

Original Research Article

Bio-computational prediction of novel epitopes on VP2 protein of infectious bursal disease virus

ABSTRACT

Aim: Recently, many viral Immunogenic peptides or epitopes have been used as potential vaccine and also immune-diagnostic candidates. In this study, we predicted different epitopic peptides on VP2 protein of infectious bursal disease virus (IBDV) by use of bioinformatics modelling which can be potential vaccine as well as diagnostic candidate for IBD, in future.

Study design: In the present study, B-cell epitopes (linear, continuous, and conformational) and T-cell epitopes were predicted on VP2 protein.

Place and Duration of Study: Bihar Animal Sciences University, Patna, and Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India between June and December 2023.

Methodology: For the prediction of linear B-cell epitopes, Bepipred Linear Epitope Prediction 2.0, SVMTriP, and BCPred tools were used, while Ellipro was used for conformational B-cell epitopes prediction. NetMHCcons 1.1 and NetMHCIIpan - 4.0 tools were used to predict strength binding affinity of peptides with MHC-I and MHC-II, respectively.

Results: As per analysis by four different tools, the peptide 'SYDLGYVRLGDIPIAIGLDPKMOVATCDSSDRPRVYTITAADDYQFSSQYQPGGV¹⁶⁴⁻²¹⁷' (Epitope_L) was predicted as the most prominent linear B-cell epitope. Two peptides, i.e. ANLNSPLKIAG (Epitope_C 1) and SSQYQPGGRTSVHGLGLTTGTDKSGGQAGDQMS (Epitope_C 2) were predicted as potent conformational B-cell epitopes. During T-cell epitopes analysis, a 9-mer GELVFQTSV²³⁶⁻²⁴⁴ peptide was predicted as MHC-I strong binder ability while, two 15-mer peptides, i.e. YTKLILSERDRLGK³⁸⁹⁻⁴⁰³ and QMLLTAQNLPASYNY⁷⁶⁻⁹⁰ were predicted as MHC-II strong binder ability.

Conclusion: Using bio-computational analysis, one linear and two conformational B-cell epitopes were predicted on VP2 protein of IBDV. During T-cell epitopes prediction one 9-mer peptide and two 15-mer peptides were predicted as MHC-I and MHC-II strong binding peptides, respectively. After assessing protective immune responses through in vitro and in vivo studies, these predicted peptides could be potential candidates for developing subunit vaccines.

Keywords: Infectious Bursal Disease Virus, VP2 protein, B-cell epitope, T-cell epitopes

1. INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious viral infection affecting young chickens, especially between 3 and 6 weeks of age. The causative agent is the infectious bursal disease virus (IBDV), a member of *Birnaviridae* family. IBDV primarily targets the bursa of Fabricius and replicates within the B cells of the bursa, causing severe immunosuppression and compromising the chicken's ability to mount an effective immune response ^{1,2}.

The IBDV has a bi-segmented double-stranded RNA genome. With the size of 3.2 kbp, segment A is the longer one, while segment B is smaller with size of 2.8 kbp ². Segment A has two partially overlapped open reading frames (ORFs). The first ORF encodes the non-structural VP5 protein of 17 kDa, and the second ORF encodes a 110 kDa polyprotein. After translation, the polyprotein is proteolytically cleaved into VPX, VP3 and VP4 proteins of 48 kDa, 32 kDa and 28 kDa, respectively. The VPX is further processed into 41 kDa VP2 protein ³. Segment B has only one ORF which encodes 95 kDa VP1 protein ⁴. The VP2 and VP3 are two structural proteins of IBDV ². The VP2 protein is the major structural protein of IBDV playing an important role in viral entry, assembly and immune recognition. It forms the bulk of the viral capsid and plays a crucial role in maintaining the structural integrity of the virus. The VP2 protein is involved in receptor binding and determines the tropism and host range of the virus. It also contains immunogenic epitopes that elicit an immune response in the host ⁵.

To prevent IBDV outbreaks, disease diagnosis, monitoring and vaccination of poultry are the common strategies ⁶. For control of infectious disease, live attenuated and inactivated vaccines are two major types of vaccines used poultry ⁷. However, both live-attenuated and inactivated vaccine have certain limitation. The live-attenuated vaccine has potential risk of reverting back to virulent pathogen, while multiple doses, alongwith adjuvant, are needed in case inactivated vaccine to achieve protective immunity ⁸.

A lot of work has been done recently on peptide-based vaccines and diagnostics. Peptide vaccines are composed of antigenic epitopic regions (short peptide sequences) which are recognized by the immune system leading to its activation. Peptides are also for development of diagnostic assays for infectious diseases ⁹. Peptide-based diagnostic techniques include enzyme-linked immunosorbent assays (ELISA), peptide microarrays, and peptide-based biosensors. Peptide-based diagnostics are affordable and having high sensitivity and specificity ¹⁰.

Considering the potential use of immunogenic peptides (epitopes) as vaccine and diagnostic assay candidates with high sensitivity and specificity, we predicted different epitopic peptides on VP2 protein of IBDV by use of bioinformatics modelling which can be potential vaccine as well as diagnostic candidate for IBD, in future.

2. MATERIALS & METHODS

2.1. Physicochemical properties prediction and 3D modelling

The amino acid (AA) sequence of VP2 protein of previously reported Indian isolate of IBDV (Acc. No.: AMQ81722; Region between 9 and 452 AA) was retrieved from the GenBank database of the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov/genbank>). Physicochemical properties like molecular weight, isoelectric point (pI), amino acid composition, estimated half-life, and instability index were determined by the ProtParam programme (<http://web.expasy.org/protparam/>). The 3D model of VP2 protein was constructed using homology modeling on the Protein Data Bank (PDB) database (<https://www.rcsb.org/>). Based on the highest sequence identity along with a lower E-value, the crystal structure of the VP2 protein of IBDV, available on PDB, was selected for further study.

2.2. Linear/continuous B-cell epitopes prediction

Possible linear B-cell epitopes on VP2 protein were predicted by the immunomedicine group, BepiPred Linear Epitope Prediction 2.0 (BepiPred-2.0), SVMTriP, and BCPred tools. The immunomedicine group tool (<http://imed.med.ucm.es/Tools/antigenic.pl>), which has approximately 75% accuracy and is based upon Kolaskar and Tongaonkar ¹¹ method was also used for epitopes prediction on VP2 protein. Linear B-cell epitopes were also predicted using BepiPred-2.0 tool (<http://tools.iedb.org/bcell/>). The BepiPred-2.0 tool is based upon a

random forest algorithm for epitopes annotated from antibody-antigen protein structures. It is a sequence-based epitope prediction tool based upon epitope data derived from solved 3D structures as well as epitopes database downloaded from IEDB¹². The SVMTriP Tool is based on machine learning algorithms (<http://sysbio.unl.edu/SVMTriP/>). SVMTriP was developed to predict antigenic epitopes using the latest sequence input from IEDB database. In the SVMTriP Tool, Support Vector Machine (SVM) in combination with Tri-peptide similarity and Propensity scores has been utilized to achieve better prediction performance¹³. The linear B-cell epitopes were also predicted using BCPREDS (B-cell epitope prediction service; <http://ailab.ist.psu.edu/bcpred/predict.html>), which joined a subsequence kernel and a support vector machine (SVM) method with the output reliability of 74.57 percentage¹⁴.

For the prediction of potential epitopic peptides with high specificity and sensitivity, consensus peptides from several epitopic peptide prediction methods should be selected¹⁵. In the current study, only those peptides or region of peptides common in three or more epitopic prediction methods were shorted as epitope.

2.3. Structure-based/conformational B-cell epitope prediction

Conformational B cell epitopes on VP2 protein was predicted using ElliPro (<http://tools.iedb.org/ellipro/>) tool based on the PDB 3D model of protein. The ElliPro is a web tool formulated by Thornton's method along with MODELLER program of a residue clustering algorithm and Jmol viewer¹⁶. The conformational B-cell epitopes predicted should have ElliPro score range between 0.5 and 0.8, and also have upper limit distance for residue clustering within 6.0 Å¹⁷.

2.4. T-cell epitope prediction

MHC-I and MHC-II binding peptides have been considered as T cell immunogens¹⁸. Since, no immune-bioinformatics tool is available to predict poultry MHC-peptide binding, in the current study only those human MHC-I/II alleles having greater than 70% identities those of poultry MHC-I/II alleles were selected, as described by Thomsen et al.¹⁹. Human HLA*B 40:06, HLA*B 41:03 and HLA*B 41:04 alleles having more than 70% similarity with poultry MHC class I alleles while DRB1:1310, DRB1:1366, DRB1:1445, and DRB1:1482 alleles having more than 70% similarity with poultry MHC class II alleles²⁰.

NetMHCcons 1.1 (<http://www.cbs.dtu.dk/services/NetMHCcons>) and NetMHCIIpan - 4.0 (<https://services.healthtech.dtu.dk/services/NetMHCIIpan-4.0/>) tools were used to predict strength binding affinity of peptides with MHC-I and MHC-II, respectively. For *in silico* prediction of MHC-I binding affinity, the VP2 protein sequence was chopped into 9 amino acids long all possible polypeptides subsequently binding affinity of all these peptide with MHC-I were estimated as IC50 concentration (in nM) as described by Valdivia-Olarte et al.²¹. Peptides were categorised into two groups based on their estimated affinities: "SB" or Strong Binder (for IC50 ≤ 2 nM) and "WB" or Weak Binder (for IC50 ≤ 2 nM ≤ 50 nM) with MHC-I. Peptides with 50 nM ≤ IC50 values were considered as non-binder of MHC-I. Similarly for prediction of MHC-II binding affinity, the VP2 protein sequence was chopped into 15 amino acids long all possible polypeptides subsequently binding affinity of all these peptide with MHC-II were estimated as % Rank as described by Andreatta et al.¹⁸. Peptide (s) having % Rank < 2 were considered as strong binders (SB) with MHC-II.

3. RESULTS

3.1. Physicochemical properties of VP2 protein

Online available ProtParam programme-based physicochemical analysis indicated that VP2 is a negatively charged protein with an isoelectric point of 5.12 and the molecular weight of 47.518 KDa. This protein contained 38 negatively charged residues (Asp + Glu) and 30 positively charged residues (Arg + Lys). The computationally calculated instability index of VP2 protein was 25.64. Glutamine (Gln; Q) was the N-terminal amino acid of this protein and its *in vitro* half-life was 0.8 hr in mammalian reticulocytes, while *in vivo* half-life in yeast, and in *E. coli* was 10 min, and 10 hr, respectively. The 3D structure of the VP2 protein of Indian isolate IBDV was predicted by homologous modeling using the PDB database. A total of eight reference templates were found on the PDB database having sequence identity by ranges between 99% and 44% and E-value range between 3.264e-288 and 1.608e-100. The

PDB code 3FBM (PDB code) (Fig. 1) having sequence identity (99%) and E-value (1.606e-287)^{22,23} was selected as reference model for further study.

3.2. Linear/continuous B-cell epitopes prediction

The complete amino acid sequence of VP2 protein was analyzed for epitope prediction using various tools. Based upon Immunomedicine Group Tool calculation, VP2 protein had an average antigenic propensity score of 1.0329 (Fig. 2). A total of 18 segments (Supplementary Table 1) of VP2 protein had antigenic greater antigenic propensity than average value of 1.0329 and these could be potential epitopes. Similarly, based upon Bepipred Linear Epitope Prediction 2.0, SVMTriP, and BCPred tools analysis of VP2 protein, 16 (Fig. 3; Supplementary Table 2), 8 (Supplementary Table 3), and 10 (Supplementary Table 4) peptides, respectively were predicted as epitopes.

As the 'consensus method' described by Yang and Yu¹⁵ for the prediction of epitopic peptides with high specificity and sensitivity, complete or partial common peptides predicted by any three or all four tools including Immunomedicine Group, Bepipred Linear Epitope Prediction 2.0, SVMTriP, and BCPred tools were shorted (Table 1). The peptide 'SYDLGYVRLGDIPIAIGLDPKMGVATCDSSDRPRVYTITAADDYQFSSQYQPGGV¹⁶⁴⁻²¹⁷' (Epitope_L) had complete or partial region of the predicted polypeptide by all four tools and was considered as the most prominent epitope on VP2 protein of IBDV.

3.3. Conformational B-cell epitopes prediction

With the help of Ellipro, conformational B-cell epitopes on VP2 protein (Table 2) were predicted by setting a minimum score of 0.8 and a maximum distance of 6.0 Å. The 3D structure of each of the Ellipro-predicted epitopes, as well as the relative orientation of the protein and peptide molecules, were all visualized in Jmol (Fig. 4). Jmol viewer also verified the placements of each projected epitope's amino acids. The top two highest-score conformational epitopes predicted by Ellipro were ANLNSPLKIAG (Epitope_C 1) and SSQYQPGGRTSVHGLGLTTGTDKSGGQAGDQMS (Epitope_C 2) with a score 0.922 and 0.901, respectively.

3.4. T-cell epitope prediction

Many 9-mer peptides on VP2 protein were predicted to bind (strongly as well as weekly) MHC-I alleles (Supplementary Table 5) out of which only four 9-mer peptides i.e. GEGVTVLSL¹⁵³⁻¹⁶¹, GELVFQTSV²³⁶⁻²⁴⁴, YERVATGSV³⁴⁶⁻³⁵⁴, and FELIPNPEL³⁶³⁻³⁷¹ were recognized to strongly binder to MHC-I alleles (Table 3) The peptide GELVFQTSV²³⁶⁻²⁴⁴ was predicted to a strong binder to all the three human substitute alleles i.e. HLA-B40:06 HLA-B41:04, and HLA-B41:03, it was considered as strongest MHC-I binding T-cell epitope.

Many 15-mer peptide segments were predicted to bind (strongly as well as weekly) with four MHC-II alleles of the human being (Supplementary Table 6) out of which seven peptides i. e. QQIVPFIRSLMPTT¹⁻¹⁵, QMLLTAQNLPASYNY⁷⁶⁻⁹⁰, YNYCRLVSRSLTVRS⁸⁸⁻¹⁰², NYCRLVSRSLTVRSS⁸⁹⁻¹⁰³, FSANIDAITSLSVGG²²²⁻²³⁶, GGELVFQTSVHGLVL²³⁵⁻²⁴⁹, and YTKLILSERDRLGIK³⁸⁹⁻⁴⁰³ were recognized as strongly binder to MHC-II alleles (Table 4). Two peptides i.e. YTKLILSERDRLGIK³⁸⁹⁻⁴⁰³ and QMLLTAQNLPASYNY⁷⁶⁻⁹⁰ were predicted to be strong binders to three different out of four human substitute alleles for MHC-II and considered as stronger MHC-II binding T-cell epitope. Moreover, GGELVFQTSVHGLVL²³⁵⁻²⁴⁹ (MHC-II binding peptide) also shares a common sequence (GELVFQTSV²³⁶⁻²⁴⁴) with the MHC-I binding T-cell epitope (Table 3).

4. DISCUSSION

Identifying strong antigenic B cell and T cell epitopic peptides and the prior knowledge of the binding interactions of these peptides with MHC alleles and host immune cells are necessary for developing an effective vaccine against any infection²⁰. Epitope identification could also contribute to the development of new diagnostics for infections²⁴. In the current study, the identification of epitopic peptides of VP2 protein of IBDV was carried out for the peptide-based vaccine as well as diagnostic development in the future. *In silico* prediction of epitopes

for quick and affordable vaccine designing has been in practice for several diseases such as Hantavirus Cardiopulmonary Syndrome, acne vulgaris, dengue, hepatitis C, etc ²⁰.

In the current study, we analyzed of the VP2 protein sequence of Indian isolate IBDV using the ProtParam program to estimate parameters determining antigenicity of protein molecule like molecular weight, pI, amino acid composition, half-life, and stability ²⁵. The VP2 protein was a negatively charged 47.518 KDa protein with a 5.12 pI value as it had higher (38 in number) content of negatively charged, hydrophilic, and polar amino acids (Asp + Glu). Hydrophilic amino acids are mainly present on the surface of antigens (Ingale, 2010) as they are accessible to adjoining aqueous environments. The stability of protein can be estimated by calculating the instability index and the protein having an instability index of less than 40 is considered as stable ²⁴. In present study, instability index valued of 25.64 indicated the stability of VP2 protein.

So far 3D structure of the VP2 protein of Indian isolate IBDV has not been determined. The 3D structure of proteins, for which no structure is available in PDB, can be determined by homology modeling ²⁴. A total of eight reference templates were found on the PDB database having sequence identity by ranges between 44% and 99%, while E-value range between 3.264e-288 and 1.608e-100. The reference model 3FBM had maximum homology with VP2 protein of Indian isolate IBDV. Therefore, reference model 3FBM was used for the prediction of epitopes.

It is commonly suggested that antigenic B-cell epitopes (linear and conformational) play the foremost role in the immunological response of the host ²⁴. We used BepiPred Linear Epitope Prediction 2.0, SVMTriP, and BCPred tools for prediction of linear B-cell epitopes. Several linear epitopes on VP2 protein were predicted using above tools. Subsequently, the consensus method ¹⁵ was used to short the most potent epitopes. Among several polypeptides, a partial or complete part of Epitope_L polypeptide matched with at least one polypeptide predicted by each of four different linear epitope prediction tools. Hence, Epitope_L was considered as the most potent linear epitope on VP2 protein. Ellipro tool-based prediction indicated Epitope_C 1 and Epitope_C 2 had scores above 0.9. Hence, Epitope_C 1 and Epitope_C 2 were considered the most potent conformational B-cell epitopes.

The binding of antigenic peptides to host MHC-I and MHC-II molecules is an essential prerequisite molecular event to trigger humoral and cell-mediated immune response ²⁴. For poultry MHC-peptide binding, currently, immune-bioinformatics tools are not available. Human HLA*B 40:06, HLA*B 41:03, and HLA*B 41:04 alleles have similarity greater than 70% with poultry MHC-I. Likewise, human DRB1:1310, DRB1:1366, DRB1:1445, and DRB1:1482 alleles have a similarity greater than 70% with poultry MHC-II ²⁰. Out of many strong MHC-I binding 9-mer peptides, the peptide GELVFQTSV²³⁶⁻²⁴⁴ was predicted as the strongest MHC-I binding T-cell epitope due to its binding ability with all three human substitute MHC-I alleles. Similarly out of many strong MHC-II binding 15-mer peptides, two peptides i.e. YTKLILSERDRLGIK³⁸⁹⁻⁴⁰³ and QMLLTAQNLPASYNY⁷⁶⁻⁹⁰ were predicted as strongest MHC-II binding T-cell epitopes due to their binding capacity with three different (out of four) human MHC-II alleles. Moreover, the MHC-II binding GGELVFQTSVHGLVL²³⁵⁻²⁴⁹ peptide was also predicted to bind MHC-I as it shared a common sequence (GELVFQTSV²³⁶⁻²⁴⁴) with the MHC-I binding epitope.

5. CONCLUSION

We predicted Epitope_L as the most prominent linear B-cell epitope on VP2 protein. Two peptide i.e. Epitope_C 1 and Epitope_C 2 were predicted as potent conformational B-cell epitopes. A 9-mer GELVFQTSV²³⁶⁻²⁴⁴ peptide was predicted as strongest MHC-I binding peptide while, two 15-mer peptides i.e. YTKLILSERDRLGIK³⁸⁹⁻⁴⁰³ and QMLLTAQNLPASYNY⁷⁶⁻⁹⁰ were as strongest MHC-II binding ability. Moreover, a stronger MHC-II binding GGELVFQTSVHGLVL²³⁵⁻²⁴⁹ peptide share common sequence with MHC-I binding GELVFQTSV²³⁶⁻²⁴⁴ peptide. These peptide should be assessed for protective immune response by *in vitro* and *in vivo* studies for development of subunit vaccine candidate. After proper validation, these peptides could be used for development of cost-effective and scalable vaccines for IBDV.

RESEARCH INVOLVING HUMAN PARTICIPANTS AND/OR ANIMALS

This article does not contain any studies with human participants or animals performed by any of the authors.

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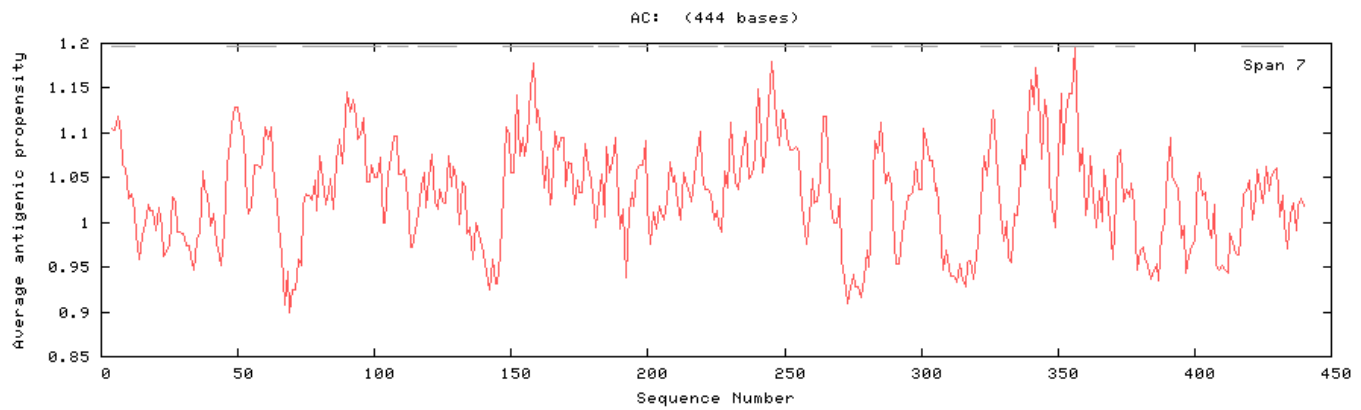
Fig. 1. 3D model of VP2 protein of IBDV.



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Fig. 2. Antigenic peptides plot

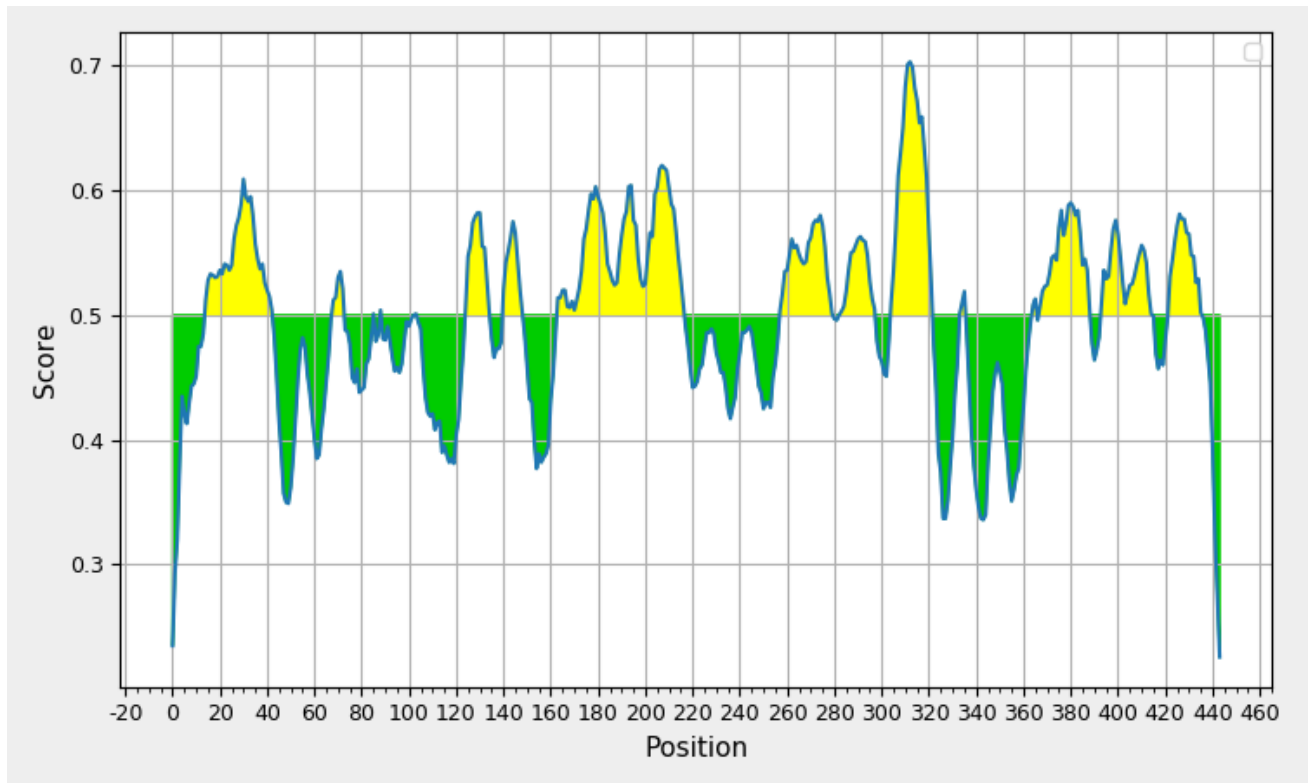
The x-axis of plot represents the residues number, and y-axis represents the average antigenic prosperity.



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Fig. 3. The graph of lineal B-cell epitopes of VP2 protein predicted by Bepipred software of IEDB.

The x-axis of graph represents the position whereas y-axis represents score of predicted amino acids in the sequence. The threshold value for epitopes prediction is 0.5 and the yellow region is representing the linear B-cell epitopes in the VP2 protein.

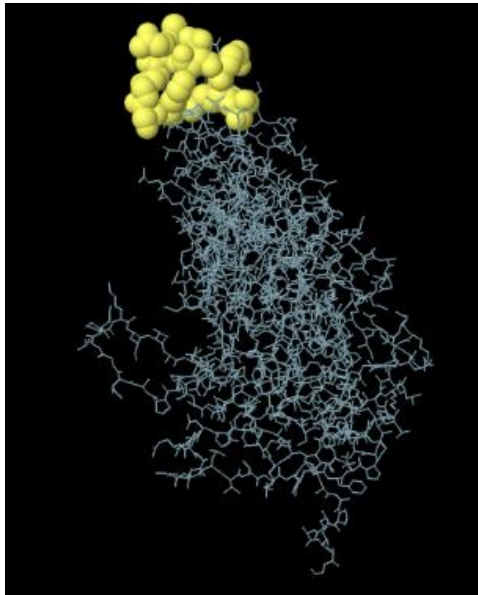


UNDETA

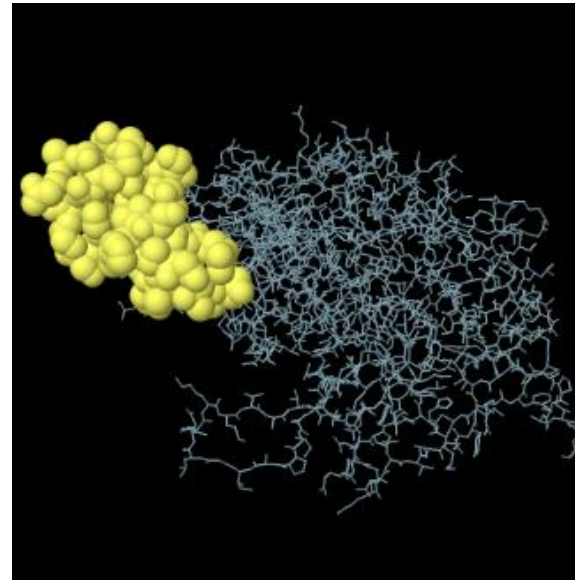
Fig. 4. Conformational B-cell epitopes of VP2 protein predicted by Ellipro tools

(A) 11 immunogenic residues with 92.2% of the residues exposed to the environment (B) 33 amino acid residues with a protrusion index of 90.1%. The immunogenic epitopes are depicted as globules on the ball and stick representation of the protein structure.

A



B



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Table 1: Shorted peptides predicted by any three or all four tools including Immunomedicine Group, BepiPred Linear Epitope Prediction 2.0, SVMTriP, and BCPred tools.

Immunomedicine Group Tool	BepiPred - 2.0	SVMTriP Tool (Score)	BCPred Predictions (Score)
DDYQFSSQYQPGGV	<u>SYDLG</u> YVRLGDPIPAIGLDPKMOVATCDSSDRPRV YTI <u>AADDYQFSSQYQPGGV</u>		<u>AADDYQFSSQYQPGGV</u> (0.958)
IGNVLV <u>GEGVT</u> VLSP <u>SYDLG</u> YVRLG DPIPAIG		<u>GEGVT</u> VLSP <u>SYDLG</u> (0.511)	
<u>DRPRV</u> YTI			
<u>DPKMOVATC</u>			
<u>DGTAVITRA</u>	<u>FDGTAVITRA</u> VAANGLTTGTDN		RAVAANGLTTGTDNL (0.996)
	TGPAS <u>SIPDDTLEKH</u> TLRSETSTYNLTVGD	<u>SIPDDTLEKH</u> TLRSET (0.936)	PTTGPAS <u>SIPDDTLEKH</u> (1.000)
<u>LAKNLVTE</u>	NPE <u>LAKNLVTE</u> YGRFDPGAMNY		VTEYGRFDPGAMNYTK (0.929)

Note: Same text color, text highlight color, underline color, and encircle color represent common peptides regions among polypeptide predicted by four different bioinformatics tools

Table 2: Conformational B-cell epitopes on VP2 protein predicted by Ellipro server.

S. No	Epitopes	Number of residues	Score

1	A:A430, A:N431, A:L432, A:N433, A:S434, A:P435, A:L436, A:K437, A:I438, A:A439, A:G440	11	0.922
2	A:S217, A:S218, A:Q219, A:Y220, A:Q221, A:P222, A:G223, A:G224, A:R249, A:T250, A:S251, A:V252, A:H253, A:G254, A:L255, A:G281, A:L282, A:T283, A:T284, A:G285, A:T286, A:D287, A:K316, A:S317, A:G318, A:G319, A:Q320, A:A321, A:G322, A:D323, A:Q324, A:M325, A:S326	33	0.901
3	A:I184, A:P185, A:A186, A:I187, A:G188, A:L189, A:D190, A:P191, A:K192, A:M193	10	0.869
4	A:V49, A:G50, A:D51, A:T52, A:T112, A:L113, A:P114, A:L120, A:N121, A:G122, A:V357, A:A358, A:T359, A:G360, A:S361	15	0.857
5	A:L33, A:E34, A:K35, A:N376, A:P377, A:E378, A:A380, A:K381, A:N382, A:L383, A:V384, A:T385, A:E386, A:Y387, A:G388	15	0.824
6	A:F390, A:D391, A:P392	3	0.809

Table 3: List of the strong MHC-I binders for VP2 protein of IBDV

Pos	Allele	peptide	1-log50k	Affinity (nM)	%Rank
235	HLA-B40:06	GELVFQTSV	0.564	111.72	0.08
362	HLA-B40:06	FELIPNPEL	0.454	367.39	0.30
345	HLA-B40:06	YERVATGSV	0.411	586.38	0.50
235	HLA-B41:04	GELVFQTSV	0.615	64.10	0.12
345	HLA-B41:04	YERVATGSV	0.548	133.13	0.30
362	HLA-B41:04	FELIPNPEL	0.523	174.64	0.40
152	HLA-B41:04	GEGVTVLSL	0.523	174.04	0.40
362	HLA-B41:03	FELIPNPEL	0.639	49.97	0.25
152	HLA-B41:03	GEGVTVLSL	0.637	51.06	0.25
235	HLA-B41:03	GELVFQTSV	0.629	55.68	0.25

Note: The same text colour of peptide represent common peptide winding capacity with different MHC-I haplotype

Table 4: List of the strong MHC-II binders for VP2 protein of IBDV

Seq	Allele	peptide	1-log50k	Affinity
388	DRB1_1482	YTKLILSERDRLGIK	0.554	125.06
0	DRB1_1482	QQIVPFIRSLMPTT	0.550	130.07
88	DRB1_1366	NYCRLVSRSLTVRSS	0.816	7.32
87	DRB1_1366	YNYCRLVSRSLTVRS	0.779	10.87
75	DRB1_1366	QMLLTAQNLPASYNY	0.774	11.58
234	DRB1_1366	GGELVFQTSVHGLVL	0.752	14.67
0	DRB1_1366	QQIVPFIRSLMPTT	0.751	14.76
388	DRB1_1366	YTKLILSERDRLGIK	0.742	16.33
88	DRB1_1310	NYCRLVSRSLTVRSS	0.808	7.97
87	DRB1_1310	YNYCRLVSRSLTVRS	0.772	11.80
388	DRB1_1310	YTKLILSERDRLGIK	0.719	20.90
75	DRB1_1310	QMLLTAQNLPASYNY	0.719	20.90
221	DRB1_1310	FSANIDAITSLVGG	0.694	25.86
234	DRB1_1310	GGELVFQTSVHGLVL	0.694	27.29
75	DRB1_1445	QMLLTAQNLPASYNY	0.520	180.56

Note: The same text colour of peptides represents common peptide winding capacity with different MHC-II haplotypes, and text highlight colour of peptides within the table and with the peptide of table 3 represent peptides having binding capacity with both MHC-I/II