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Mapping of Epitopes in Potential Antigenic Proteins of Bovine Rhinotracheitis Virus Using Immunoinformatics Tools

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Abstract

Infectious Bovine Rhinotracheitis (IBR), caused by Bovine Herpesvirus type 1 (BHV-1), is a globally significant cattle disease that impacts both respiratory and reproductive health. As per the recommendations of World organization of animal Health, several PCR based assays and ELISA based assays were developed, however due to higher cost and complexity associated with the tests, regular surveillance programmes and onsite screening still remains a challenge. This current study aimed at proposing a potential antigen suitable for developing Antibody detection assays. Based on proteomics data, highly expressed proteins were shortlisted and analysed for various Bioinformatics and Immunoinformatics tools. B-cell epitopes from major IBRV envelope glycoproteins such as gB, gC, gD, gE and latency associated protein BICP0 were analysed using IEDB tools. Suitable epiptopes from each protein were utilized and developed a fusion protein suitable for detection of antibodies in both active infection as well as latent infection in the herds. Future experimental validation will confirm the utility of these epitopes for large-scale IBRV antibody detection and herd surveillance.

Keywords: Infectious Bovine Rhinotracheitis, B-cell epitope, fusion proteins, peptide-based ELISA, immunoinformatics

1. Introduction

Infectious Bovine Rhinotracheitis (IBR) is an economically significant viral disease of cattle caused by Bovine Herpesvirus type 1 (BHV-1), also referred to as IBR Virus (IBRV). The disease is found to affects the respiratory and reproductive systems resulting in considerable economic losses due to reduced milk yield, abortions, infertility. (Ackermann & Engels, 2006; Muylkens et al., 2007; WOAH, 2023). A major challenge in controlling IBR is the ability of BHV-1 to establish lifelong latency with recurrent reactivation (Thiry 2006). The asymptomatic viral shedding is known to be the major bottleneck in detection and eradication of IBR from herds (Raaperi et al., 2014; Nandi 2009; Patel 2017).

Among the current diagnostic methods for IBRV such as virus isolation, molecular detection using PCR/qPCR, and serological assays, Virus isolation is highly specific but time-consuming and requires high-containment laboratories (OIE, 2021). Though Molecular assays are sensitive and rapid, they were found to be not suitable for field level screening due to higher cost. Molecular assays were found to detect only active infections, failing to identify latently infected animals (Bachanek-Bankowska et al., 2020). The conventional ELISA and gE-based DIVA ELISA kits that are being widely used yet can exhibit cross-reactivity and batch variability (Schrijver et al., 2021; Lemaire et al., 2020).

These limitations highlight the need for improved antibody-based diagnostic assays. Epitope-based approaches, targeting well-defined B-cell epitopes from viral glycoproteins, can improve specificity, reproducibility, and cost-effectiveness (He et al., 2021; Li et al., 2023).

Based on earlier proteomics studies of *Barber et al.,* (2017) and Jones & Chowdhury (2007) Envelope glycoproteins gB, gC, gD, and gE and BICP0 were found to associated with strong antibody responses in active infection as well as latency.

This study aims to employ an integrated immunoinformatics workflow to predict and prioritize B-cell epitopes for the development of peptide-based antibody detection assays for IBRV.

2. Methodology

2.1.Retrieval of IBRV Antigenic Protein Sequences

Full-length amino acid sequences of BHV-1 glycoproteins gB, gC, gD, gE and BICP0 were retrieved from NCBI. Redundant or incomplete sequences were removed, and representative reference sequences were selected in FASTA format.

2.2. Gene Ontology annotation of target proteins

Protein sequences of Infectious Bovine Rhinotracheitis Virus (IBRV) were retrieved in FASTA format from the NCBI protein database for subsequent analysis. Gene Ontology (GO) annotation was performed to identify the associated molecular functions and biological processes of the selected antigenic target proteins. Functional annotation data were obtained from the UniProt Knowledgebase, which provides curated and comprehensive GO terms relevant to protein characterization.

2.3. Retrieval of the amino acid sequences as suitable epitope domains from target proteins

Amino acid sequences of target proteins in the FASTA format were submitted to Immune epitope database tools of IEDB Resource (Vita et al., 2019). BepiPred-2.0 algorithm (http://tools.iedb.org/bcell/) (Jespersen et al., 2017) was selected and analyzed for potential epitopes at 0.5 threshold value. The largest domain of amino acids with a minimum of 20 amino acids were selected and noted.

2.4. Production of multiepitopic fusion protein using $(H_4)_2$ linkers.

Linkers were found to play a crucial role in facilitating the proper folding of fusion proteins. The (H4)2 linker, consisting of two tandem repeats of four histidine residues was known to be widely utilized and found to enhance the stability and expression efficiency of chimeric proteins (Chen et al., 2012). Hence, in this study, we have utilized the $(H_4)_2$ linker to fuse the selected individual epitope domains from each protein.

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2.5. Prediction of stability of the fusion protein using Expasy Protparam Tool.

The amino acid sequence of fusion proteins developed using linkers were analyzed using Expasy Protparam Tool (https://web.expasy.org/protparam/). The parameters such as duration of Stability of protein when expressed in various cell lines and Instability index were recorded.

3. Results and Discussion

3.1. Retrieval of Amino acid sequences of target proteins from NCBI

Amino acid sequences were retrieved in FASTA format and presented in Table.1.

Table. 1. Potential antigenic proteins of Bovine herpes virus 1

Envelope glycoprotein gE >NP_045372.1 envelope glycoprotein E [Bovine alphaherpesvirus 1] MQPTAPPRRRLLPLLLPQLLLFGLMAEAKPATETPGSASVDTVFTARAGAPVFLPGPAARPDV RAVRGWSVLAGACSPPVPEPVCLDDRECFTDVALDAACLRTARVAPLAIAELAERPDSTGDK EFVLADPHVSAQLGRNATGVLIAAAAEEDGGVYFLYDRLIGDAGDEETQLALTLQVATAGA QGAARDEEREPATGPTPGPPPHRTTTRAPPRRHGARFRVLPYHSHVYTPGDSFLLSVRLQSEF FDEAPFSASIDWYFLRTAGDCALIRIYETCIFHPEAPACLHPADAQCSFASPYRSETVYSRLYE QCRPDPAGRWPHECEGAAYAAPVAHLRPANNSVDLVFDDAPAAASGLYVFVLQYNGHVEA WDYSLVVTSDRLVRAVTDHTRPEAAAADAPEPGPPLTSEPAGAPTGPAPWLVVLVGALGLA GLVGIAALAVRVCARRASQKRTYDILNPFGPVYTSLPTNEPLDVVVPVSDDEFSLDEDSFADD DSDDDGPASNPPADAYDLAGAPEPTSGFARAPANGTRSSRSGFKVWFRDPLEDDAAPARTPA APDYTVVAARLKSILR
Envelope glycoprotein gB >NP_045331.1 envelope glycoprotein B [Bovine alphaherpesvirus 1] MAARGGAERAAGAGDGRRGQRRHLRPGRVLAALRGPAAPGAGGARAALAAALLWATWA LLLAAPAAGRPATTPPAPPPEEAASPAPPASPSPPGPDGDDAASPDNSTDVRAALRLAQAAGE NSRFFVCPPPSGATVVRLAPARPCPEYGLGRNYTEGIGVIYKENIAPYTFKAYIYKNVIVTTTW AGSTYAAITNQYTDRVPVGMGEITDLVDKKWRCLSKAEYLRSGRKVVAFDRDDDPWEAPL KPARLSAPGVRGWHTTDDVYTALGSAGLYRTGTSVNCIVEEVEARSVYPYDSFALSTGDIIY MSPFYGLREGAHREHTSYSPERFQQIEGYYKRDMATGRRLKEPVSRNFLRTQHVTVAWDWV PKRKNVCSLAKWREADEMLRDESRGNFRFTARSLSATFVSDSHTFALQNVPLSDCVIEEAEA AVERVYRERYNGTHVLSGSLETYLARGGFVVAFRPMLSNELAKLYLQELARSNGTLEGLFA AAAPKPGPRRARRAAPSAPGGPGAANGPAGDGDAGGRVTTVSSAEFAALQFTYDHIQDHVN TMFSRLATSWCLLQNKERALWAEAAKLNPSAAASAALDRRAAARMLGDAMAVTYCHELG EGRVFIENSMRAPGGVCYSRPPVSFAFGNESEPVEGQLGEDNELLPGRELVEPCTANHKRYFR FGADYVYYENYAYVRRVPLAELEVISTFVDLNLTVLEDREFLPLEVYTRAELADTGLLDYSEI QRRNQLHELRFYDIDRVVKTDGNMAIMRGLANFFQGLGAVGQAVGTVVLGAAGAALSTVS GIASFIANPFGALATGLLVLAGLVAAFLAYRYISRLRSNPMKALYPITTRALKDDARGATAPG

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EEEEEFDAAKLEQAREMIKYMSLVSAVERQEHKAKKSNKGGPLLATRLTQLALRRRAPPEY QQLPMADVGGA
Envelope glycoprotein D >NP_045370.1 envelope glycoprotein D [Bovine alphaherpesvirus 1] MQGPTLAVLGALLAVAVSLPTPAPRVTVYVDPPAYPMPRYNYTERWHTTGPIPSPFADGREQ PVEVRYATSAAACDMLALIADPQVGRTLWEAVRRHARAYNATVIWYKIESGCARPLYYME YTECEPRKHFGYCRYRTPPFWDSFLAGFAYPTDDELGLIMAAPARLVEGQYRRALYIDGTVA YTDFMVSLPAGDCWFSKLGAARGYTFGACFPARDYEQKKVLRLTYLTQYYPQEAHKAIVDY WFMRHGGVVPPYFEESKGYEPPPAADGGSPAPPGDDEAREDEGETEDGAAGREGNGGPPGP EGDGESQTPEANGGAEGEPKPGPSPDADRPEGWPSLEAITHPPPAPATPAAPDAVPVSVGIGIA AAAIACVAAAAAGAYFVYTRRRGAGPLPRKPKKLPAFGNVNYSALPG
Envelope glycoprotein gC >NP_045314.1 envelope glycoprotein C [Bovine alphaherpesvirus 1] MGPLGRAWLIAAIFAWALLSARRGLAEEAEASPSPPPSPSPTETESSAGTTGATPPTPNSPDAT PEDSTPGATTPVGTPEPPSVSEHDPPVTNSTPPPAPPEDGRPGGAGNASRDGRPSGGGRPRPPR PSKAPPKERKWMLCEREAVAASYAEPLYVHCGVADNATGGARLELWFQRVGRFRSTRGDD EAVRNPFPRAPPVLLFVAQNGSIAYRSAELGDNYIFPSPADPRNLPLTVRSLTAATEGVYTWR RDMGTKSQRKVVTVTTHRAPAVSVEPQPALEGAGYAAVCRAAEYYPPRSTRLHWFRNGYP VEARHARDVFTVDDSGLFSRTSVLTLEDATPTAHPPNLRCDVSWFQSANMERRFYAAGTPA VYRPPELRVYFEGGEAVCEARCVPEGRVSLRWTVRDGIAPSRTEQTGVCAERPGLVNLRGVR LLSTTDGPVDYTCTATGYPAPLPEFSATATYDASPGLIGSPVLVSVVAVACGLGAVGLLLVA ASCLRRKARARL
BICP0 Latency associated protein [Bovine alphaherpesvirus 1] >Q69259MRDLGHKSPAHARAVTFGLGMTAATAARKLSMQAGGRKYTAVRCGGLRVAEWV GGEPAAAIIAARRCRRCSGRAARPGSGPGDRLAAAPGPRCCGGGSPGGFLAPRRPLPFRCAAS SAPSSRGRGPGRPAPWRSLAFSPGRGCHCGRPRPGRLRPGPERRPAGGRRQGRGRRETGSGL GWK

3.2. Gene ontology/Functional annotation of Target proteins using UniprotKB.

Gene ontology annotation using UniprotKB revealed that the test proteins are associated with antigenicity, Virulence and cell – cell attachment and presented in Table.2.

Table. 2. Gene ontology/Functional annotation of Target proteins using UniprotKB

Target genes	Name of Protein	Function
Gene US8 Gene ID: 1487407	Envelope glycoprotein gE	Important for the cell-to-cell spread of the virus, spread of the virus by sorting nascent virions to cell junctions

Gene US6 Gene ID: 1487406	Envelope glycoprotein gD	Envelope glycoprotein that binds to host cell entry receptors, promoting the virus entry into host cells. May trigger fusion with host membrane
Gene UL 44 Gene ID: 4783424	Envelope glycoprotein gC	Involved in Host-virus interaction, Inhibition of host complement factors by virus Viral attachment to host adhesion receptor, Viral attachment to host cell,Viral immunoevasion and Virus entry into host cell.
Gene UL 27 Gene ID: 4783419	Envelope glycoprotein gB	Envelope glycoprotein that forms spikes at the surface of virion envelope. Essential for the initial attachment to heparan sulfate moieties of the host cell surface proteoglycans. Involved in fusion of viral and cellular membranes leading to virus entry into the host cell.
LRORF2 GeneID:1487364	Latency Reactivation gene BICP0	latency-related region protein

3.3. Retrieval of the amino acid sequences as suitable epitope domains from target proteins

According to studies by Buus *et al.* (2012) and Stave *et al.* (2013) 6-12 amino acid and amino acid lengths >20 amino acids were found to be suitable epitope domains can serve as antigens. Using IEDB tools the test proteins were analyzed and the appropriate domains were selected and presented in Table.3.

Table.3. Epitope domains of target proteins using IEDB Tools.

S.No	Name of the Protein	From	To	Amino Acid sequence	No. of AA
1	gE	168	196	AGDEETQLALTLQVATAGAQGAARDEERE	29
2	gB	756	805	KTDGNMAIMRGLANFFQGLGAVGQAVGT VVLGAAGAALSTVSGIASFIAN	50
3	gC	322	339	TVDDSGLFSRTSVLTLED	18
4	gD	282	322	EAREDEGETEDGAAGREGNGGPPGPEGDG ESQTPEANGGAE	41

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5	BICP0	116	142	SSAPSSRGRGPGRPAPWRSLAFSPGRG	27

3.4. Engineering of multi-epitopic fusion protein using $(H_4)_2$ Linker

Epitope domains of all the target proteins in Table.3 were fused in few different combinations using $(H_4)_2$ Linkers are presented in Table.4.

Table.4. Fusion proteins generated by using $(H_4)_2$ Linkers

Fusion Protein	Amino Acid sequence of fusion proteins
gE – $(H_4)_2$- gB- $(H_4)_2$ - gC- $(H_4)_2$ - gD- $(H_4)_2$. - BICP0	AGDEETQLALTLQVATAGAQGAARDEERE HHHHHHHHKTDGNMAIMRGLANFFQGLGAVGQAVGTVV LGAAGAALSTVSGIASFIAN HHHHHHHH TVDDSGLFSRTSVLTLEDHHHHHHHHEAREDEGETEDGA AGREGNGGPPGPEGDGESQTPEANGGAEHHHHHHHH SSAPSSRGRGPGRPAPWRSLAFSPGRG
gB- $(H_4)_2$ - gC- $(H_4)_2$ - gD- $(H_4)_2$. - BICP0 – $(H_4)_2$ -gE	KTDGNMAIMRGLANFFQGLGAVGQAVGTVVLGAAGAAL STVSGIASFIAN TVDDSGLFSRTSVLTLEDHHHHHHHH EAREDEGETEDGAAGREGNGGPPGPEGDGESQTPEANGG AEHHHHHHHH SSAPSSRGRGPGRPAPWRSLAFSPGRGHHHHHHHH AGDEETQLALTLQVATAGAQGAARDEERE
gC- $(H_4)_2$ - gD- $(H_4)_2$. - BICP0 – $(H_4)_2$ - gE - $(H_4)_2$ gB	EAREDEGETEDGAAGREGNGGPPGPEGDGESQTPEANGG AEHHHHHHHH SSAPSSRGRGPGRPAPWRSLAFSPGRGHHHHHHHH AGDEETQLALTLQVATAGAQGAARDEEREHHHHHHHH KTDGNMAIMRGLANFFQGLGAVGQAVGTVVLGAAGAAL STVSGIASFIAN TVDDSGLFSRTSVLTLED
gD- $(H_4)_2$ -BICP0 – $(H_4)_2$ -gE - $(H_4)_2$ gB- $(H_4)_2$ gC	SSAPSSRGRGPGRPAPWRSLAFSPGRGHHHHHHHH AGDEETQLALTLQVATAGAQGAARDEEREHHHHHHHH KTDGNMAIMRGLANFFQGLGAVGQAVGTVVLGAAGAAL STVSGIASFIAN TVDDSGLFSRTSVLTLEDHHHHHHHH EAREDEGETEDGAAGREGNGGPPGPEGDGESQTPEANGG AE

3.5. Prediction of stability of the fusion protein using Expasy Protparam Tool.

The multi-epitopic fusion proteins that were generated by using linkers were analysed for protein stability using Expasy – Protparam tools. Stability of the engineered proteins when expressed in

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Mammalian cells, Yeast cells and E.coli were analyzed along with the Instability index of test proteins were presented in Table. 5.

Table.5. Predicted stability of the test proteins using Expasy Protparam Tool.

S.No.	Fusion orientation of Epitope domains (N-C Terminal)	Estimated half-life of the protein when expressed in			Instability Index
		Mammalian reticulocytes In Vitro	Yeast- in vivo	Escherichia coli- in vivo	
1	gE – $(H_4)_2$- gB- $(H_4)_2$ - gC- $(H_4)_2$ - gD- $(H_4)_2$. - BICP0	4.4 hours	>20 hours	>10 hours	27.25
2	gB- $(H_4)_2$ - gC- $(H_4)_2$ - gD- $(H_4)_2$. - BICP0 – $(H_4)_2$ - gE	1.3 hours	3 min	3 min	26.44
3	gC- $(H_4)_2$ - gD- $(H_4)_2$. - BICP0 – $(H_4)_2$ - gE - $(H_4)_2$ gB	1 hours	30 min	>10 hours	27.93
4	gD- $(H_4)_2$. - BICP0 – $(H_4)_2$ - gE - $(H_4)_2$ gB- $(H_4)_2$ gC	1.9 hours	>20 hours	>10 hours	28.33

All four glycoproteins were stable (instability index <40). Among the above four engineered Multi-epitopic fusion proteins, the sequence of amino acids for gE – $(H_4)_2$- gB- $(H_4)_2$ - gC- $(H_4)_2$ - gD- $(H_4)_2$. - BICP0 exhibited a stability of 4.4 hours, if the proteins is expressed in Mammalian reticulocytes In Vitro, >20 hours in Yeast- in vivo and >10 hours in Escherichia coli- in vivo. Instability index of 27.25 indicates that fusion protein is in stable configuration.

The combined linear and conformational approach increases reliability and reduces cross-reactivity. Peptide-based assays offer advantages over full-protein ELISA, including enhanced specificity, reproducibility, and scalability. These peptides provide a foundation for next-generation serological tests, complementing existing gE-based DIVA ELISAs (He et al., 2021; Li et al., 2023).

4. Conclusion and Future Prospects

An integrated immunoinformatics approach enabled systematic prediction of B-cell epitopes from IBRV glycoproteins for peptide-based antibody detection assay development. Multi-epitopic fusion proteins was identified, providing candidates for sensitive and specific ELISA assays. Experimental validation of

predicted peptides in ELISA to assess immunoreactivity. Development of this multiplexed peptide-based ELISAs to enhance diagnostic throughput and specificity. This study establishes a robust computational framework for the rational design of next-generation antibody detection assays for IBRV.

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