysis (Table 5). Demonstrated the population coverage percentages for each epitope and their epitopes sets. The epitopes 57-REYCLHAKL-65, 458- LLMGVALAW -466, 356-MLITPNPTI-364 and 130-YVYDANKIV-138 interacted with most frequent MHC-I alleles and they demonstrated high percentage against the whole world population coverage with epitope set 83.14%. Strikingly the first three epitopes that interacted with MHC-I in addition to the epitope 130-YVYDANKIV-138 were interacted with most frequent MHC-II alleles and they demonstrated high percentage against the whole world population coverage with epitopes set of 99.98%. The overall MHC-I and MHC-II population coverage epitopes set was 100%

Table 5: The population coverage against the whole world for the predicted epitopes. The overall population coverage epitope set for all predicted epitopes in MHC-1 and MHC-11 was 100%. PC (population coverage)

MHC I			MHC II			MHCI/MHCII	
Epitope	**PC**	**Interacted alleles**	**Epitope**	**PC**	**Interacted alleles**	**Epitope**	**PC**
MLITPNPTI	44.14%	3	VALAWLGLN	99.03%	14	MLITPNPTI	44.14%
YVYDANKIV	73.06%	9	VIGEHAWDF	99.44%	67	YVYDANKIV	73.06%
LLMGVALAW	18.06%	4	WFNDLALPW	99.87%	62	VALAWLGLN	99.03%
REYCLHAKL	25.17%	5	YVYDANKIV	99.44%	94	VIGEHAWDF	99.44%
Epitope set	83.14%		Epitope set	99.98%		WFNDLALPW	99.87%
						YVYDANKIV	99.44%
						Epitope set	100.00%

Discussion

The main goal of vaccine design is prevent or minimize the possibility of infection. Historically, live attenuated vaccine though it provides the needed immunity to have been used for induction of antigen-specific responses that protect the host against infectionsbut it may cause infection or allergy because it contains the necessary and much unnecessary proteins because some vaccine formulations can contain necessaryseveral proteins based on microbial pathogens, this protein vaccine can makeprotected the immune system of host. Vaccine mainly contains two classes of epitopes: a B-cell epitopes and a T-cell epitopes. The combination of these epitopes, vaccine is able to either induce specific humoral or cellular immune against specific pathogens [40, 44]. In this study envelope E glycoprotein used as a target in the designing of peptide based vaccine against louping ill virus, which is wide spread among sheep in British Isles area, which led to severe encephalomyelitis and death.

To determine a potential and effective peptide antigen for B cell, peptide should pass the threshold scores in Bepipred linear epitope prediction, Emini surface accessibility, Kolaskar and Tongaonkar antigenicity prediction methods. Four epitopes selected (Table 2) according to conservancy and threshold passed demonstrate high affinity to interact with B cell.

Vaccine against T cell is more promising, effective, and long lasting than b cell because the antigen can easily escape the antibody memory response [45, 46]. The T cell predicted epitopes is measured by binding affinity between the peptide and MHC alleles, The conserved epitopes illustrated in (Table 3, Table 4) was found to interact with some of HLA-A, HLA-B and HLA-C alleles for MHC class I, as well as some of HLA-DRB1, HLA-DP and HLA-DQ alleles for MHC class II. 43 and 299 conserved T cell epitopes were predicted to interact with both MHC-1 and MHC-II alleles respectively. Among them, the population coverage of the conserved MHC II epitope (99.98%) higher than MHC I (83.14%). Six epitopes (Table 5) demonstrated good binding affinity and high population coverage against MHC-1 or MHC-II alleles. The epitope 130-YVYDANKV-138 was found positioned in the region from 1 to 496 that considered as a unique domain because this epitope successfully interacted with both MHC-1 and MHC-II alleles.

In a study by jiang et al. described, the single amino acid codon change in position 308 to 311 in louping ill virus can reduce neurovirulence of infection [47]. The substitutions change from the amino acid aspartate to asparagine at amino acid position 308, which represented a potential glycosylation site, was the most effective substitution in reducing neurovirulence. The envelope E glycoprotein fall into area from 1 to 496, this demonstrates importance of E protein and epitope selection in this study for virulence of virus.

Conclusion

Nine epitopes were predicted eliciting B and T cells and proposed as vaccine candidates against louping ill virus. The need to develop an effective and safe vaccine is strongly recommended to prevent louping ill virus infection. Vaccine design using an insilico prediction method is highly appreciated as it selects specific epitopes in protein than conventional peptide vaccine development methods. In this study, three epitopes (205-TAEHLP-210,336-KPCR-339 and 349-SPDV-352) were successfully proposed as B cell epitopes. The epitopes 130-YVYDANKV-138 and 356-MLITPNPTI-364 were proposed as a peptide vaccine since they interacted with the highest number of MHC-1 alleles. The core epitopes 130-YVYDANKV-138, 219-WFNDLALPW-227, 415-VIGEHAWDF-423 and 462-VALAWLGLN-470 interacted with higher number of MHC-II alleles and proposed as vaccine since they demonstrated high affinity to MHC-II alleles. The population coverage epitopes set for MHC-I and MHC-II alleles was 83.14% and 99.98%, respectively. While the epitopes set for all T cell, proposed epitopes was 100%. However, in vitro and in vivo trials are required to achieve the effectiveness of these epitopes as vaccine candidates.

Acknowledgments

Authors would like to thank the staff members of department of Molecular Biology and Bioinformatics, College of Veterinary Medicine, University of Bahri/ Sudan for their cooperation and support.

Competing Interest

The authors declared that they have no competing interests.

Funding

No funding was received.

References

1. Spickler, Rovid A (2009) Ovine Encephalomyelitis; Infectious Encephalomyelitis ague Sheep. The Center of Food Security and Puplic Health 2009: 1-4
2. Westawaye GE, Brinton MA, Gaidamovichs SYa, MC Horzinek, A Igarashi, et al. (1985) Flaviviridae. Intervirology 24: 183-192.
3. Smith CEG, Varma MGR. Louping ill (1981) CRC Handbook Series in Zoonosis. Section B: Viral Zoonosis, vol. I. Boca Raton, El: CRC Press 1981: 191-200
4. Gilbert L (2016) Louping ill virus in the UK: a review of the hosts, transmission and ecological consequences of control. Experimental and Applied Acarology 68: 363-374.
5. McGuire K, Holmes EC, Gao GF, Reid HW, Gould EA (1998) Tracing the origins of louping ill virus by molecular phylogenetic analysis. Journal of General Virology 79: 981-988.
6. Davidson M, Williams H, Macleod JA (1991) Louping ill in man: a forgotten disease. Journal of Infection 23: 241-249.
7. Jeffries CL, Mansfield KL, Phipps LP, Wakeley PR, Mearns R, et al. (2014) Louping ill virus: an endemic tick-borne disease of Great Britain. Journal of General Virology 95: 1005-1014.
8. Rocchi M, Sargison N (2015) Ticks and Tickborne Diseases. The Moredun Foundation News Sheet 6.
9. Macleod J, Gordon WS (1932) Studies in louping ill (an encephalitis of sheep) II. Transmission by the sheep tick Ixodesricinus. J Comp PatholTher 45: 24o-256.
10. Gao GF, Zanotto PM, Holmes EC, Reid HW, Gould EA (1997) Molecular variation, evolution and geographical distribution of louping ill virus. Actavirologica 41: 259-263.
11. Westaway EG (1980) Replication of flaviviruses; in Schlesinger. The togavirus 1980: 531-581.
12. Westaway EG, Ng ML (1980) Replication of flaviviruses; separation of translation site of kunjun virus protein and of cell protein. Virology 106: 107-122.
13. Bressanelli S, Stiasny K, Allison SL, Stura EA, Duquerroy S, et al. (2004) Structure of a flavivirus envelope glycoprotein in its low-pH-induced membrane fusion conformation. EMBO J 23: 728-738.
14. Lindenbach BD, Thiel HJ, Rice CM (2007) Flaviviridae the viruses and their replication. Philadelphia, USA: Lippincott

William & Wilkins 2007: 1101-1152.
15. Zhang X, Jia R, Shen H, Wang M, Yin Z, et al. (2017) Structures and Functions of the Envelope Glycoprotein in Flavivirus Infections. Viruses 9: 338.
16. Yu L, Nomaguchi, Padmanabhan M, Markoff RL (2008) Specific requirements for elements of the 50 and 30terminal regions in flavivirus RNA synthesis and viral replication. Virology 374: 170-185.
17. Melian EB, Hall-Mendelin, S Du, Owens F, Bosco-Lauth N, et al. (2014) Programmed ribosomal frameshift alters expression of west nile virus genesand facilitates virus replication in birds and mosquitoes. PLoS Pathog 10: e1004447.
18. Tomar S, Mudgal R, Fatma B (2017) Flavivirus Protease: An Antiviral Target. Satya P. Gupta (ed). "Viral Proteases and Their Inhibitors, 1nd ed. India: Academic Press 2017: 137-161.
19. Zhu B, Jing Y, Ping L, Rong J, Yang X, et al. (2012) Induction of antigen-specific immune responses in mice by recombinant baculovirus expressing premembrane and envelope proteins of West Nile virus. Virol J 9: 1-11.
20. Roby JA, Setoh YX, Hall RA, Khromykh AA (2015) Post-translational regulation and modifications of flavivirus structural proteins. J Gen Virol 96: 1551.
21. Peng JG, Wu SC (2014) Glutamic acid at residue 125 of the prM helix domain interacts with positively charged amino acids in E protein domain II for Japanese encephalitis virus-like-particle production. J Virol 88: 8386-8396.
22. Rey FA, Heinz FX, Mandl C, Kunz C, Harrison SC (1995) The envelope glycoprotein from tick-borne encephalitis virus at 2A resolution. Nature 25: 291-298.
23. Winkler G, Heinz FX, Kunz C (1984) Epitope mapping of flavivirus glycoproteins. J Chromatogr 297: 63-73.
24. Cherrier MV, Kaufmann B, Nybakken GE, Lok SM, Warren JT, et al. (2009) Structural basis for the preferential recognition of immature flaviviruses by a fusion-loop antibody. EMBO J 28: 3269-3276.
25. Kellman EM, Offerdahl DK, Melik W, Bloom ME (2018) Viral Determinants of Virulence in Tick-Borne Flaviviruses. Viruses 10: 329.
26. Hurrelbrink RJ, McMinn PC (2003) Molecular determinants of virulence: the structural and functional basis for flavivirus attenuation. Advances in virus research 60: 1-43.
27. Wayne D, Crill G, Jen J Chang (2004) Localization and characterization of flavivirus envelope glycoprotein cross-reactive epitopes. Journal of virology 78: 13975-13986.
28. Heinzf XT, Guirakhoo F, Bergeri R, Kunz (1984) Immunogenicity of tick-borne encephalitis virus glycoprotein fragments: epitope-specific analysis of the antibody response. J gen Virol 65: 1921-1929.
29. George F, Gao I, Wen R, Jiang I, Mohammed H, et al. (1993) Sequencing and antigenic studies of a norwegian virus isolated from encephalomyelitis sheep confirm the existence of louping ill virus outside great britain and Ireland. Journal of General Virology 74: 109-114.
30. Heinz F, Stiasny K (2012) Flaviviruses and flavivirus vaccines. Vaccine 30: 4301-4306.
31. Brotherston JG, Boyce JB (1970) Development of a non-infective protective antigen against louping-ill (arbovirus group b). J Comp Pathol 80: 377-388.
32. Edward DG (1948) Immunization against louping-ill. immunization of man." From the Wellcome Veterinary Research Station, Frant Sussex 1948: 372-378.
33. Williamis H, Thorburn H (1961) "The serological response of sheep to infection with louping-ill virus." The Bacteriology Laboratory, Northern Regional Hospital Board, Inverness 59: 437-447.
34. Marina N, Fleeton, Brian J, Sheahan, Ernest A, et al. (1999) Atkins and Peter Liljestro,"Recombinant Semliki Forest virus particles encoding the prME or NS1 proteins of louping ill virus protect mice from lethal challenge." Journal of General Virology 80: 1189-1198.
35. Marina N Fleeton, Peter Liljestro M, Brian J Sheahan, Gregory J (2000) Atkins Recombinant Semliki Forest virus particles expressing louping ill virus antigens induce a better protective response than plasmid-based DNA vaccines or an inactivated whole particle vaccine." Journal of General Virology 81: 749-758.
36. Taylor PD, Flower DR (2007) Immunoinformatics and computational vaccinology: a brief introduction. In Silico Immunology 2007: 23-46.
37. Springer, Boston MA, Skwarczynski M, Toth I (2016) Peptide-based synthetic vaccines. Chemical Science 7: 842-854.
38. Badawi MM, Fadlalla AA, Alam SS, Mohamed WA, Nasr-Eldin Osman DA, et al. (2016) Immunoinformatics predication and in silico modeling of epitope-based peptide vaccine against virulent Newcastle disease viruses. J Infectious Dis Microbiol 4: 61-71.
39. Yassir A Almofti, Khoubieb Ali Abd-elrahman, SaharAbd Elgadir Gassmallah, Mohammed Ahmed Salih (2018) "Multi Epitopes Vaccine Prediction against Severe Acute Respiratory Syndrome (SARS) Coronavirus Using Immunoinformatics Approaches." American Journal of Microbiological Research 6: 94-114.
40. Idris ST, Salih S, Basheir M, Elhadi A, Kamel S, et al. (2018) In silico Prediction of Peptide based Vaccine against Fowlpox Virus (FPV). Immunome Res 14: 154.
41. Hall T (1999) A BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95-98.
42. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ (2015) The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc 10: 845-858.
43. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, et al. (2007) UCSF Chimera a visualization system for exploratory research and analysis. J Comput Chem X 25: 1605-1612.
44. Purcell AW, McCluskey J, Rossjohn J (2007) More than one reason to rethink the use of peptides in vaccine design. Nat Rev Drug Discov 6: 404-414.
45. Black M, Trent A, Tirrell M, Olive C (2010) Advances in the design and delivery of peptide subunit vaccines with a focus on toll-like receptor agonists. Expert Rev Vaccines 9: 157-173.
46. Sesardic D (1993) Synthetic peptide vaccines. J Med Microbiol 39: 241-242.
47. Jiang WR, Lowe1 A, Higgs S, Reid H, Gould EA (2018) Single Amino Acid Codon Changes Detected in Louping Ill Virus Antibody- Resistant Mutants with Reduced Neurovirulence. J Gen Virol 74: 931-935.fig