Awadelkareem and Hamdoun
Translational Medicine Communications (2024) 9:28
https://doi.org/10.1186/s41231-024-00190-y

Translational Medicine Communications

RESEARCH Open Access

Insilco vaccine design of spike and hemagglutinin esterase proteins of bovine coronavirus

Eman A. Awadelkareem[1*] and S. Hamdoun[2]

Abstract

Background Bovine coronavirus (BCoV) is a widespread, fatal illness in cattle that has a large economic impact in particularly disease-prone hosts. BCoV does not have an effective vaccine. As a result, the objective of this study was to use immunoinformatics and computational tools to design a multi-epitope vaccine against Spike and haemagglutinin proteins of Bovine coronavirus. BCoV proteins were also subjected to protein analysis.

Methods A variety of tools of bioinformatics were used for data analysis. Conserved B and T cell epitopes against BCoV target proteins were predicted using the Immune Epitope Database (IEDB). Proteins were characterized utilizing a variety of servers, including Protparam, PSIPRED and the GOR IV servers. The antigenicity, allergenicity, and toxicity of the anticipated epitopes were assessed as well.

Results Several MHCI epitopes were predicted from S and HE proteins. As top epitopes, the peptides 77NMALKGTLL85 and 56SYMDLNPAL65 were proposed from Spike and hemagglutinin proteins, respectively. These epitopes exhibited high scores of antigenicity, no allergenicity, no toxicity, and a strong connection to Bola alleles. Moreover, three epitopes (1204YYYPE1208, 379TCQPQ384, and 720QLQPINY726) from Spike glycoprotein were selected as surface, linear, and antigenic epitopes using B cell scales. The methods dropped to anticipate effective and safe epitope(s) to cover all B cell scales from HE protein.

Conclusion Three B cell epitopes (1204YYYPE1208, 379TCQPQ384, and 720QLQPINY726) were predicted from Spike protein (S) of BCoV only. MHC I epitopes of S and HE proteins of BCoV predicted two epitopes (77NMALKGTLL85 and 56SYMDLNPAL65 respectively) to have a strong link to Bola alleles, as well as high antigenicity and safety. The predicted epitopes' activity should be tested experimentally as a multi-epitope vaccine against BCoV using in vitro and in vivo trials.

Keywords BCoV, Glycoproteins, Bola, Immunoinformatics tools, Multi-epitope

*Correspondence:
Eman A. Awadelkareem
emanawadelkareem1@gmail.com
[1]Department of Molecular Biology and Bioinformatics, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan
[2]Faculty of Pharmacy, University of Khartoum, Khartoum, Sudan

Introduction

Bovine respiratory disease (BRD) is caused by pathogenic organisms and is typically exacerbated by stress factors such as the environment, nutrition, transportation, and commingling with cattle from various herds [1]. Bovine coronavirus (BCoV) is now generally recognized as a major cause of neonatal calf diarrhea [2]. The disease affects the respiratory tract of cattle and has been linked with different syndromes in cattle: calf diarrhea, winter dysentery in adult cattle, and respiratory infection in cattle of different age groups [3–8]. BCoV can significantly economically impact the veterinary industry [9]. The bovine coronavirus (BCoV) is a member of a family of microorganisms that causes intestinal and respiratory disease in a variety of mammalian and avian species. BCoV's function in calf hood diarrhea is well-established, and it remains a problem in calf-rearing enterprises [10]. Along with wild ruminant CoVs, porcine hemagglutinating encephalomyelitis virus, equine coronavirus, HCoV-OC43, HECoV-44, and canine respiratory coronavirus, bovine coronavirus (BCoV) belongs to the species Betacoronavirus 1 (subgenus Embecovirus) of the Betacoronavirus genus. In the Bovine Respiratory Disease Complex, BCoV is a major pathogen. Viral particles are big (100–150 nm), pleomorphic, and contain four key structural proteins: a membrane (M) glycoprotein, an envelope (E) glycoprotein, a spike (S) glycoprotein, and a hemagglutinin-esterase (HE) glycoprotein [5, 11, 12].

Bovine coronavirus BCoV is pleomorphic and enclosed, with a diameter varying from 65 to 210 nm with a double layer of short (hemagglutinin) and long (spike) surface projections. The big genome is made up of 27 to 32 kb of single-stranded positive-sense RNA that codes for five main structural proteins. The 50-kDa nucleocapsid (N) is the most conserved among them, making it a popular target for viral RNA detection techniques. 29 The presence of a surface hemagglutinin-esterase (HE) glycoprotein (120–140 kDa) is unique to some group 2 CoVs, including BCoV and wild ruminant CoVs. To reverse hemagglutination, the HE operates as a receptor-destroying enzyme (esterase) [11, 12].

Bovine coronavirus (BCoV), like other coronavirus (CoVs), has an outer-surface S glycoprotein (190 kDa). It is made up of two subunits, one of which carries the dominant neutralizing epitopes and the other of which mediates viral membrane fusion. Because HE and S proteins elicit neutralizing antibodies that can prevent viral attachment and infectivity, they are significant for immunity and vaccine development [13, 14].

Bovine coronavirus (BCoV) respiratory illness has little control methods. The vaccinations for BCoV are approved for the prevention of newborn intestinal illness [9, 15, 16]. There are three inactivated vaccines approved for the treatment of neonatal enteric disease, and they are given to pregnant cows/heifers throughout pregnancy to increase humoral immunity in the newborn calf. A modified live virus vaccination against BCoV, the vaccine was found to be safe as four colostrum-deprived newborn calves remained healthy after oronasal administration of ten doses of the vaccine that can induce an active antibody and cell-mediated immunity at the same time [9, 15].

Three antigenic groups of coronaviruses have been identified, and all BCoV strains classified around the world belonged to the 2a subgroup [9]. The International Commission for Virus Taxonomy (ICTV) has recommended a revision of the Coronaviridae family to include the Alpha, Beta, and Gammacorona-virus genera in a new subfamily Coronavirinae. BCoV is classified as a member of the Betacoronavirus genus, which is part of the Corona-virinae subfamily, Coronaviridae family, and Nidovirales order [17–20].

Recently, prevention and control of the infectious diseases have been among the top public health priorities. However, controlling disease due to pathogens that move between animals and humans has been challenging. Such zoonotic pathogens have been responsible for the majority of new human disease threats and a number of recent international epidemics [21].

Developing a medication or vaccine using traditional methods was a labor-intensive and time-consuming process that also carried the risk of toxicity and safety. By combining computational and experimental approaches, significant progress in building insilico tools not only accelerates drug discovery and vaccine design, but also decreases cost and time [22]. There are various possible advantages of peptide vaccinations over conventional organism vaccines. Most crucially, it permits the immune response to concentrate solely on relevant epitopes while avoiding those that trigger non-protective responses, immune evasion, or undesirable side effects such autoimmunity [23]. Furthermore, peptide vaccines are safe and cost-effective approach. Because of its ease of manufacture and simple composition, peptide vaccines are generally inexpensive to produce [24].

The main objective of this study was to develop a multi-epitope vaccine against Bovine coronavirus (BCoV) glycoprotein Spike (S) and hemagglutinin-esterase (HE) glycoproteins, using several immunoinformatics tools.

Materials and methods

Retrieval of protein sequences

The sequences of spike (S) and hemagglutinin esterase (HE) proteins of bovine coronavirus (BCoV) were obtained from the National Center for Biotechnology Information's (NCBI) GeneBank database in October 2021 (http://www.ncbi.nlm.nih.gov/protein/).

Multiple sequence alignment and construction of phylogenetic tree

Multiple sequence alignment (MSA) was achieved on the recovered spike (S) and hemagglutinin proteins (HE) bovine coronavirus (BCoV) using ClustalW program in BioEdit software version 7.2.5 [25]. The maximum likelihood parameter was used to create phylogenetic trees of S and HE protein sequences from bovine coronavirus (BCoV) using MEGA7.0.26 (7170509) software [26].

Structural analysis, physicochemical and antigenic assessments of the target proteins

The antigenicity qualities of the reference sequences S (NP_150077.1) and HE (NP_150076.1) proteins of BCoV were assessed using the VaxiJen v2.0 server [27]. The physico-chemical properties of the target protein sequences of BCoV were evaluated using the online tool ProtParam, which included GRAVY (Grand average of hydropathicity), half-life, molecular weight, stability index, and amino acids atomic composition [28]. Secondary structures of BCoV, S and HE proteins were predicted using PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/) and the GOR IV servers (https://npsa-prabi.ibcp.fr/cgi-bin/secpred sopma.pl) [29] The existence of disulphide bonds was expected using DIANNA v1.1. (http://clavius.bc.edu/~clotelab/DiANNA/). TMHMM, an online tool (http://www.cbs.dtu.dk/services/TMHMM/). Existed used to predict the trans-membrane topologies of S and HE proteins [30]. DD-BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) and PFAM (https://pfam.xfam.org/) [31–34] were used to search the defined conserved domains in the targeted protein sequences. Blastp in NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using reference sequence (refseq - protein) database was used to compare spike reference sequences of different coronaviruses in human and animals against IBV spike protein sequence. COBALT multiple alignments were also used to construct the phylogenetic tree (https://www.ncbi.nlm.nih.gov/blast/treeview/treeView.cgi) [31] 2.4 Prediction of B-cell epitopes The free online server, Immune Epitope Database (IEDB) (http://tools.iedb.Org/mhci/) [35] was used to predict B epitopes in S and HE proteins. Linear B-cell epitopes were forecasted using BepiPred from IEDB [36]. Emini surface accessibility prediction tool was used to predict surface epitopes [37]. However, the antigenic epitopes were inspected using kolaskar and Tongaonkar antigenicity method [38]. 2.5 Prediction of MHC I The MHC Class-I prediction tool was utilized using Immune Epitope Database (IEDB) (http://tools.iedb.org/mhci/). Cow alleles (BoLAHD6, BoLA-JSP.1, BoLA-T2a, BoLA-T2b, and BoLA-T2c) were used to predict CTL epitopes of MHC Class-I target proteins [39]. The reference sequence of the S and HE proteins of bovine coronaviruses (BCoV) were submitted in FASTA format; the Percentile ranks of binding affinity to specific cow MHC-I molecules were taken within the range of 0–2.

Assessment of B and T-cell epitopes

Conservation analysis

The conservation of anticipated epitopes was determined using Epitope Conservancy Analysis in the Immune Epitope Database (IEDB) (http://tools.iedb.org/conservancy/) [40]. The conservation score was calculated using a sequence identity criterion of 100%. 2.6.2Antigenicity, allergenicity and Toxicity The antigenicity qualities of the conserved predicted epitopes of S and HE proteins of BCoV were assessed using the VaxiJen v2.0 server (http://www.jenner.ac.uk/VaxiJen) [27]. Based on a training set containing known allergens and non-allergens from various species, AllerTop v.2.0 categorizes the allergens of a protein utilizing amino acid E-descriptors, k nearest neighbor's machine learning algorithms, and the auto- and cross-covariance (ACC) transformation (http://www.ddg-pharmfac.net/AllerTOP) [41]. The ToxinPred server (http://crdd.osdd.net/raghava/toxinpred/) was also utilized to assess epitopes toxicity as well as mutation and physico-chemical characteristics. ToxinPred predicts the peptides' toxicity based on the patterns found in hazardous peptides. As a result, motifs were utilized in the prediction algorithms [42]. 2.6.3 Structural modelling Three-dimensional (3D) structures of the reference Spike S protein were modeled using phyer 2 server [43]. Whereas, Raptor X online server [44, 45] was used to model the reference HE protein of BCoV. Visualization of the result was performed using Chimera software 1.8 [46]. 3. Results: 3.1 Multiple sequence alignment Multiple sequence alignment of all retrieved sequences of S and HE proteins using ClustalW through BioEdit software showed high conservancy between the aligned sequences.

Antigenicity, physicochemical properties and structural analysis of the target proteins of BCoV

VaxiJen predicted scores of 0.4786 and 0.5367 for S and HE proteins respectively, indicating that the sequences were effectively antigenic. Using Expasy tools in protparam, physical and chemical properties of S and HE protein sequences from bovine coronavirus (BCoV) revealed that both targeted proteins were stables (Table 1).

The spike S protein, showed that it contained.

1363 amino acids (aa) with a molecular weight of 150235.48 kDa, while, HE protein contained 424 amino acids (aa) with a molecular weight of 47617.94 kDa (Table 1).

The secondary structure of BCoV spike S and HE proteins were analyzed through PSIPRED and GOR IV server Fig. 1.

Table 1 Physical and chemical properties of S and HE protein sequences of BCoV using protparam server

NO.	Epitopes	MW	PI	Asp + Glu	Arg + Lys	Ext. coefficient	Instability index (II)	Aliphatic index (AI)	Grand average of hydropath city (GRAVY)
1	S	150235.48	5.31	110	89	194,250	stable.	85.49	0.001
2	HE	47617.94	5.45	33	26	73,535	stable.	84.08	0.002

Fig. 1 (**a**): Secondary structure of Spike (S); (**b**) Secondary structure of Heamagglutinase Estrase (HE) proteins using GOR IV server

DiANNA1.1 tool calculated 28 and 7 disulphides bond (S–S) positions in S and HE proteins respectively. The trans-membrane protein topology was investigated via online Tool TMHMM. Residues from 1 to 1307 were found to be exposed to the surface, residue from 1308 to 1330 were found inside trans-membrane-region and residues from 1331 to 1363 were buried within the core-region of the S protein. In HE protein, residues from 1 to 392 were found to be exposed to the surface, residue from 383 to 415 were found inside trans-membrane-region and residues from 416 to 424 were buried within the core-region. The closest homologs for S and HE proteins, when comparing different coronaviruses in humans and animals with S and HE protein sequences of BCoV, are Rabbit coronavirus and Rabbit coronavirus HKU14 protein, respectively, followed by spike surface glycoprotein of Human coronavirus and hemagglutinin-esterase of Human coronavirus OC43. Based on multiple alignments of COBALT, a phylogenetic tree of BCoV viruses and other coronaviruses on humans and animals was constructed (Tables 2 and 3).

Prediction of B-cell epitopes

Utilizing the Bepipred Linear Epitope Prediction program, several epitopes were predicted using B cell prediction methods. The conservation of predicted epitopes was determined using Epitope Conservancy Analysis in the Immune Epitope Database (IEDB) (http://tools.iedb.org/conservancy/). The conservation scores were calculated using a sequence identity criterion of 100%.

Twenty-five linear conserved epitopes from spike S and fifteen epitopes from HE proteins were identified when the predicted epitopes were shortened. Between locations 379 and 1208 of spike S protein, three epitopes ($_{379}TCQPQ_{384}$, $_{720}QLQPINY_{726}$ and $_{1204}YYYPE_{1208}$) with

Table 2 Blastp similarity search of Spike S protein of BCoV against other spike proteins of coronaviruses in human and animals

Accession No.	Name	Percent Identity
NP_150077.1	Bovine coronavirus (Spike)	100.00
YP_005454245.1	Rabbit coronavirus	94.06
YP_009555241.1	Human coronavirus	91.81
YP_009755834.1	Rodent coronavirus	73.81
YP_009113025.1	Betacoronavirus HKU24	67.65
YP_003029848.1	Rat coronavirus Parker	64.96
YP_173238.1	Human coronavirus HKU1	63.88
YP_209233.1	Murine hepatitis virus strain JHM	64.10
YP_009824982.1	Murine hepatitis virus	63.47
NP_045300.1	Murine hepatitis virus	63.47

Table 3 Blastp similarity search of HE protein of BCoV against other HE proteins of other coronaviruses in human and animals

Accession NO.	Name	Identity
NP_150076.1	Bovine coronavirus (H E)	100.00%
YP_005454244.1	Rabbit coronavirus HKU14	96.23%
YP_009555240.1	Human coronavirus OC43	94.34%
YP_009755833.1	Rodent coronavirus	77.91%
YP_003029847.1	Betacoronavirus HKU24	70.91%
YP_.003029847.1	Rat coronavirus Parker	60.00%
YP_209232.1	Murine hepatitis virus strain JHM	59.08%
YP_173237.1	Human coronavirusHKU1	57.26%
YP_009824981.1	Murine hepatitis virus	57.19%
YP_008798234.1	Porcine torovirus	32.96%

varying lengths were identified as linear, surface, and antigenic epitopes (Table 4; Fig. 2).

In HE protein, however, no epitopes have been discovered as linear, surface, or antigenic.

Prediction of MHC1 epitopes

Only seven epitopes showed a high linkage with MHCI bovine alleles for spike glycoprotein ($_{77}NMALKGTLL_{85}$, $_{11}TAFAVIGDL_{19}$, $_{986}AAAGVPFYL_{994}$, $_{865}TQLQVANSL_{873}$, $_{1103}SQQLSDSTL_{1111}$, $_{1300}YEYYVKWPW_{1308}$, and $_{972}TLAATSASL_{980}$) and three epitopes in HE protein showed a high linkage with MHCI bovine allele ($_{56}SYMDLNPAL_{65}$, $_{186}YKVEADFYL_{194}$ and $_{360}SSVWPLYPY_{368}$).

Assessment of B-cell and MHC I epitopes

Antigenicity, allergenicity and toxicity of MHCI epitopes

VaxiJen v2.0 server, AllerJen v2.0, and ToxiPred were used to estimate the possible antigenicity, allergenicity, and toxicity of MHCI predicted epitopes. The S protein, B cell epitope $_{720}QLQPINY_{726}$, and MHC1 epitope

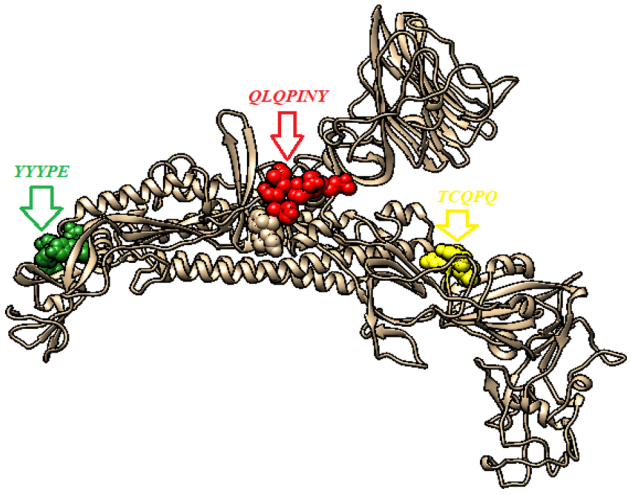

Fig. 2 The position of proposed B cell epitopes in the 3D structure of reference spike (S) protein of BCoV using Chimera software 1.8

$_{77}NMALKGTLL_{85}$ showed significant antigenicity, no allergencity, and no toxicity. While using the VaxiJen v2.0 server, the HE protein MHC1 epitope $_{56}SYMDLNPAL_{65}$ showed a high antigenicity score as well as no allergencity or toxicity (Tables 5 and 6; Figs. 3 and 4.)

Discussion

To improve protection against viral infections, a single antigen or a group of antigens must be presented to the immune system [23]. In silico vaccine design can be thought of as picking good virus protein fragments and then putting them together to make a final vaccine. As a subunit of the final vaccine, a fragment with numerous merits can be picked. An ideal subunit, for example, would have several B-cell and T-cell epitopes, as well as a high antigenicity to elicit human and animals protective responses [47]. An antigenic epitope is a basic unit that triggers a cellular or humoral immune response. As a result, a multi-epitope vaccination composed of a series of or overlapping peptides is a highly effective strategy to prevent and treat malignancies and viral infections [23, 48]. Epitopes capable of inducing both B-cell and T-cell immunity are known to be effective vaccine candidates [49]. However, in this study no overlapping between B-cell and T-cell Epitopes was detected. Peptide-based vaccinations are developed using an emerging computational method that combines immunoinformatic prediction with rigorous experimental validation, making epitope identification within protein antigens much

Table 4 List of B cell epitopes of BCoV proteins predicted by different B cell methods

Epitopes	Length	Position	Emini 1.000	Kolesckar 1046	Antigenicity	Allergecity	Toxinicity
YYYPE	5	1204–1208	3.776	1.08	-	Non -allergen	Non-Toxin
TCQPQ	5	379–384	1.315	1.083	-	Non -allergen	Non-Toxin
QLQPINY	7	720–726	1.678	1.062	2.2411	Non -allergen	Non-Toxin

Table 5 List of MHC1 epitopes of S protein of BCoV

Peptide	Allele	Antigenicity	Allergeicity	Toxicity	Position	Percentile Rank
NMALKGTLL	BoLA-D18.4	1.0105	Non-allergen	Non-Toxin	77–85	0.61
	BoLA-HD6					
	BoLA-JSP.1					
	BoLA-T2b					
	BoLA-T2C					
TAFAVIGDL	BoLA-D18.4	0.5833	Non-allergen	Non-Toxin	19 – 11	0.08
	BoLA-HD6					
	BoLA-JSP.1					
	BoLA-T2b					
	BoLA-T2C					
AAAGVPFYL	BoLA-D18.4	0.5636	Non-allergen	Non-Toxin	986–994	1.5
	BoLA-HD6					
	BoLA-JSP.1					
	BoLA-T2b					
	BoLA-T2C					
TQLQVANSL	BoLA-D18.4	0.5012	Non-allergen	Non-Toxin	865–873	0.56
	BoLA-HD6					
	BoLA-T2b					
	BoLA-T2C					
SQQLSDSTL	BoLA-D18.4	0.4347	Non-allergen	Non-Toxin	1103–1111	1.3
	BoLA-HD6					
	BoLA-T2b					
YEYYVKWPW	BoLA-D18.4	0.9858	Non-allergen	Non-Toxin	1300–1308	0.94
	BoLA-HD6					
	BoLA-JSP.1					
	BoLA-T2b					
TLAATSASL	BoLA-D18.4	0.9408	Non-allergen	Non-Toxin	972–980	1.4
	BoLA-HD6					
	BoLA-JSP.1					
	BoLA-T2C					

Table 6 List of MHC1 epitopes of HE protein of BCoV

Peptide	Allele	Antigenicity	Allergeicity	Toxicity	Position	Percentile Rank
SYMDLNPAL	BoLA-D18.4	**1.7505**	Non-allergen	Non-Toxin	56–65	1.5
	BoLA-HD6					
	BoLA-JSP.1					
	BoLA-T2b					
	BoLA-T2C					
YKVEADFYL	BoLA-D18.4	0.6267	Non-allergen	Non-Toxin	186–194	0.42
	BoLA-HD6					
	BoLA-JSP.1					
	BoLA-T2C					
SSVWPLYPY	BoLA-D18.4	0.5663	Non-allergen	Non-Toxin	360–368	0.21

easier [50]. These vaccines are designed to accurately induce immune responses against antigens using essential epitope peptides rather than the full antigen, and hence have various advantages over traditional vaccines, including improved specificity, increased safety, reduced costs, and fewer adverse events [51].

In the present study, spike (S) and hemagglutinin-esterase (HE) glycoproteins of BCoV were analyzed using a variety of online and offline immunoinformatics tools. Protparam protein characterization of the BCoV spike S and HE glycoproteins indicated that both proteins are positive in nature and stables.

A protein whose instability index is smaller than 40 is predicted as stable, a value above 40 predicts that the protein may be unstable.

The proteins also showed good antigenic characteristics using the Vaxijen 2.0v server.

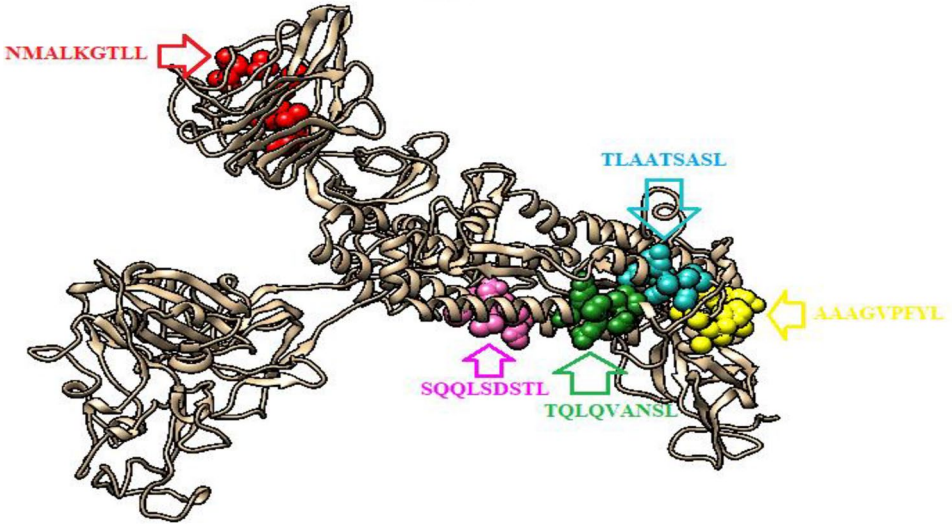

Fig. 3 The position of proposed MHC1 epitopes in the 3D structure of reference spike (S) protein of BCoV using Chimera software 1.8

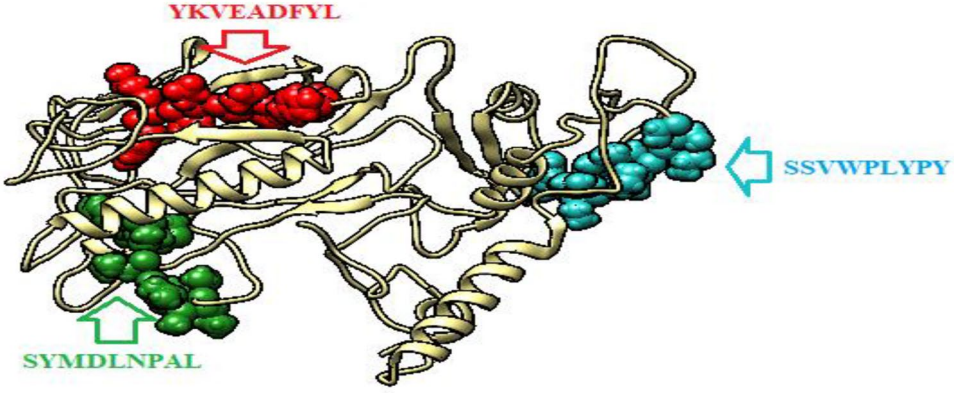

Fig. 4 The position of proposed MHC1 epitopes in the 3D structure of reference HE protein of BCoV using Chimera software 1.8

ClustalW was used to align sequences. The aligned sequences of the two analyzed proteins (S and HE) showed strong conservancy between sequences. On the other hand, a few regions had different amino acids in various sequences. We analyzed the evolutionary divergence of each protein.

B-cell epitope prediction is critical for the development of vaccine components and immunodiagnostic reagents. Continuous epitopes are used in the majority of epitope prediction algorithms Linear B cell epitopes have been shown to play an important function in viral neutralization [52, 53]. Based on amino acid qualities such as hydrophilicity, surface accessibility, flexibility, and antigenicity, the IEDB prediction algorithm was utilized to predict linear, surface, and antigenic epitopes [23]. It is known that both cellular and humoral immune responses are essential against coronaviruses infection [54]. The development of neutralizing antibodies, in addition to T cell-mediated immunity, is required to protect against virus infection, such as Coronaviruses. While cytotoxic lymphocytes can kill infected cells, antibodies have the ability to destroy infected cells while also preventing the infectious virus from infecting the cell (neutralization) [54, 55]. In this study, three shortened conserved epitopes $_{379}TCQPQ_{384}$, $_{720}QLQPINY_{726}$ and $_{1204}YYYPE_{1208}$ were predicted as surface, linear, and antigenic epitopes using B cell prediction algorithms. The top epitope was $_{720}QLQPINY_{726}$ due to high safety and antigenicity.

BoLA is called bovine leukocyte antigen (the MHC in cattle) and is located on chromosome 23; it shares the overall structure of other mammals' MHC. MHC is an adaptive immune system component that addresses both immunological and evolutionary biological issues at the same time [56].

In the bovine genome project, the bovine MHC-II locus was not sequenced completely [57, 58]. As a result, the analysis was completed with the BoLA MHC-I alleles Only. Cell-mediated immunity induced by cytotoxic T lymphocytes (CTLs) is vital for the defense against viral diseases. CTLs are responsible for the immune

elimination of intracellular, pathogens such as viruses because these cells recognize the presented endogenous antigenic peptides by the MHC class I molecules.

The induction of CTL is highly dependent on how effectively the antigenic peptide is delivered into the major histocompatibility complex (MHC) class I presentation pathway in antigen presenting cells (APCs) [24]. MHCI prediction methods on the other hand, revealed seven, high linkage epitopes with MHCI allele from S protein ($_{77}NMALKGTLL_{85}$, $_{11}TAFAVIGDL_{19}$, $_{986}AAAGVPFYL_{994}$, $_{865}TQLQVANSL_{873}$, $_{1103}SQQLSDSTL_{1111}$, $_{1300}YEYYVKWPW_{1308}$, and $_{972}TLAATSASL_{980}$). In HE protein, only three epitopes showed a high linkage with MHCI allele ($_{56}SYMDLNPAL_{65}$, $_{186}YKVEADFYL_{194}$ and $_{360}SSVWPLYPY_{368}$). The predicted epitopes exhibited high levels of antigenicity, no allergenicity, and no toxicity for both spike S and HE proteins.

Conclusion

Three B cell epitopes were predicted from spike glycoprotein of BCoV in this investigation, but the best epitope was $_{720}QLQPINY_{726}$ of BCoV because of high antigenicity. However, the HE did not predict B cell epitopes. Several T cell epitopes were predicted from S and HE glycoproteins of BCoV. The predicted epitopes exhibited high safety and antigenicity as well as strong binding with bola alleles. The proposed epitopes should be included into a multi-epitopes vaccine against BCoV and evaluated using in vivo and in vitro trails. By generating humoral and cellular responses, this vaccine could be used as a peptide vaccine to reduce BCoV infection in bovines. The peptide vaccination against BCoV's spike protein (S) and (HE) which designed to cover all strains in different serotypes, might be effectively replace existing vaccines, reducing recurrent outbreaks and their associated significant economic losses.

Acknowledgements
Author want to acknowledgement Sudanese Veterinarian Association for capacity building and Training, Khartoum, Sudan for their support and engorgement. In addition, all classmate and staff of master program of Bioinformatics, Department of Molecular Biology and Bioinformatics, Collage of Veterinary Medicine, University of Bahri, Khartoum, Sudan.

Author contributions
Eman, A. Awadelkareem and Hamdoun S designed this study, accomplished the data collection and analyze and results' writing. Eman, A. Awadelkareem interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

Funding
Author want to acknowledgement Sudanese Veterinarian Association for capacity building and Training, Khartoum, Sudan for their support and engorgement.

Data availability
All the data supporting the findings are contained within the manuscript.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Published online: 11 October 2024

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