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Immunoinformatics Prediction of Peptide-Based Vaccine Against African Horse Sickness Virus

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Abstract

Background: African horse sickness (AHS) is a viral disease of equidae. It is transmitted by hematophagous *Culicoides midges* (Diptera, Ceratopogonidae) and causes severe disease in horse that can lead to death. African Horse Sickness Virus (AHSV) is a double-stranded RNA (dsRNA) virus with ten genome segments encoding seven structural proteins (VP1-VP7) and four non-structural proteins (NS1, NS2, NS3, NS3A). The aim of this study is to analyze (VP2) protein of the African Horse Sickness Virus (AHSV) strains reported in the National Center for Biotechnology Information database (NCBI) database to select all possible epitopes that can be used to design a peptide vaccine.

Materials and methods: A total of 27 outer capsid protein (VP2) sequences of African Horse Sickness Virus (AHSV) were retrieved from the National Center for Biotechnology Information database (NCBI) (https://www.ncbi.nlm.nih.gov/protein/?term=VP2+African+horse+sickness+virus) in the 7th of September 2016. On them, several tests were conducted using Immune Epitope Analysis Database (IEDB) to detect the highly conserved immunogenic epitopes of B and T cells from which all possible epitopes that can be used as a therapeutic peptide vaccine to be selected.

Results and Discussion: Regarding epitopes that would elicit an antibody immune response, "**FSPEYY**, **DKVVEDPESY** and **YDTDQNVV** "were proposed to stimulate B cell. While 5 epitopes for each MHC I and II were addressed as potentially promising epitopes as they bound the highest number of alleles, all these epitopes were found to have a high binding affinity and the lowest binding energy to equine MHC class I molecule (ELA-A3) haplotype in the structural level. The epitopes "**YAYCLILAL and YTFGNKFLL"** were represented because they were bound to the largest number of alleles. In spite of binding to 4 alleles the epitope **WFFDYYATL** was represented because it has the lowest global energy. To our knowledge there is no epitope based vaccine for the African Horse Sickness Virus (AHSV) using in silico approaches.

Keywords: African horse sickness; Diptera; Protein; Immunogenicity; Virus

Introduction

African horse sickness (AHS) is a viral disease of equidae (Horses, Bonnies, zebras and donkeys) transmitted by hematophagous *Culicoides midges* (Diptera, Ceratopogonidae) and classified as an A-list infectious disease of the Office International des Epizooties (OIE) with important economic consequence for the horse trade [1-3]. The disease caused by this virus is characterized by mild to high fever, respiratory symptoms, severe weight loss, lethargy, rough hair, apathy and can eventually lead to death [4-7]. The morbidity and mortality rate is very high in infected animals and reaches up to 95% in horses [3,8-11].

(AHS) disease is endemic in sub-Saharan Africa. There were sporadic outbreaks in North Africa, Spain in 1969, Portugal in 1987 and the Middle East with few outbreaks recorded in India and Pakistan [10-13]. In the last decade the disease has emerged in different African countries such as the (AHSV-2) in Senegal and Nigeria in 2007, Ethiopia in 2008 and 2010 and Ghana in 2010, (AHSV-4) in Kenya in 2007 and (AHSV-7) in Senegal in 2007 [11,12] as well as in Sudan that has frequently reported AHS disease in imported race and breeding horses [14]. More recently outbreaks were recorded in Southern Africa in 2013 and 2014 [11,12] and the recent effects of climate change further increase the risk of virus introduction into Europe, where the insect vector also occurs [8,11].

African horse sickness virus (AHSV) is considered as the etiological agent of African Horse Sickness. It has nine antigenically different identified serotypes that belongs to the Orbivirus genus of the Reoviridae family [3,8,9,15,16]. (AHSV) is a double-stranded RNA (dsRNA) virus with ten genome segments encoding seven structural proteins (VP1-VP7) and four non-structural proteins (NS1, NS2, NS3, NS3A) [1,6]. There are two major outer capsid structural proteins in the African horse sickness virus; (VP2) and (VP5) [10,17]. (VP2) is the major protective antigen of (AHSV) that is responsible for the serotype formation. The majority of neutralizing epitopes are located on (VP2) which can be considered as the main target of immune response to the virus [11-13,17,18].

Previous studies targeted developing or producing an effective vaccine has used different types of approaches. Polyvalent live attenuated vaccine (LAV) that is used to control the disease in Africa was proposed to be

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unsafe due to the probability of re-assortment between AHSV serotypes. In addition, serotypes 5 and 9 are normally excluded from the (LAV) formulations as serotype 5 is difficult to attenuate and partially cross-reacts with serotype 8 and serotype 9 partially cross-reacts with serotype 6 [1,9,11,12,19-21]. A recent Approach applied is the recombinant technology that was used in the development of recombinant modified vaccinia ankara (MVA-VP2) vaccine which is highly protective against (AHSV), but the scale of it is limited to only two serotypes; (AHSV-4) and (AHSV-9) [5,9,10,13,17,18,22]. Therefore, it is important to consider alternative approaches for (AHSV) vaccine development.

Accession number	Collection	Country
AKP19875.1	1963	South Africa
AHI44018.1	1963	South Africa
AKP19876.1	1975	South Africa
AJU57393.1	1975	South Africa
ALM00096.1	1975	South Africa
ALM00095.1	1987	USA
ALM00102.1	1990	South Africa
YP_052941.1	1998	CNEVA, Paris
AAC40994.1	1998	CNEVA, Paris
ALG63774.1	1998	South Africa
ALM00099.1	1998	South Africa
ALM00098.1	1998	South Africa
ALM00097.1	1998	South Africa
ALM00093.1	1998	South Africa
O71024.1	1998	CNEVA, Paris
ALM00103.1	1999	South Africa
ALM00101.1	2000	South Africa
ALM00091.1	2000	South Africa
ALM00092.1	2001	South Africa
ALM00094.1	2003	South Africa
ABI96882.1	2006*	South Africa
ALM00100.1	2009	South Africa
AIX10308.1	2010	Ethiopia
AKP19874.1	2012	South Africa
AFL20816.1	2012	USA
AHZ12849.1	2014	South Africa
AJU57323.1	2014*	South Africa

Table 1: Virus Strains retrieved, their accession numbers and their area of collection; *specific year of collection are not available, the written year refer to the year of submission in the NCBI database.

The aim of this study is to analyze (VP2) protein sequences of (AHSV) strains reported in the (NCBI) database using Immune Epitope Analysis Database (IEDB) to select all possible epitopes that can be used to design a peptide vaccine.

The identification of specific epitopes derived from infectious disease has significantly advanced the development of peptide - based vaccines. Peptides elicit more desirable manipulation of immune response through the use of the B-cell epitopes which mainly induce antibody production and the T-cell epitopes that induce cellular response and cytokine secretion as cytotoxic T-cells. This approaches regarding the molecular basis of antigen recognition and HLA binding motifs to host class I and class II MHC proteins is highly supported by the immunoinformatics which aids in designing epitopes based vaccine motifs that serve as therapeutic candidates for many infectious diseases [5,7,11,12,23-26].

Materials and Methods

Protein sequence retrieval

A total of 27 outer capsid protein (VP2) sequences of African Horse Sickness Virus (AHSV) were retrieved from the National Center for Biotechnology Information database (NCBI) (https://www.ncbi.nlm.nih.gov/protein/?term=VP2+African+horse+sickness+virus) in the 7th of September 2016. These sequences were collected from different geographic regions of the world and most of these sequences were from South Africa. Retrieved sequences and their accession numbers as well as the collection area are listed in Table 1.

Determination of conserved regions

The retrieved sequences were aligned by multiple sequence alignment (MSA) using BioEdit software (version 7.2.5.0) [27], to obtain the conserved regions. Which were considered candidate epitopes were analyzed by different prediction tools at the Immune Epitope Database IEDB analysis resource (http://www.iedb.org/), Figure 1.

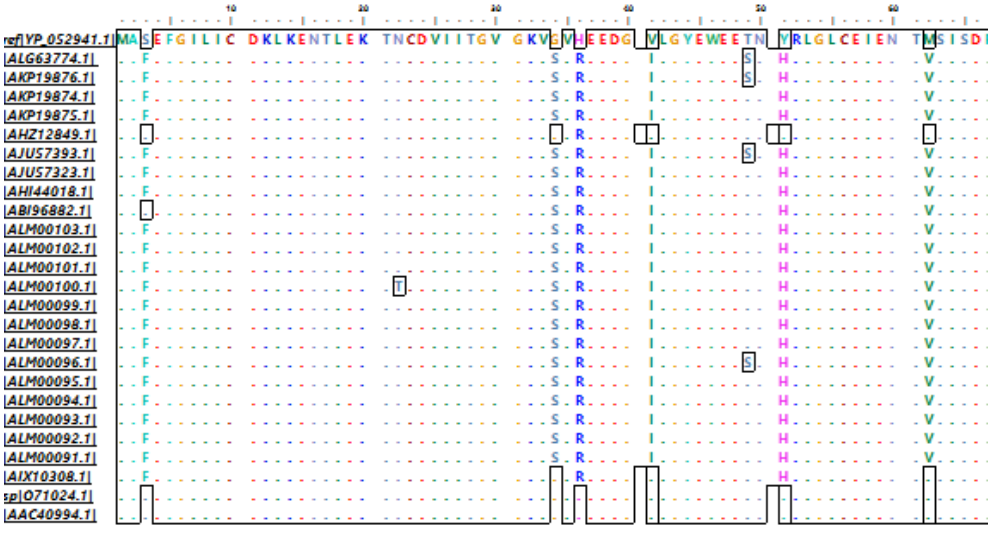

Figure 1: Multiple sequence alignment; *Dots indicate the conservancy, while letters shows the mutation or change in amino acid.

B-cell epitope prediction

Candidate epitopes were analyzed by several B-cell prediction methods which determine the antigenicity, hydrophilicity, flexibility and surface accessibility [28]. The method for predicting B-cell epitopes is the hidden Markov model. The linear predicted epitopes were obtained by using BepiPred linear epitope prediction with a threshold value of -0.165 [29]. The surface accessible epitopes were predicted from the conserved region with a threshold value of 1.000 using the Emini surface accessibility prediction tool [30]. Using the kolaskar and tongaonker antigenicity we determined the antigenic sites with a threshold value of 1.000 Figure 2, [31].

MHC Class I binding predictions

MHC I prediction tool has been used to analyze the referential (VP2) protein of the (AHSV) binding to MHC class I molecules (http://tools.iedb.org/mhci/). This prediction tool consists of 9 different prediction methods available in the Immune Epitope Database (IEDB) namely the covariance matrix SMMPMBEC, Artificial Neural Network (ANN), Stablized Materix Method (SMM), CombLib-Sidney2008, Consensus,

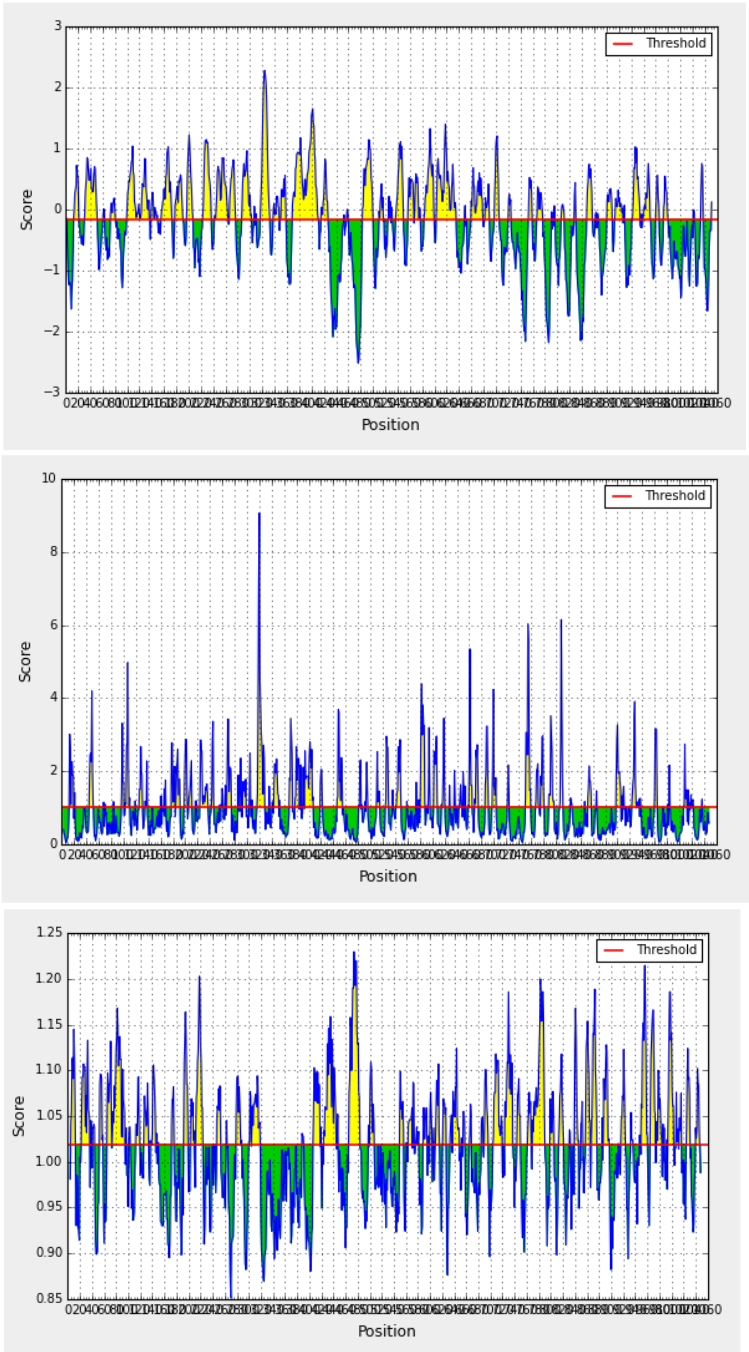

Figure 2: Prediction of B-cell epitopes by different scales; *Prediction of B-cell epitopes by Bepipred linear epitope method; **Prediction of B-cell epitopes by Emini surface accessibility method; Prediction of B-cell epitopes by Kolaskar & Tongaonkar Antigenicity method; *Yellow areas above threshold (red line) are proposed to be a part of B cell epitope, while green areas are not.

NetMHCpan, PickPocket, NetMHCcons and IEDB Recommended. Artificial neural network (ANN) was chosen as it is perfectly suitable in the incorporated such higher order connections when predicting the binding affinity [32,33]. Analysis was done using HLA alleles [34]. For all the alleles, peptide length was set to 9 amino acids prior to the prediction. The half maximal inhibitory concentration (IC50) values of the peptide's binding to MHC I molecules was calculated and the ones that had binding affinity (IC50) less than 100 nM were suggested to be promising candidate epitopes.

MHC Class II binding predictions

The referential protein sequence was submitted in the (IEDB) MHC II binding prediction tool (http://tools.immuneepitope.org/mhcii/). For the screening of promising epitopes, human allele reference set(HLA DR, DQ, DP) was used [35-37]. In MHC II binding analysis at the (IEDB) there are seven prediction methods including IEDB recommended, Consensus, Net MHCII pan, NN-align, SMM-align, combinatorial library and Sturniolo methods. NN-align method was used with a half maximal inhibitory concentration (IC50) 0f 1000 nM. Peptides less or equal to the (IC50) value were proposed to be promising MHC II epitopes [38,39].

Homology modeling

Raptor X protein structure prediction server available at (http://raptorx.uchicago.edu/StructurePrediction/predict/) was utilized for the creation of the 3D structure of the outer capsid protein (VP2) using the reference sequence (YP_052941.1) as an input – and Chimera 1.8 [40] was the tool used to visualize the selected epitopes belonging to the B cell, MHC class I and MHC class II.

Docking of the selected epitopes with MHC class 1 alleles

The reference MHC class I of Equas Caballus ELA-A3 haplotype (NP_001116853.1) was retrieved in FASTA format in the 7th of September 2016 from the National Center for Biotechnology Information (NCBI) and uploaded to the Raptor X (http://raptorx.uchicago.edu/StructurePrediction/predict/) to obtain the 3D structure. Pep fold available at the mobyle web server was used to predict the peptides structure. The five peptides that bound the largest number of alleles were selected for Pep-Fold server [41,42] which produced five models (3D peptide structure) for each peptide. The proposed models for each of the selected MHC class I binding peptides were docked with the refrence MHC class I protein using Patch dock server with a Clustering Root Mean Square Deviation value of 1.5 (clustering RMSD=1.5) and a complex type of Protein –small ligand [43,44]. The results were refined to the best five using the fast interaction refinement in molecular docking server Fire Dock [44] and visualized using Chimera 1.8 [40].

Results

Alignment and determination of conserved regions

Multiple sequence alignment was done for all retrieved sequences using Bioedit software to determine the conserved region so as to predict the only conserved epitopes that might act as a peptide vaccine.

B-cell epitope prediction

The major outer capsid protein (VP2) was subjected to Bepipred linear epitope prediction, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity methods in the IEDB. In the Bepipred linear epitope prediction with a maximum score of 2.285, a minimum score of -2.520 and an average of -0.165 at a hypothetical threshold of 0.023, epitopes that had values equal or more than the threshold were considered to be a linear antibody binders (B-cell linear epitopes). When Emini surface accessibility method was applied, it gave a minimum score of 0.037, a maximum score of 9.074 and an average score of 1.000 at a hypothetical threshold of 1.000. Those with scores equal or great than the threshold were regarded to be potential surface B cell epitopes. The average of antigenic propensity of the protein was predicted to be 1.019, the maximum value was 1.230 and the minimum value was 0.851. Epitopes of values equal or more than the hypothetical threshold 1.019 were proposed to be potential antigenic determinants. The result is summarized in Table 2 and the position of peptides at the molecular level is presented in Figure 3.

Predicted T-cell epitopes and interaction with MHC Class I

Table 3 shows T-cell MHC class-I predicted epitopes for various HLA alleles. These 9 sequenced epitopes were obtained from the IEDB MHC I binding epitope prediction tool using ANN method and the reference major outer capsid protein (VP2) as an input. There were 32 human MHC class-I alleles for which the epitopes were predicted. Sucsseful candidates –those that had a half maximal inhibitory concentration (IC50) <100 nM were determined and the best 5 are listed Table 3 with the best 3 shown at the structural level of (VP2) protein, Figure 4.

Predicted T- cell epitopes and interaction with MHC Class II

The reference major outer capsid protein (VP2) was analyzed by IEDB MHC II binding prediction tool using human alleles (25 alleles). Based on the NN-align method with half maximal inhibitory concentration (IC50) ≤ 1000 nM, epitopes are listed in Table 4 and

Peptides	Start	End	Length	Emini	Antigenicity
TLEK	17	20	4	1.377	0.985
EEDG	37	40	4	1.656	0.861
RVDRDEKSI	103	111	9	2.378	0.978
FSPEYY*	125	130	6	1.497	1.057
YERIE	184	188	5	1.644	0.978
HETDPTY	198	204	7	3.023	0.981
TRTS	232	235	4	1.827	0.926
QGVVK	247	251	5	0.481	1.117
SDNAE	257	261	5	1.604	0.914
MEEEEL	269	274	6	1.44	0.913
KYATRSGMRE	288	297	10	2.825	0.937
KKKDEGDDETA	319	329	11	8.853	0.903
KVDINPNHQTWKD	374	386	13	2.711	0.974
RAQQ	397	400	4	1.983	0.992
NKPLK	404	408	5	2.09	0.99
KYGT	421	424	4	1.495	0.969
GQDIEGFKKGSNSS	485	498	14	1.147	0.948
DKVVEDPESY*	589	598	10	2.122	1.037
QKNPAEV	603	611	7	1,702	1.012
GYDTDQNVV**	616	624	9	0.822	1.026
YDTDQNVV*'***	617	624	8	1.15	1.045
KMTE	628	631	4	1.653	0.879
RGQKV	699	703	5	1.27	1.015
DVDYGK	850	855	6	1.259	1.013
ERRGEPR	925	931	7	5.249	0.895

Table 2: List of B cell epitopes predicted by different scales. *Candidates that showed high probability to be B-cell epitopes; **Peptide that when shortened from (616-624) to (617-624) gave a high score in Emini; ***Peptide after being shortened.

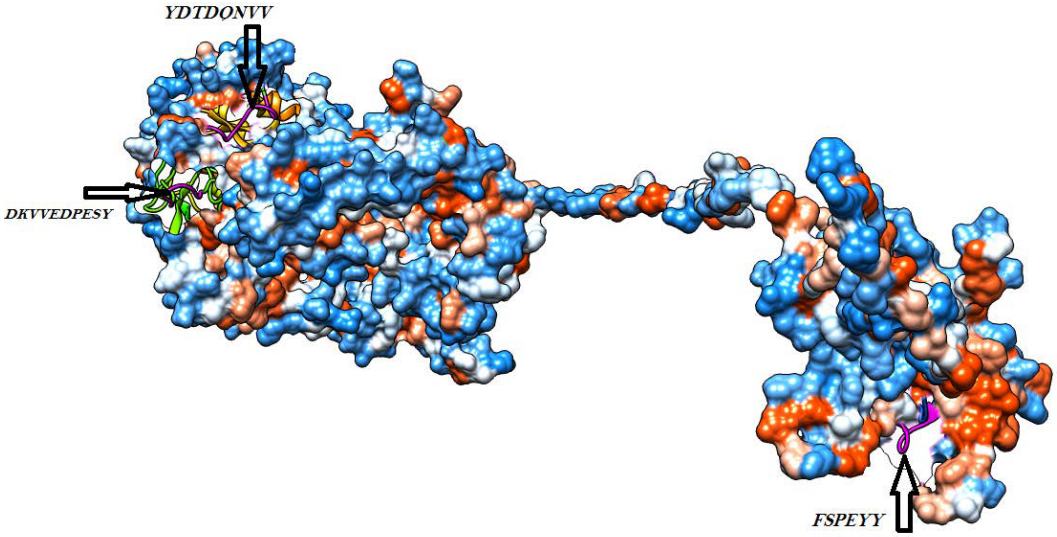

Figure 3: Proposed B-cell epitopes in (VP2) protein; Proposed epitopes of B-cell that are conserved in all obtained sequence of virulent strain are shown in the structural level of (VP2) protein of AHSV.

Peptide	Start	End	Allele	ANN-IC50*	Percentile rank
YTFGNKFLL	1035	1043	HLA-A*02:01	41	1.4
			HLA-A*02:06	33	1.9
			HLA-A*68:02	8	0.4
			HLA-C*03:03	21	1.5
			HLA-C*07:01	75	0.7
			HLA-C*12:03	46	1.7
			HLA-C*14:02	54	1.6
			HLA-C*15:02	64	0.6
YAYCLILAL	472	480	HLA-A*02:01	52	1.7
			HLA-A*02:06	37	2
			HLA-B*35:01	97	1.6
			HLA-B*39:01	11	0.2
			HLA-C*03:03	3	0.2
			HLA-C*12:03	4	0.2
			HLA-C*14:02	10	0.4
			HLA-C*15:02	88	0.9
FPTYKHYYL	775	783	HLA-B*07:02	90	1
			HLA-B*08:01	24	0.3
			HLA-B*35:01	78	1.4
			HLA-B*35:03	62	0.2
			HLA-C*03:03	57	2.4
KMTEGVTYF	628	636	HLA-A*02:06	31	1.8
			HLA-A*32:01	76	0.7
			HLA-B*15:01	66	0.9
			HLA-B*58:01	43	0.7
			HLA-C*14:02	96	2.3
WFFDYYATL	914	922	HLA-A*23:01	72	0.5
			HLA-C*03:03	33	1.8
			HLA-C*07:02	33	0.2
			HLA-C*12:03	64	2
			HLA-C*14:02	7	0.3

Table 3: List of best epitopes that had binding affinity with the human MHC class I alleles; *Inhibitory concentration needed for binding MHC I according to the IEDB ANN method, the lower is better.

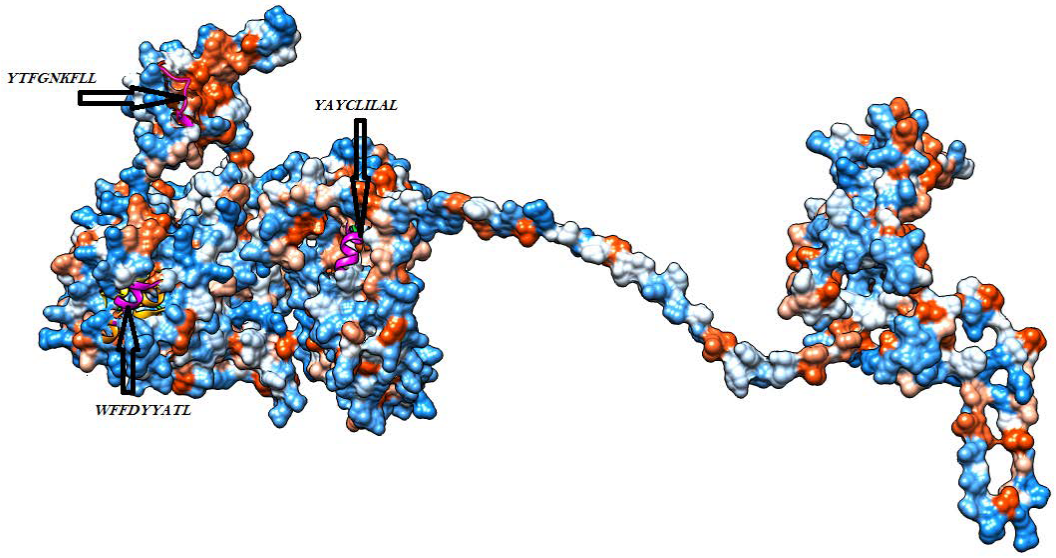

Figure 4: Proposed T-cell epitopes that interact with MHC I; Proposed epitopes of MHC I that are conserved in all obtained sequence of virulent strain are shown in the structural level of (VP2) protein of AHSV.

Epitope (core)	Start	End	Peptide	Allele	NN-IC50	Percentile rank
YKYESLLLG	688	696	ASTYKYESLLLGKNR	HLA-DRB1*04:01	44	3.23
				HLA-DRB1*01:01	29	15.11
				HLA-DRB1*04:04	551.1	36.44
				HLA-DRB1*04:05	215.6	17.46
				HLA-DRB1*11:01	60.3	9.8
				HLA-DPA1*01:03/DPB1*02:01	12.9	1.54
				HLA-DPA1*02:01/DPB1*01:01	8.6	0.31
				HLA-DPA1*03:01/DPB1*04:02	7.7	0.51
				HLA-DQA1*01:02/DQB1*06:02	988.2	39.96
				HLA-DQA1*05:01/DQB1*03:01	810	44.11
			STYKYESLLLGKNRG	HLA-DRB1*04:01	65	5.19
				HLA-DRB1*01:01	44.9	19.97
				HLA-DRB1*04:04	539.3	36.04
				HLA-DRB1*11:01	52	8.76
				HLA-DPA1*01/DPB1*04:01	32.6	2.11
				HLA-DPA1*01:03/DPB1*02:01	20.3	2.42
				HLA-DPA1*02:01/DPB1*01:01	10.2	0.46
				HLA-DPA1*02:01/DPB1*05:01	43.4	0.61
				HLA-DPA1*03:01/DPB1*04:02	9.3	0.75
				HLA-DQA1*05:01/DQB1*03:01	965.5	47.33
			TYKYESLLLGKNRGQ	HLA-DRB1*04:01	78.8	6.37
				HLA-DRB1*04:04	766.7	42.34
				HLA-DPA1*01/DPB1*04:01	88.8	4.86
				HLA-DPA1*01:03/DPB1*02:01	72.2	6.69
				HLA-DPA1*02:01/DPB1*01:01	21.8	1.82
				HLA-DPA1*02:01/DPB1*05:01	53.6	0.84
				HLA-DPA1*03:01/DPB1*04:02	14.2	1.46
			HASTYKYESLLLGKN	HLA-DRB1*01:01	43.3	19.54
				HLA-DRB1*04:04	582.2	37.38
				HLA-DRB1*04:05	225	17.97
				HLA-DRB1*11:01	122.1	15.48
				HLA-DPA1*01:03/DPB1*02:01	11.6	1.36
				HLA-DPA1*02:01/DPB1*01:01	8.8	0.33
				HLA-DPA1*03:01/DPB1*04:02	9.1	0.72

				HLA-DQA1*05:01/DQB1*03:01	715.3	41.88
			IHASTYKYESLLLGK	HLA-DRB1*01:01	55.7	22.57
				HLA-DRB1*04:04	718.2	41.15
				HLA-DRB1*04:05	341.8	23.54
				HLA-DRB1*11:01	201.4	20.37
				HLA-DRB5*01:01	440.8	31.37
				HLA-DPA1*01:03/DPB1*02:01	10.5	1.21
				HLA-DPA1*02:01/DPB1*01:01	9.6	0.4
				HLA-DPA1*03:01/DPB1*04:02	11	0.99
				HLA-DQA1*05:01/DQB1*03:01	816.9	44.26
			GIHASTYKYESLLLG	HLA-DRB1*01:01	89.8	28.71
				HLA-DRB1*04:04	664.4	39.8
				HLA-DRB1*04:05	320.4	22.65
				HLA-DRB3*01:01	861.2	16.27
				HLA-DRB5*01:01	652.6	36.78
				HLA-DPA1*03:01/DPB1*04:02	16.9	1.85
				HLA-DQA1*05:01/DQB1*03:01	818.9	44.3
				HLA-DPA1*02:01/DPB1*01:01	12.7	0.73
				HLA-DPA1*01:03/DPB1*02:01	10.7	1.24
			YKYESLLLGKNRGQK	HLA-DPA1*01/DPB1*04:01	180.6	7.99
				HLA-DPA1*01:03/DPB1*02:01	450.8	20.33
				HLA-DPA1*02:01/DPB1*01:01	83.3	8.85
				HLA-DPA1*02:01/DPB1*05:01	229.5	5.08
				HLA-DPA1*03:01/DPB1*04:02	28.5	3.4
VRDYVVQLL	950	958	CGGVRDYVVQLLPMR	HLA-DRB1*03:01	342.5	11.2
				HLA-DRB1*09:01	779.6	33.68
				HLA-DRB4*01:01	36.2	2.46
				HLA-DPA1*02:01/DPB1*01:01	51.2	5.42
				HLA-DQA1*01:01/DQB1*05:01	230.8	4.9
			NCGGVRDYVVQLLPM	HLA-DRB1*01:01	269.3	46.21
				HLA-DRB1*07:01	975.1	43.78
				HLA-DRB4*01:01	47.1	3.46
				HLA-DPA1*02:01/DPB1*01:01	98.6	10.27
				HLA-DQA1*01:01/DQB1*05:01	194.7	4.23
				HLA-DQA1*05:01/DQB1*02:01	300.9	6.77
			SNCGGVRDYVVQLLP	HLA-DRB1*01:01	372	51.86
				HLA-DRB4*01:01	95.3	7.43
				HLA-DPA1*02:01/DPB1*01:01	176.7	16.26
				HLA-DPA1*03:01/DPB1*04:02	677.7	27.06
				HLA-DQA1*01:01/DQB1*05:01	230.5	4.89
				HLA-DQA1*01:02/DQB1*06:02	993.2	40.06
				HLA-DQA1*05:01/DQB1*02:01	597	13.17
			GSNCGGVRDYVVQLL	HLA-DRB1*04:05	933.9	40.1
				HLA-DRB1*07:01	916	42.81
				HLA-DRB4*01:01	354.6	21.87
				HLA-DPA1*02:01/DPB1*01:01	316.7	23.82
				HLA-DQA1*01:01/DQB1*05:01	309.9	6.26
				HLA-DQA1*05:01/DQB1*02:01	736.7	15.87
			GGVRDYVVQLLPMRK	HLA-DRB1*13:02	965.7	25.43
				HLA-DRB4*01:01	30.6	1.97
				HLA-DPA1*02:01/DPB1*01:01	43.9	4.57
				HLA-DPA1*02:01/DPB1*05:01	749.8	14.37
				HLA-DQA1*01:01/DQB1*05:01	410.4	7.79

FKQEIQANF	511	519	PNFKQEIQANFGINL	HLA-DRB1*04:01	961.2	42.07
				HLA-DRB1*03:01	103.5	5.14
				HLA-DRB1*04:04	611.1	38.27
				HLA-DRB1*04:05	219.7	17.68
				HLA-DRB1*08:02	451.4	10.97
				HLA-DRB3*01:01	165.9	6.12
				HLA-DRB4*01:01	518.6	28.04
				HLA-DRB5*01:01	295.2	26.4
				HLA-DPA1*02:01/DPB1*01:01	421.3	28.04
			FPNFKQEIQANFGIN	HLA-DRB1*04:01	974.8	42.38
				HLA-DRB1*03:01	49.7	2.85
				HLA-DRB1*01:01	415.3	53.72
				HLA-DRB1*04:04	706.6	40.87
				HLA-DRB1*04:05	147.1	13.19
				HLA-DRB1*07:01	459.1	32.07
				HLA-DRB1*08:02	711.1	16.87
				HLA-DRB1*09:01	304.9	17.94
				HLA-DRB3*01:01	73.1	3.66
				HLA-DRB5*01:01	168.5	20.2
				HLA-DPA1*02:01/DPB1*01:01	454.1	29.21
			MFPNFKQEIQANFGI	HLA-DRB1*04:01	985.3	42.63
				HLA-DRB1*03:01	67.1	3.66
				HLA-DRB1*01:01	641.6	60.64
				HLA-DRB1*04:04	684.9	40.31
				HLA-DRB1*04:05	155.6	13.77
				HLA-DRB1*07:01	347.4	28.18
				HLA-DRB1*08:02	836.1	19.37
				HLA-DRB1*09:01	434.9	23.23
				HLA-DRB3*01:01	72.2	3.62
				HLA-DRB5*01:01	221.2	23.09
				HLA-DPA1*02:01/DPB1*01:01	388	26.77
			QMFPNFKQEIQANFG	HLA-DRB1*03:01	146.6	6.56
				HLA-DRB1*04:04	677.1	40.11
				HLA-DRB1*04:05	203.8	16.97
				HLA-DRB1*07:01	545.1	34.55
				HLA-DRB1*09:01	671.9	30.83
				HLA-DRB3*01:01	87.9	4.13
				HLA-DRB5*01:01	385.1	29.63
			NFKQEIQANFGINLN	HLA-DRB1*03:01	205.1	8.16
				HLA-DRB1*04:05	361.1	24.35
				HLA-DRB3*01:01	367.4	9.8
				HLA-DRB4*01:01	676.7	32.86
				HLA-DRB5*01:01	425.7	30.91
			IQMFPNFKQEIQANF	HLA-DRB1*03:01	215.2	8.38
				HLA-DRB1*04:05	190.1	15.99
				HLA-DRB1*09:01	503.9	25.66
				HLA-DRB3*01:01	85.4	4.05
				HLA-DPA1*01:03/DPB1*02:01	981.1	30.19
			FKQEIQANFGINLNI	HLA-DRB1*03:01	447.3	13.19
				HLA-DRB1*04:05	556	30.98
				HLA-DRB3*01:01	812.5	15.71
				HLA-DRB5*01:01	759.1	39

	934	942	RLSFKYEGLTTWIGS	HLA-DRB1*04:01	145.2	11.46
				HLA-DRB1*04:04	512.7	35.13
				HLA-DRB1*11:01	886.9	38.97
				HLA-DRB3*01:01	117.5	4.96
				HLA-DRB5*01:01	656.2	36.87
				HLA-DPA1*01/DPB1*04:01	34.1	2.2
				HLA-DPA1*01:03/DPB1*02:01	14.4	1.73
				HLA-DPA1*02:01/DPB1*01:01	151.4	14.5
			PRLSFKYEGLTTWIG	HLA-DRB1*04:01	185.8	14.12
				HLA-DRB1*04:04	554.2	36.55
				HLA-DRB1*11:01	770.7	36.96
				HLA-DRB3*01:01	118.1	4.98
				HLA-DRB5*01:01	729.5	38.42
				HLA-DPA1*01/DPB1*04:01	37.5	2.41
				HLA-DPA1*01:03/DPB1*02:01	14.2	1.71
			EPRLSFKYEGLTTWI	HLA-DRB1*04:01	330.4	21.88
				HLA-DRB1*01:01	332.9	49.94
				HLA-DRB1*04:04	760.7	42.19
				HLA-DRB1*04:05	842.8	38.21
				HLA-DRB1*07:01	591.5	35.8
				HLA-DRB3*01:01	112.2	4.82
				HLA-DRB5*01:01	731.9	38.46
				HLA-DPA1*01/DPB1*04:01	37.6	2.41
				HLA-DPA1*01:03/DPB1*02:01	13.8	1.66
FKYEGLTTW			GEPRLSFKYEGLTTW	HLA-DRB1*04:01	501.4	28.88
				HLA-DRB1*04:04	876.8	44.84
				HLA-DRB3*01:01	141.4	5.56
				HLA-DRB5*01:01	997.3	43.2
				HLA-DPA1*01/DPB1*04:01	57.5	3.45
				HLA-DPA1*01:03/DPB1*02:01	20.2	2.41
				HLA-DPA1*02:01/DPB1*01:01	232.4	19.61
			SFKYEGLTTWIGSNC	HLA-DRB1*04:04	349.1	28.74
				HLA-DRB1*08:02	950	21.57
				HLA-DRB3*01:01	496.9	11.7
				HLA-DPA1*01/DPB1*04:01	219.7	9.06
				HLA-DPA1*01:03/DPB1*02:01	59	5.8
				HLA-DPA1*02:01/DPB1*01:01	423.6	28.12
				HLA-DPA1*03:01/DPB1*04:02	59.4	6.63
			FKYEGLTTWIGSNCG	HLA-DRB1*04:04	404	31.13
				HLA-DPA1*01/DPB1*04:01	471.6	14.4
				HLA-DPA1*01:03/DPB1*02:01	213.1	13.38
				HLA-DPA1*02:01/DPB1*01:01	816.7	38.97
				HLA-DPA1*03:01/DPB1*04:02	624.4	26.11
			LSFKYEGLTTWIGSN	HLA-DRB1*04:04	510.3	35.05
				HLA-DRB3*01:01	259	7.99
				HLA-DPA1*01/DPB1*04:01	65.1	3.81
				HLA-DPA1*01:03/DPB1*02:01	21.4	2.54
				HLA-DPA1*02:01/DPB1*01:01	246.3	20.38
				HLA-DPA1*03:01/DPB1*04:02	32.9	3.93

	500	508	SSAILETVIQMFPNF	HLA-DRB1*03:01	517.2	14.34
				HLA-DRB1*04:05	234.8	18.5
				HLA-DRB1*07:01	225.5	22.72
				HLA-DRB3*01:01	759.1	15.06
				HLA-DPA1*01:03/DPB1*02:01	250.3	14.68
				HLA-DPA1*02:01/DPB1*01:01	74.8	8.02
				HLA-DQA1*04:01/DQB1*04:02	597.1	9.45
				HLA-DQA1*05:01/DQB1*02:01	268.8	6
			NSSAILETVIQMFPN	HLA-DRB1*03:01	554.3	14.92
				HLA-DRB1*04:05	231.6	18.34
				HLA-DRB1*07:01	164.7	19.26
				HLA-DRB3*01:01	828.5	15.9
				HLA-DPA1*01/DPB1*04:01	660.2	17.39
				HLA-DPA1*01:03/DPB1*02:01	314	16.69
				HLA-DPA1*02:01/DPB1*01:01	94.2	9.88
				HLA-DQA1*05:01/DQB1*02:01	267.2	5.96
			SNSSAILETVIQMFP	HLA-DRB1*03:01	687.8	16.8
				HLA-DRB1*04:05	225.9	18.02
				HLA-DRB1*07:01	97.4	13.94
				HLA-DRB3*01:01	910.4	16.85
				HLA-DRB4*01:01	108.1	8.38
				HLA-DPA1*01/DPB1*04:01	647.8	17.22
				HLA-DPA1*01:03/DPB1*02:01	289.9	15.95
				HLA-DPA1*02:01/DPB1*01:01	94.9	9.94
ILETVIQMF				HLA-DQA1*04:01/DQB1*04:02	346.8	5.12
				HLA-DQA1*05:01/DQB1*02:01	229.1	5.04
			GSNSSAILETVIQMF	HLA-DRB1*03:01	806.2	18.32
				HLA-DRB1*04:05	279.5	20.78
				HLA-DRB1*07:01	59	9.83
				HLA-DRB4*01:01	253.4	17.19
				HLA-DPA1*01:03/DPB1*02:01	362.9	18.1
				HLA-DPA1*02:01/DPB1*01:01	106.2	10.94
				HLA-DQA1*04:01/DQB1*04:02	380	5.71
				HLA-DQA1*05:01/DQB1*02:01	377.9	8.55
			SAILETVIQMFPNFK	HLA-DRB1*07:01	241.9	23.59
				HLA-DRB4*01:01	102.6	7.97
				HLA-DPA1*01:03/DPB1*02:01	273.9	15.46
				HLA-DPA1*02:01/DPB1*01:01	85.4	9.06
				HLA-DPA1*02:01/DPB1*05:01	474.4	9.92
				HLA-DQA1*04:01/DQB1*04:02	814.2	12.98
				HLA-DQA1*05:01/DQB1*02:01	337	7.61
			AILETVIQMFPNFKQ	HLA-DPA1*01/DPB1*04:01	696.7	17.91
				HLA-DPA1*01:03/DPB1*02:01	291.8	16.01
				HLA-DPA1*02:01/DPB1*01:01	79.7	8.51
				HLA-DQA1*05:01/DQB1*02:01	408.4	9.22
			ILETVIQMFPNFKQE	HLA-DPA1*01/DPB1*04:01	76.66	18.89
				HLA-DPA1*01:03/DPB1*02:01	338	17.4
				HLA-DPA1*03:01/DPB1*04:02	289.8	18.06
				HLA-DQA1*05:01/DQB1*02:01	508.2	11.34

Table 4: List of best epitopes that had binding affinity with the human MHC class II alleles; *Inhibitory concentration needed for binding MHC II to the IEDB NN-align method, the lower is better.

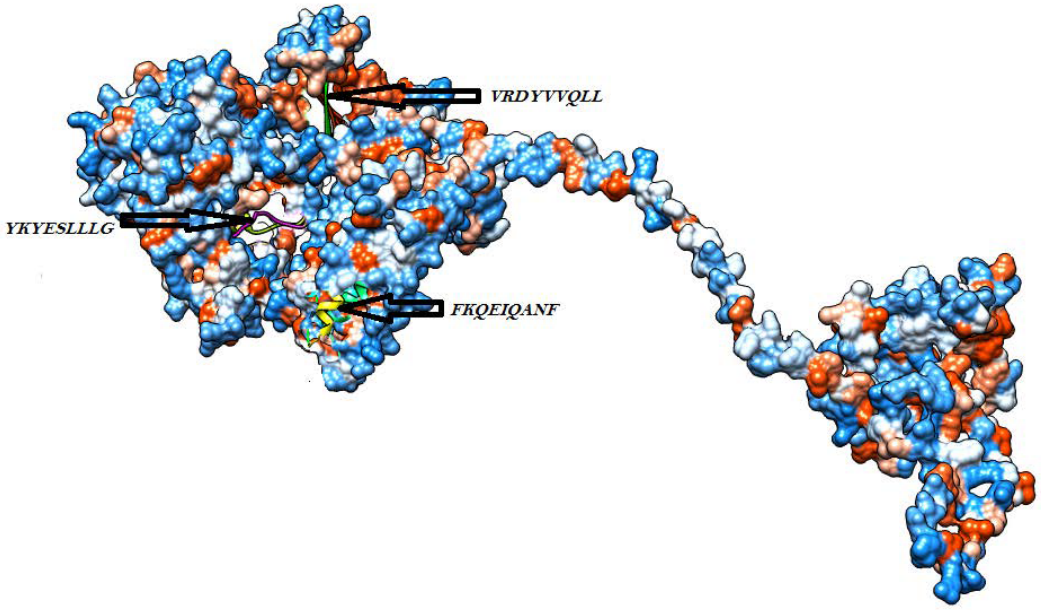

Figure 5: Proposed epitopes of T-cell that interact with MHC II**;** Proposed epitopes of MHC II that are conserved in all obtained virulent strain are shown in the structural level of (VP2) protein of AHSV.

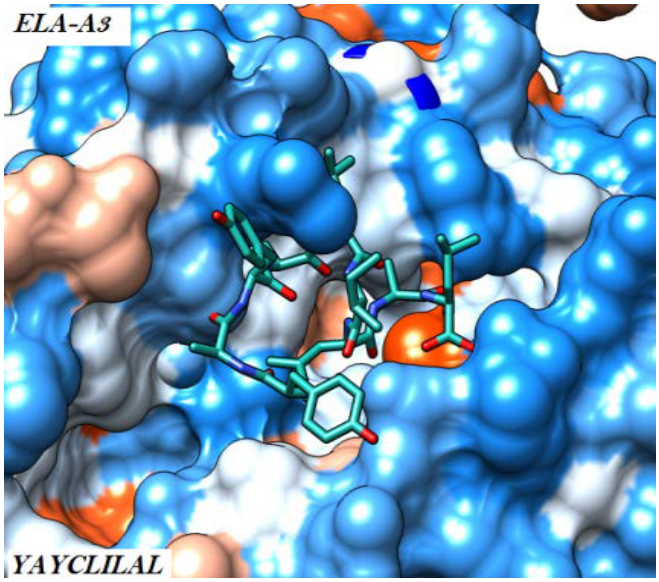

Figure 6: Interaction of YAYCLILAL with MHC I allele of horse;* The ligand is shown in a stick structure while the receptor is shown in the rounded ribbon structure.

position of the best 3 in the structural level of (VP2) protein is shown in Figure 5.

Docking

All docked epitopes were found to have binding affinity to equine MHC I molecule (ELA-A3) haplotype Table 5. illustrates the binding energy score for the suggested epitopes as a global energy as obtained from patch dock for all epitopes. (Figures 6-8) visualize the binding interactions between MHC I receptor and epitopes in the structural level.

MHC I binding peptides	Global energy
YTFGNKFLL	-27.94
YAYCLILAL	-39.28
WFFDYYATL	-57.56
KMTEGVTYF	-38.24
FPTYKHYYL	-42.10

Table 5: Docking results of most promising predicted and modeled MHC-I binding epitopes with the equine MHC-I using patch dock server; *Global energy, it is the energy required for the binding of epitopes with MHC molecule, lower energy indicates good and stable binding.

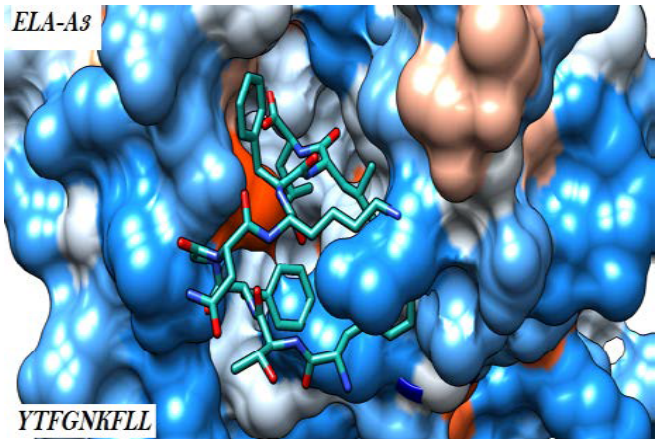

Figure 7: Interaction of YTFGNKFLL with MHC I allele of horse**;** *the ligand is shown in a stick structure while the receptor is shown in the rounded ribbon structure.

Discussion

The equine disease caused by the African Horse Sickness Virus (AHSV) often causes a mortality rate exceeding 90% in susceptible horse populations [45-47]. Attempts for protection and prevention

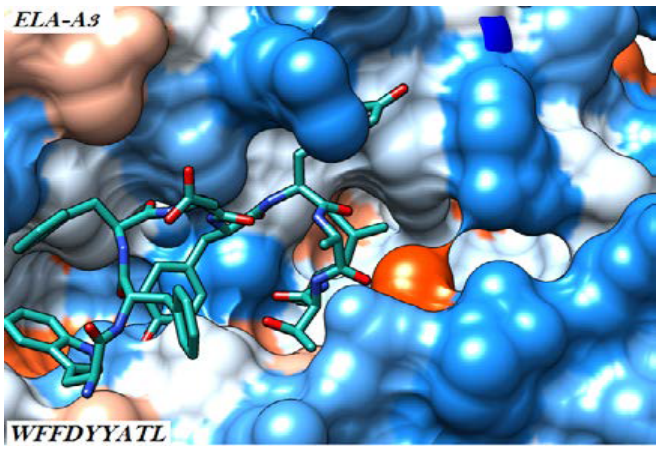

Figure 8: Interaction of WFFDYYATL with MHC I alleles of horse; *the ligand is shown in a stick structure while the receptor is shown in the rounded ribbon structure.

from African horse sickness diseases has started as early as 1930's; when a prophylactic vaccination was carried out in South Africa in 1933 using a bivalent live AHS vaccine. Other live attenuated vaccines were developed through years and currently there are commercially available for most AHSV serotypes [45,46,48-51]. These vaccines are cheap and effective in producing a protective immunity following single inoculation, yet they have limitations; the biggest of which is the reassortment of the vaccine virus with wild-type virus strains in the mammalian host and/or insect [47,48,52].

The safety of inactivated vaccines if produced properly- is an advantage that can overweigh their expensiveness and requirement for repetition due to loss of immunogenicity that accompanies thorough inactivation. This was the case when the killed (AHS) vaccine was developed against serotype -4 of the virus during its invasion into the non-enzootic region of the Iberian Peninsula in the 1980s and the formalin-inactivated vaccine "Equipest®" that was commercially available during the 1987-1991 (AHSV) epidemic in Spain, Portugal and Morocco [49,52].

Killed vaccines are no longer available and other approaches have been considered to develop vaccines that overcome the limitations of the previous ones. Virus like particle vaccines have been synthesized by baculoviruse system, baculo synthesized virus consisting of (VP2, VP3, VP5 and VP7) were tested [45]. A recombinant vaccine of the 2 major outer capsid proteins (VP2a and VP5) with a third major inner one (VP7) was developed through baculoviruse. This vaccine is believed to not only prevent the disease, but also to reduce the propagation of the virus by vectors, but it is not yet available in the market [45]. (VP2) and (VP5) genes of (AHSV-4) expressed by the Venezuelan equine encephalitis virus have been considered for a candidate recombinant (AHS) vaccine, however, they failed to induce neutralizing antibody in horse [47,50]. Canarypox virus vectored (ALVAC®) vaccine expressing the (VP2) and (VP5) of (AHSV-4) was also studied against African horse sickness virus (AHSV) infection and it gave promising results [52]. More recently a pilot study has investigated the immunogenicity of recombinant Modified Vaccinia Ankara virus as a vector for the (AHSV-4): (VP2, VP7) and the non-structural protein (NS3) antigens, the results revealed the great potential for the (MVAVP2) in eliciting protective immunity against lethal (AHSV) infections [13,47].

Peptide vaccine have shown its preventive and therapeutic success in a considerable number of human and animal viruses and diseases including e.g, Influenza virus , Hepatitis B virus, Human Immune Deficiency virus (HIV), Ebola virus, cancer and others [53-57]. In this study we aimed to predict possible peptide vaccine for the African horse sickness virus and we targeted strain 6 out of 9 known strains for this virus [58] as the data we collected from the reference sequence alignment suggested to do. Since the dominant antigenic sites eliciting serotype specific neutralizing immunoglobulin's are mainly positioned on the major outer capsid protein (VP2) [59,60] it was the antigenic protein form which we proposed our candidate peptides.

Regarding epitopes that would elicit an antibody immune response "B-cell epitopes", FSPEYY, DKVVEDPESY and YDTDQNVV were proposed as they got results above thresholds in Bepipred linear epitope prediction, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity prediction methods and have shown 100% conservancy among the (AHSV-VP2) retrieved sequences on the 7th of September 2016.

As we are targeting a virus the importance of MHC I and II in evading and controlling the infection is crucial [61]. Till the 7th of September 2016, the IEDB T-cell prediction tools haven't entered equine alleles as data yet, thus we used human as a model using his alleles to predict possible horse MHC I and II binding candidates since it showed similarity [34-37]. There were 5 epitopes that was addressed as potentially promising epitopes as they bound the highest number of alleles. 'YTFGNKFLL' epitope was found to bind 8 MHC I Alleles 'HLA-A*02:01, HLA-A*02:06, HLA-A*68:02, HLA-C*03:03, HLA-C*07:01, HLA-C*12:03, HLA-C*14:02 and HLA-C*15:02' and 'YAYCLILAL' epitope was found to bind 8 MHC I alleles as well 'HLA-A*68:02, HLA-A*02:01, HLA-A*02:06, HLA-B*35:01, HLA-B*39:01, HLA-C*03:03, HLA-C*12:03, HLA-C*14:02, HLA-C*15:02'. Other 3 epitopes 'WFFDYYATL, KMTEGVTYF and FPTYKHYYL' showed affinity for 5 MHC I alleles. As a matter of confirmation, YTFGNKFLL, YAYCLILAL and WFFDYYATL peptides were docked with the equine MHC molecule using the patchdock server. All epitopes were found to have a high binding affinity and low binding energy to equine MHC class I molecule ELA-A3 haplotype in the structural level. The epitopes YAYCLILAL, YTFGNKFLL were visualized because they bind the largest number of alleles. In spite of binding to 4 alleles the epitopes WFFDYYATL was also visualized as it had the lowest global energy (-57.56) suggesting its importance as a component of the designed peptide vaccine.

For the MHC II binding peptides, 312 conserved epitopes were predicted to interact with different human MHC II alleles. We postulated the best five; the 9-mer peptide (core) YKYESLLLG that showed affinity for 14 MHC- II molecules including (HLA-DRB1*04:01, HLA-DRB1*01:01, HLA-DRB1*04:04, HLA-DRB1*04:05, HLA-DRB1*11:01, HLA-DRB3*01:01, HLA-DRB5*01:01, HLA-DPA1*01/DPB1*04:01, HLA-DPA1*01:03/DPB1*02:01, HLA-DPA1*02:01/DPB1*01:01, HLA-DPA1*02:01/DPB1*05:01, HLA-DPA1*03:01/DPB1*04:02, HLA-DQA1*01:02/DQB1*06:02, HLA-DQA1*05:01/DQB1*03:01), followed by VRDYVVQLL, FKQEIQANF and FKYEGLTTW that showed to interact with 13 human MHC II alleles and ILETVIQMF that was predicted to bind 12 human MHC II alleles.

Up to our knowledge no peptide vaccine has been developed yet which would highlight this work to be considered for further investigation *in vivo*.

Conclusion

We targeted determining the possible peptide vaccine moieties

through an immunoinformatics approach as it enables achieving effective, cost-efficient development of vaccines. We chose the peptide vaccines to work on as they impart important advantages by which they circumvent major issues making them important practical approach considered in developing vaccines of many important human and veterinary diseases.

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