Analysis of the B glycoprotein of Bovine Alphaherpesvirus 1 and 5 as a vaccine candidate against Infectious Bovine Rhinotracheitis

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Analysis of the B glycoprotein of Bovine Alphaherpesvirus 1 and 5 as a vaccine candidate against Infectious Bovine Rhinotracheitis

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COLABIOCLI Confederación Latinoamericana de Bioquímica Clínica
CNB Colegio Nacional de Bacteriología
www.congresocolabiocli.com

Disease produced

Infectious Bovine Rhinotracheitis (IBR) and Bovine Meningoencephalitis

The disease is characterized by symptoms in the upper respiratory tract, including purulent rhinorrhea, conjunctivitis, fever, prostration, and loss of appetite.

Infection in the genital tract can cause pustular vulvovaginitis or balanoposthitis, leading to reduced reproductive performance in animals

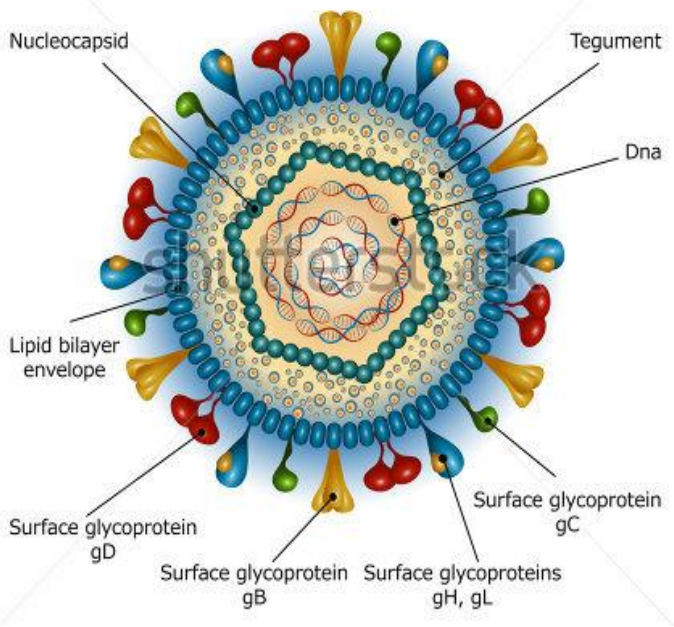
Responsible for serious economic losses due to restrictions on international cattle trade, abortions (25-60%), weight loss and reduced milk production.

Neural problems

Etiological agent

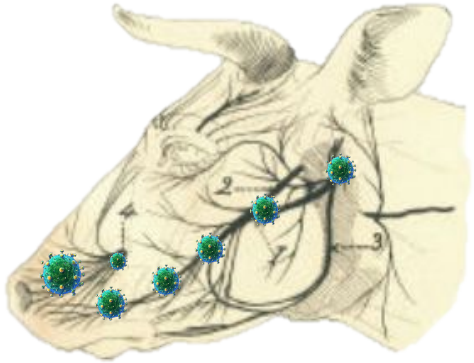
Alphaherpesvirus Bovino (BoHV) 1 y 5

Características	
Family	*Herpesviridae*
Subfamily	*Alphaherpesvirinae.*
Gender	*Varicellovirus*
Genoma	DNA double chain
Structure	Icosahedral capsid. Envelope



Patil, S. S. et al. 2018; Carter et al., 2005

Pathogeny



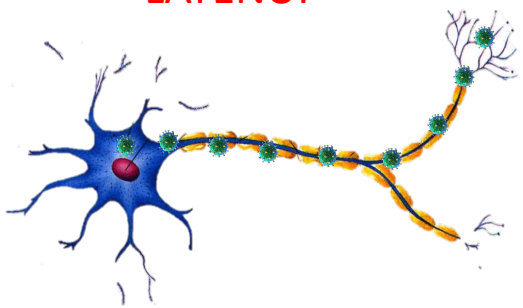
Neuroinvasion

BoHV-1 / BoHV-5

Epithelial tissue surfaces

BoHV-1 / BoHV-5

LATENCY



Olfactory and trigeminal nerve

Ascends central nervous system

BoHV-5

Acute meningoencephalitis

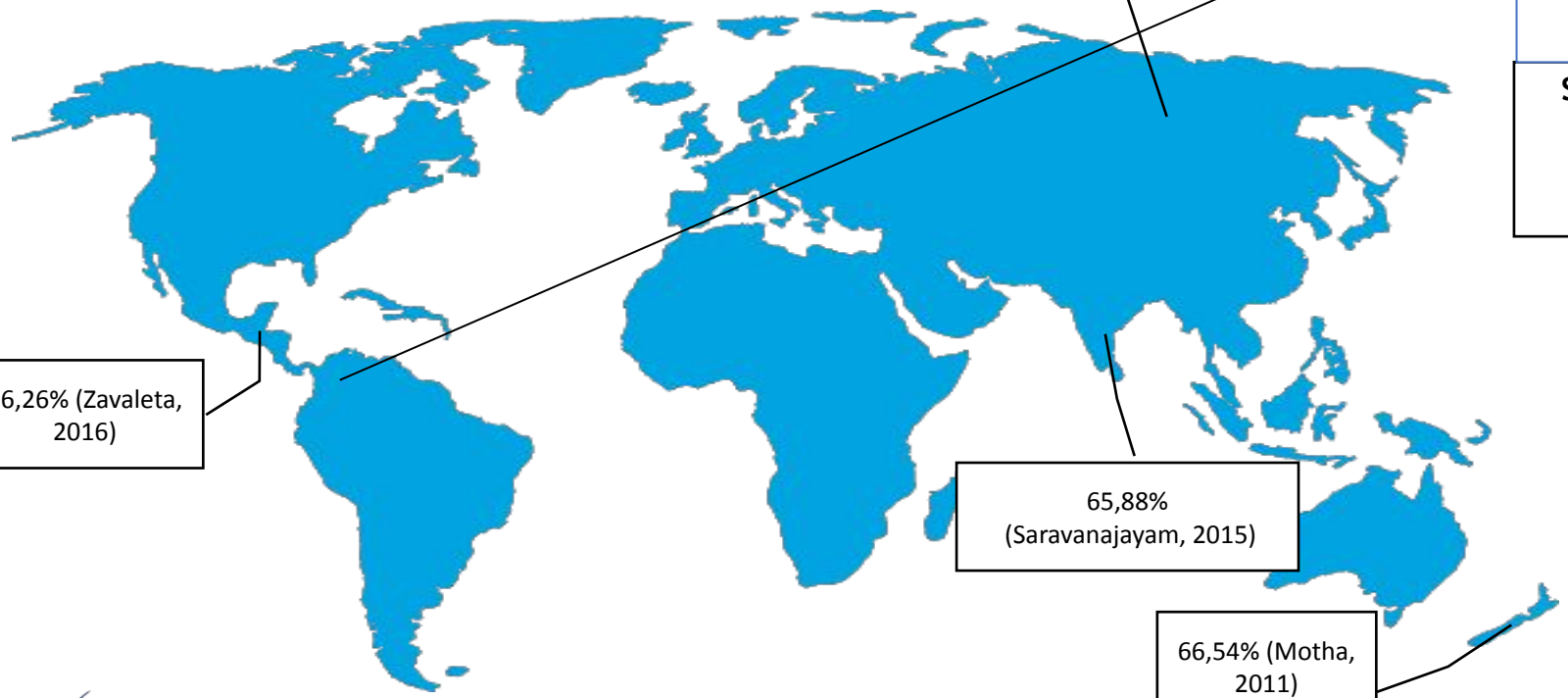
BoHV-5

Gerdts et al., 2000; D´Offay et al., 1993; Roels et al., 2000; Muylkens et al., 2007

Epidemiology

BoHV-1 — World

BoHV-5 — South America

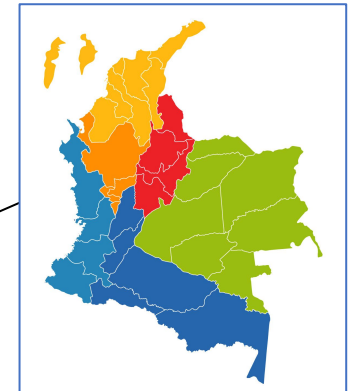


96,26% (Zavaleta, 2016)

40% (Xuelong, 2018)

65,88% (Saravanajayam, 2015)

66,54% (Motha, 2011)



Seroprevalence study (VECOL 2017) 57-84%

Straub, 2001; Metzler et al. 1986; Ackermann et al., 2006; VECOL, 2018.

Current vaccines

The World Organisation for Animal Health (OIE) reports that vaccination against BoHV-1/5 can be effective in reducing clinical manifestations and, consequently, economic losses. However, it does not provide complete protection against infection.

Inactivated	Modified live virus
Vecol S.A	Zoetis
Novartis	Fort Dodge
Virbac	Pfizer
Tecnovax	

For this reason, a wide variety of vaccine agents have been developed in recent years, ranging from classical inactivated vaccines to those utilizing new technologies.

OIE, 2020; Ruiz-Saenz et al., 2017

BoHV glycoproteins

Viral release

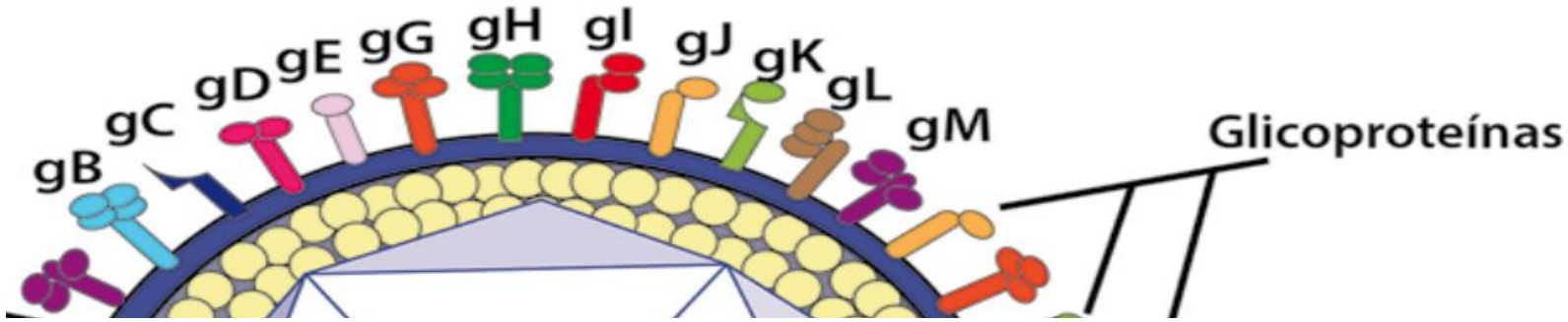
Antigen

Attachment

Cell-cell infecton

Penetration

viral envelope



Baranowski et al., 1996, Schwyzer y Ackermann, 1996; Bryant et al., 2003

Glycoprotein evidence

Babiuk et al., 1987
gB/gC/gD BoHV-1 Native

Zhu et al., 1997
gD BoHV-1 *P. pastoris*

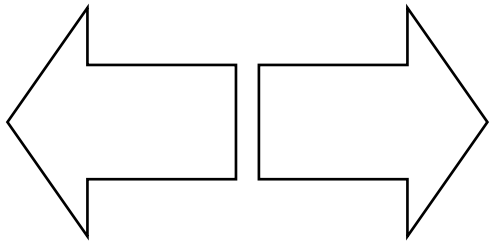
Zhu et al., 1999
gD + IL-6 Bovina BoHV-1 *P.pastoris*

Ioannou, et al., 2002
gD BoHV-1 *E.coli*

Araujo et al., 2018
gD BoHV-1/5 *P. pastoris*

Wen et al., 2020
gD/gB/gC + P2 IL-6 BoHV-1 *E.coli*

Hou et al., 2022
gB BoHV-1 *E.coli*



Fisher et al., 2007
BoHV-5i + Propoleo

Dummer et al., 2014
gD BoHV-5 *P. pastoris*

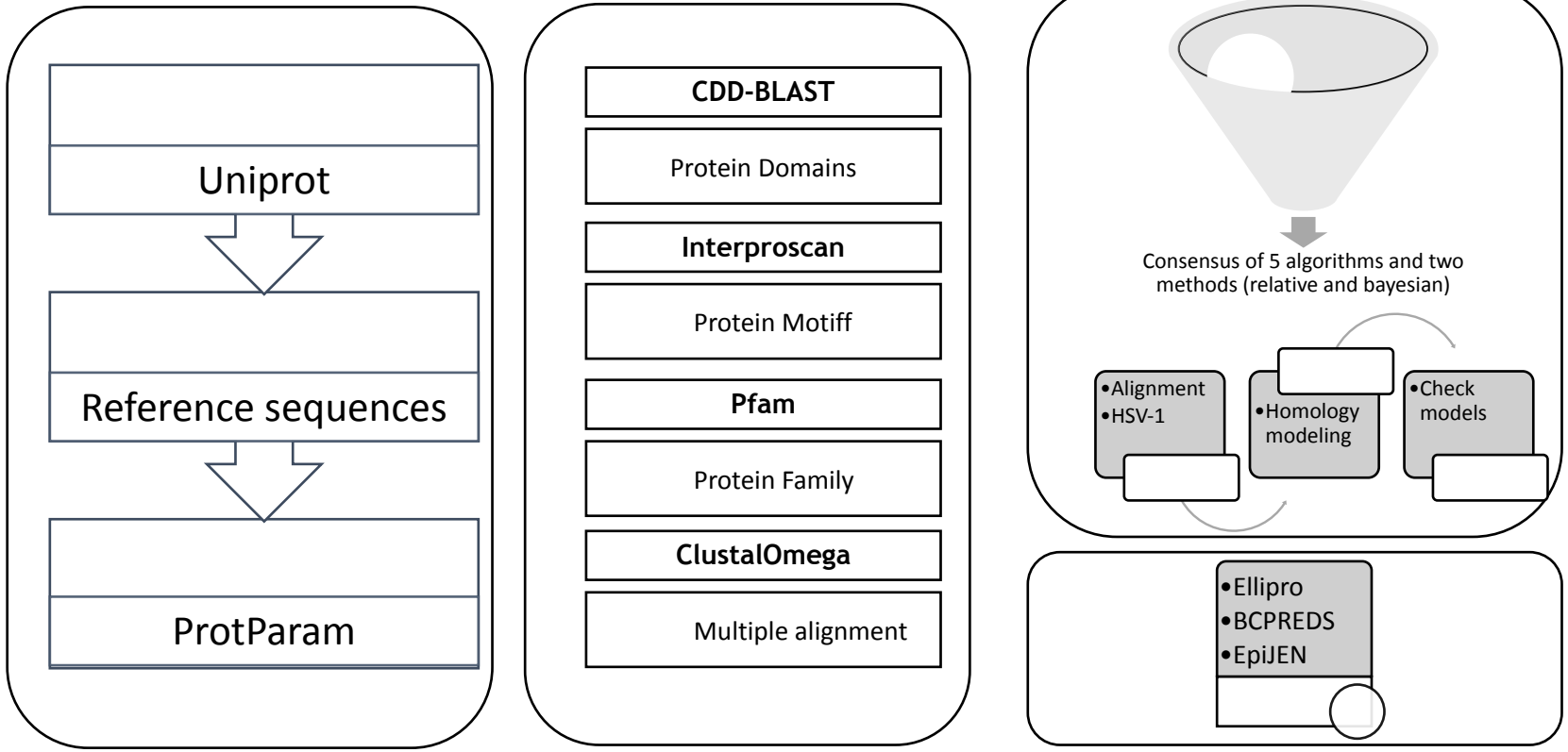
Araujo et al., 2018
gD BoHV-1/5 *P. pastoris*

Santos et al., 2021
gD + Esporas *B. Toyonensis* BoHV-5 *P. pastoris*

Araujo et al., 2022
gD BoHV-5 *P. pastoris*

Could be the B glycoprotein of BoHV-1/5 a vaccine candidate against IBR??

Bioinformatic approach



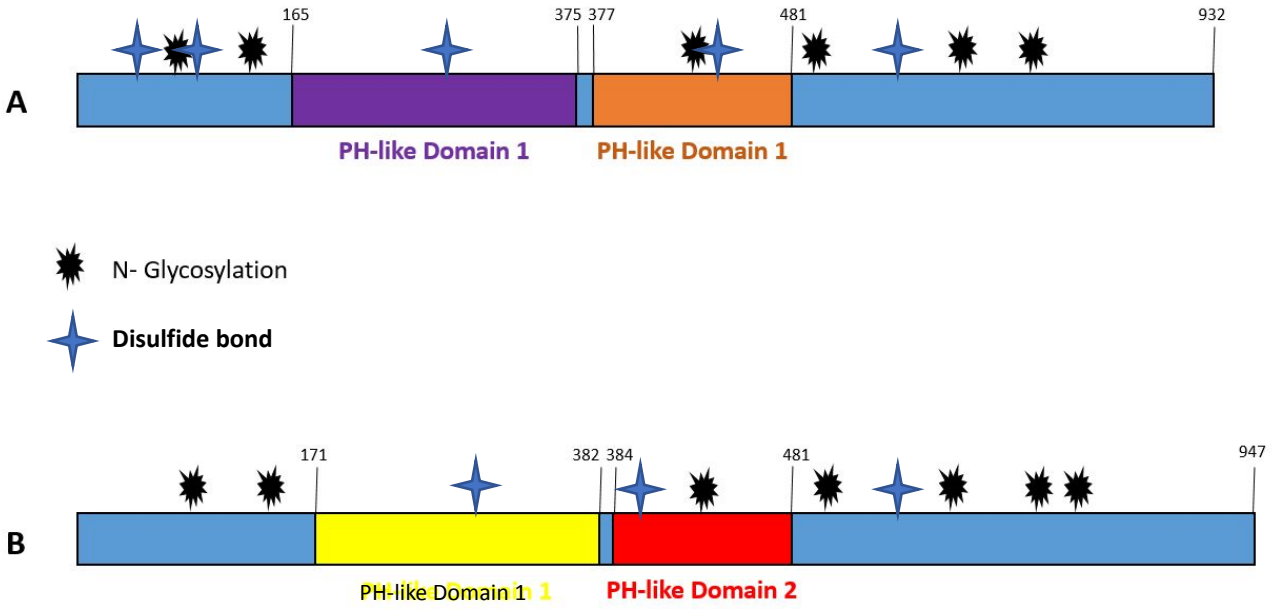
Whitbeck et al., 1988; Gasteiger et al., 2005; Doytchinova et al., 2006

Bioinformatic results

GLYCOPROTEIN	gB		gC		gD		gN		gM		gE		gH		gI	
VIRUS STRAIN	1	5	1	5	1	5	1	5	1	5	1	5	1	5	1	5
Amount of aa	932	947	521	486	417	417	96	95	438	419	575	599	842	848	380	387
Molecular weight(Kda)	101.19	102.45	55.38	51.44	44.92	44.62	10.26	10.31	45.51	43.17	61.16	63.54	88.37	88.99	39.91	39.67
pI theoretical	8.46	8.73	8.61	8.97	5.18	5.10	9.77	9.77	10.47	9.81	4.71	4.81	8.66	8.88	10.97	8.60
Total number of negatively charged residues	104	103	51	45	49	47	6	6	18	24	77	82	71	77	24	33
Total number of positively charged residues	109	111	56	53	38	36	11	11	36	35	47	58	77	85	49	38
Instability index	45.86	47.18	57.63	59.80	49.91	50.27	44.12	53.15	38.98	30.77	53.84	53.22	38.49	37.97	65.92	47.79
Aliphatic index	78.56	77.84	68.43	70.99	63.12	68.47	98.65	104.84	114.09	116.06	79.74	72.62	93.61	92.29	81.42	80.75
Average hydropathicity (GRAVY)	**(-0.273)**	**(-0.272)**	(-0.352)	(-0.261)	(-0.377)	(-0.286)	0.477	0.558	0.543	0.604	(-0.161)	(-0.311)	0.239	0.212	(-0.029)	0.052
Alignment idendity (%)	**91.878**		75.287		79.905		78.125		78.475		69.772		85.984		70.951	
Funtion	**Attachment**		Attachment		Penetration		Morphogenesis		Morphogenesis		Cell-cell infection		Cell-cell infection		Cell-cell infection	
emPAI	0.778		3.786		0.551				1.683		0.413		0.688		0.389	

Physicochemical properties of glycoproteins

Bioinformatic results



Schematic representation of the B glycoproteins of BoHV-1 and BoHV-5

1A. BoHV-1 B glycoprotein. PH-like domain 1 found between 165 to 375 and PH-like domain 2 found between 377 to 481; N-glycosylation sites (105, 153, 441, 483, 640 and 706). 1 B. BoHV-5 B glycoprotein. PH-like domain 1 found between 171 to 382 and PH-like domain 2 found between 384 to 481; N-glycosylation sites (111, 159, 448, 490, 594, 654 and 720).

Bioinformatic results

```
Glycoprotein B of BoHV-1 LAQAAGENSRFFVCPPPSGATVVRLAPARPCPEYGLGRNYTEGIGVIYKENIAPYTFKAY  174
Glycoprotein B of BoHV-5 LAQAAGENSRFYVCPPPSGATVVRLAPARPCPEYELGRNYTEGIGAIYKENIAPYTFKAY  180
                         ***********:********************** **********.**************

Glycoprotein B of BoHV-1 IY-KNVIVTTTWAGSTYAAITNQYTDRVPVGMGEITDLVDKKWRCLSKAEYLRSGRKVVA  233
Glycoprotein B of BoHV-5 IYYKNVIVTTTWAGSTYAAITNQYTDRVPVGLGEITDLVDKKWRCLSKAEYLRSGRKVVA  240
                         ** ****************************:**************************

Glycoprotein B of BoHV-1 FDRDDDPWEAPLKPARLSAPGVRGWHTTDDVYTALGSAGLYRTGTSVNCIVEEVEARSVY  293
Glycoprotein B of BoHV-5 FDRDEDPWEAPLKPARLSAPGVRGWHTTDEVYTALGSAGLYRTGTSVNCIVEEVEARSVY  300
                         ****:************************:******************************

Glycoprotein B of BoHV-1 PYDSFALSTGDIIYMSPFYGLREGAHREHTSYSPERFQQIEGYYKRDMATGRRLKEPVSR  353
Glycoprotein B of BoHV-5 PYDSFALSTGDIIYMSPFYGLRDGAHREHTSYSPERFQQIEGYYKRDMATGRRLKEPVSR  360
                         **********************:************************************

Glycoprotein B of BoHV-1 NFLRTQHVTVAWDWVPKRKNVCSLAKWREADEMLRDESRGNFRFTARSLSATFVSDSHTF  413
Glycoprotein B of BoHV-5 NFLRTQHVTVAWDWVPKRKNVCSLTKWREADEMLRDESRGNFRFTARSLSATFVSDGHTF  420
                         ************************:******************************.***

Glycoprotein B of BoHV-1 ALQNVPLSDCVIEEAEAAVERVYRERYNGTHVLSGSLETYLARGGFVVAFRPMLSNELAK  473
Glycoprotein B of BoHV-5 ALQNVPLSDCVTEEAGAAVERVYRERFNATHVLSGGLETYLARGGFVVAFRPMLSNALAK  480
                         *********** *** **********:*.******.******************** ***

Glycoprotein B of BoHV-1 LYLQELARSNGTLEGLFAA--------AAPKPGPRRARRAAPSAPGGPGAANGPAGDGDA  525
Glycoprotein B of BoHV-5 LYLQELARSNGTLEGLFAAGGSGAAAAAAPKPVPRRARRSASPTPPAP-AASGDGGDGDA  539
                         ******************       ***** ******:*  :* .* **.* .*****
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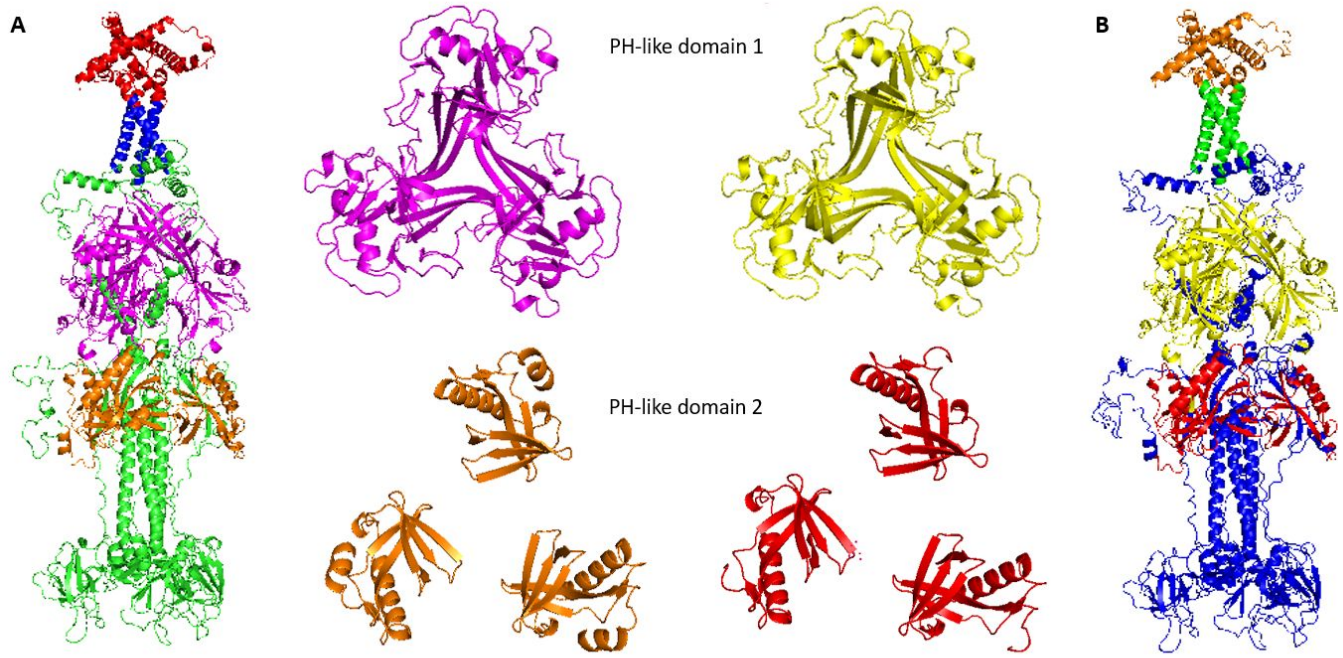
Sequence alignment of the B-glycoprotein domains of BoHV-1 and BoHV-5. "*" Represents identical aa, red and blue lines represent PH-like domain 1 and PH-like domain 2 respectively.

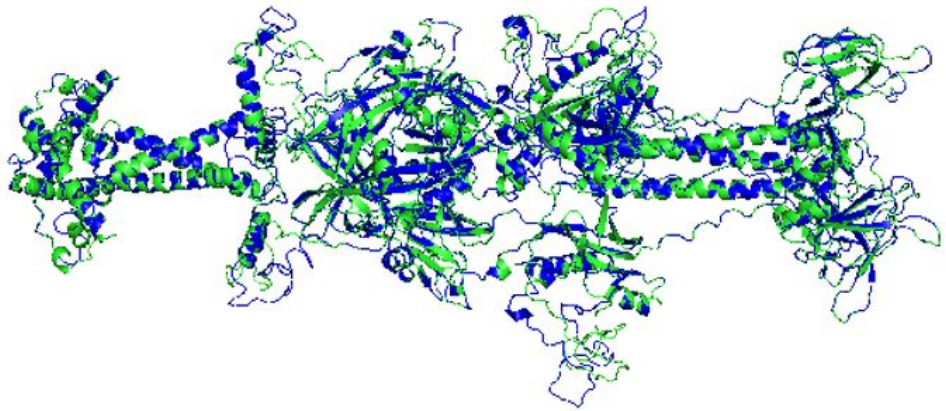
Bioinformatic results



Modeling of the B glycoproteins of BoHV-1 and BoHV-5
4A. BoHV-1 glycoprotein B. Green: general structure. Magenta: domain similar to PH 1. Orange: domain similar to PH 2.
4B. BoHV-5 glycoprotein B. Blue: general structure. Yellow: domain similar to PH 1. Red: domain similar to PH 2.

Bioinformatic results

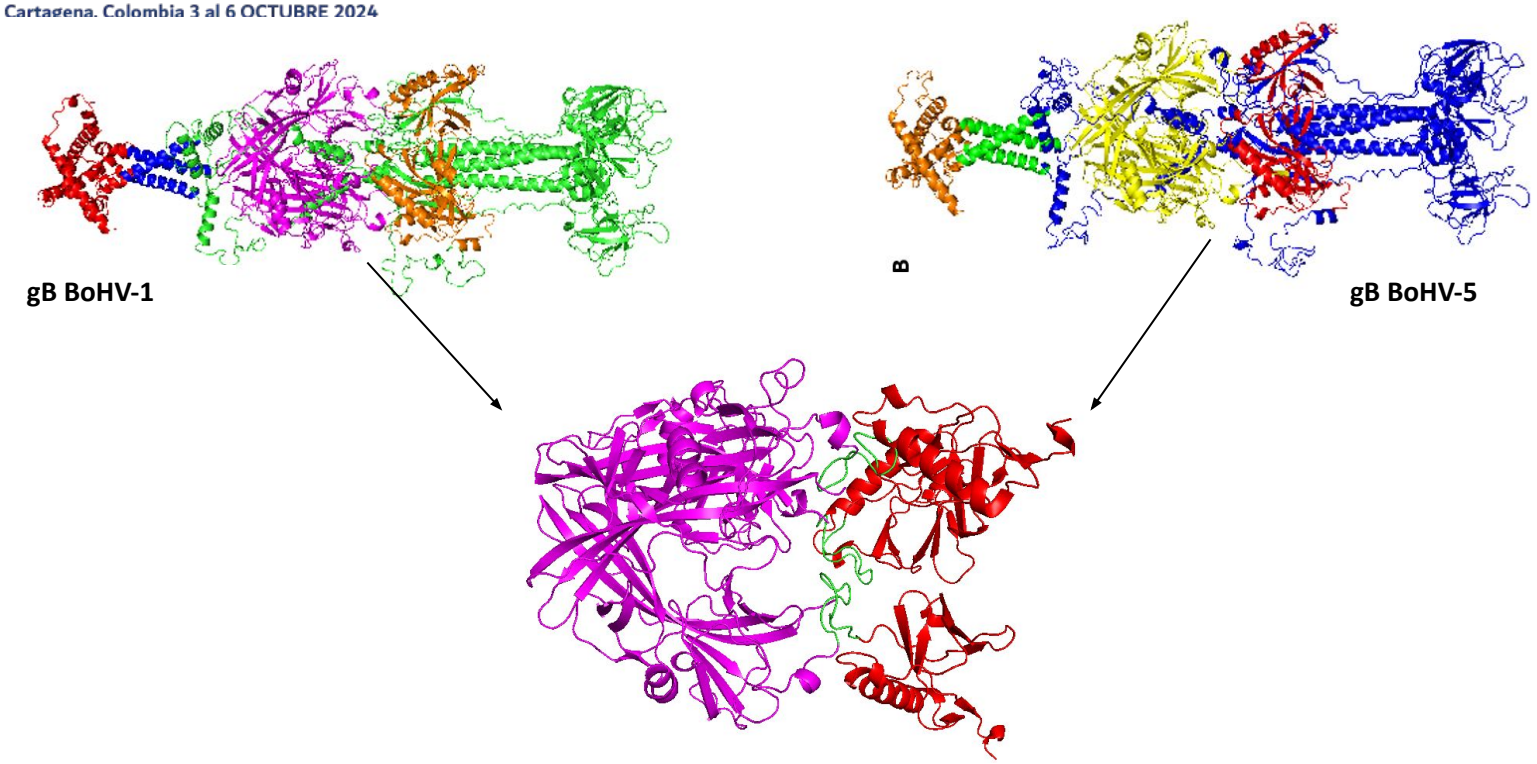
C



Modeling of the B glycoproteins of BoHV-1 and BoHV-5

4C. Overlapping patterns of BoHV-1 B-glycoprotein (green) overlaid with the BoHV-5 B-glycoprotein pattern (blue). RMSD value (0.081)

Vaccine design



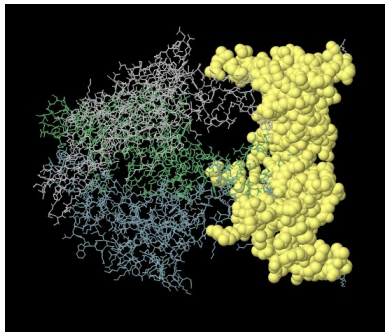
Domain 1 (BoHV-1) + Linker (GS) x8 + Domain 2 (BoHV-5)

3D modeling of the vaccine candidate.

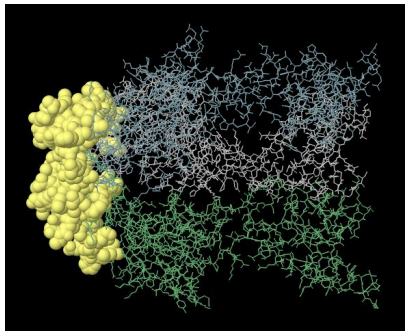
Vaccine design

A. Ellipro

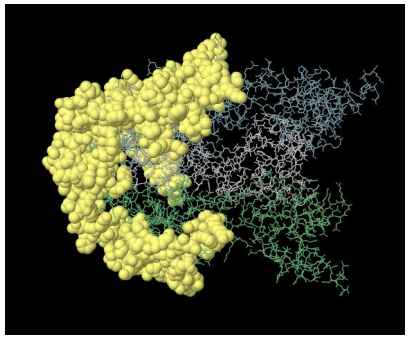
0,782



0,781



0,779



B. BCpreds

Position	Epitope	Score
273	SDCVIEEAEAAVERVYRERY	1
210	KNVCGSGSGSGSGSGSGSGS	1
119	NCIVEEVEARSVYPYDSFAL	1
169	QQIEGYYKRDMATGRRLKEP	1
231	KWREADEMLRDESRGNFRFT	1
86	ARLSAPGVRGWHTTDDVYTA	1
148	PFYGLREGAHREHTSYSPER	0.999
252	RSLSATFVSDSHTFALQNVP	0.99
314	AFRPMLSNELAKLYLQELAR	0.986
27	TYAAITNQYTDRVPVGMGEI	0.952
6	YTFKAYIYYKNVIVTTTWAG	0.829

C. EpiJen

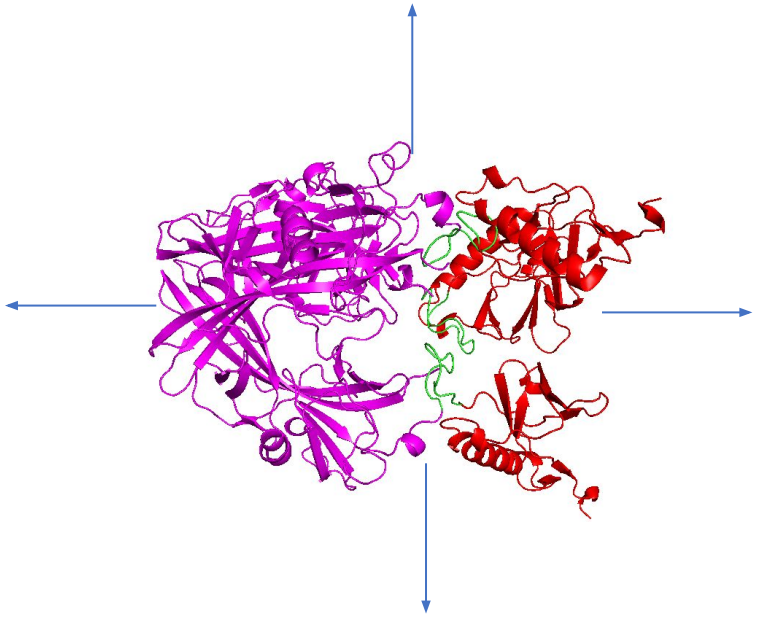
Starting position	Peptide	Predicted -logIC_{50} (M)	Predicted IC_{50} Value (nM)
137	ALSTGDIIY	9.175	0.67
6	YTFKAYIYY	8.772	1.69
20	TTTWAGSTY	8.622	2.39
142	DIIYMSPFY	8.367	4.30
155	GAHREHTSY	8.25	5.62
297	VLSGSLETY	7.523	29.99
11	YIYYKNVIV	7.436	36.64
113	RTGTSVNCI	7.341	45.60
3	IAPYTFKAY	7.115	76.74
104	TALGSAGLY	6.953	111.43
319	LSNELAKLY	6.906	124.17
103	YTALGSAGL	6.89	128.82
305	YLARGGFVV	6.88	131.83
98	TTDDVYTAL	6.789	162.55
294	GTHVLSGSL	6.73	186.21
27	TYAAITNQY	6.353	443.61
120	CIVEEVEAR	6.264	544.50

Epitope prediction. A. Prediction of B-lymphocyte discontinuous epitopes. **B**. Prediction of continuous epitopes B lymphocytes. **C**. Prediction of continuous epitopes T lymphocytes

Vaccine design

Independent folding of each domain thanks to the Linker

Physicochemical characteristics to be considered good Ag

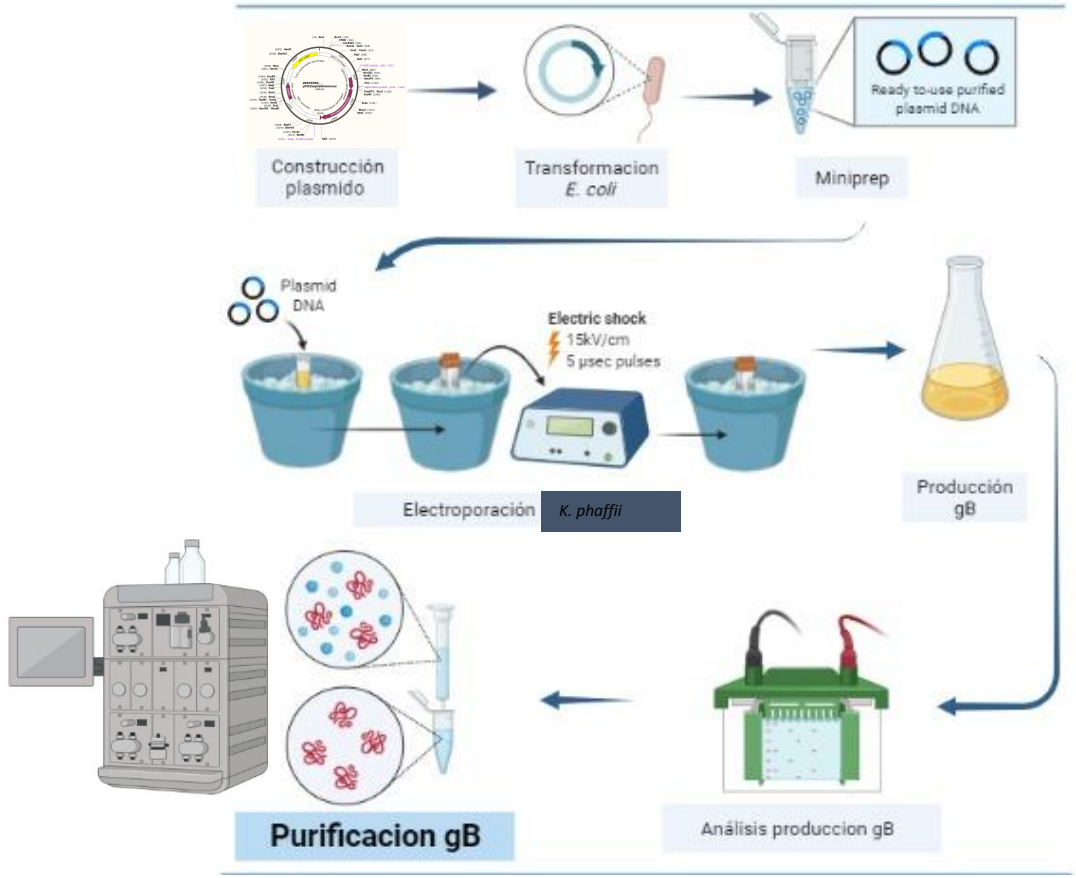


Importance in viral cycle, attachment process

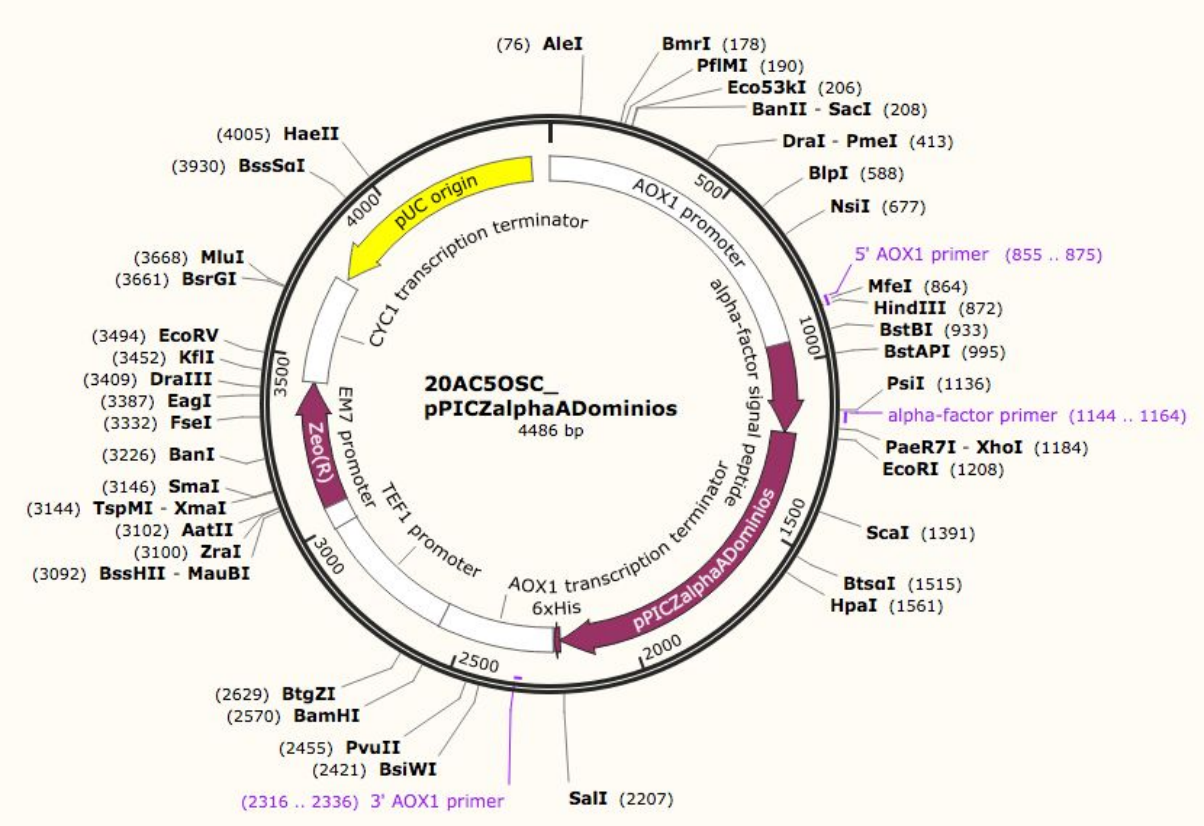
Recognizable epitopes:
B lymphocytes: continuous and discontinuous
T lymphocytes: Continuous

Parham 2010; Dummer 2014; Araujo 2018

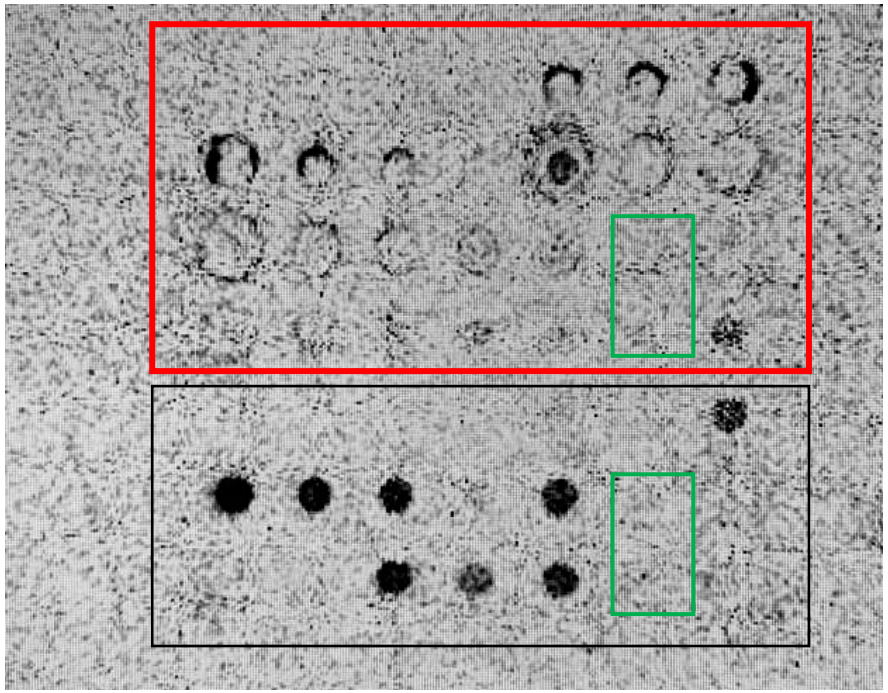
Vaccine production



Vaccine production



Vaccine production



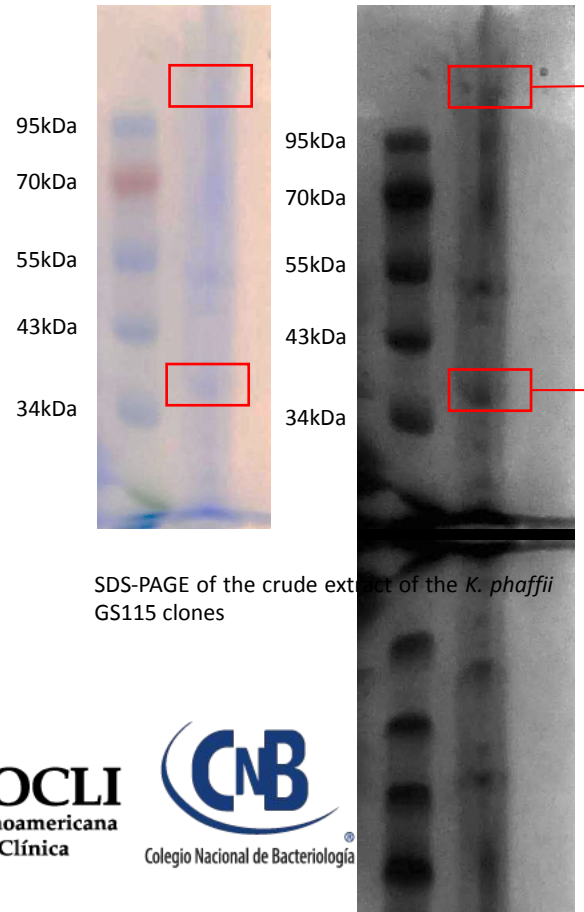
Dot blot of the crude extract of the *K. phaffii* GS115 clones

0 Hours

72 Hours

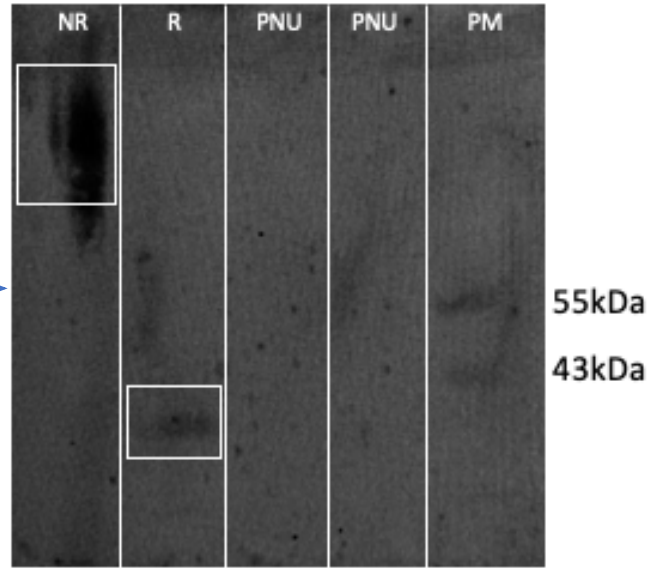
Control

Vaccine production



SDS-PAGE of the crude extract of the *K. phaffii* GS115 clones

NR = Non-reducing
R= Reducing
PNU= Unbound protein
MP=Weight marker



Western blotting analysis of DgB purification with MAb Anti-6xHis HRP conjugate

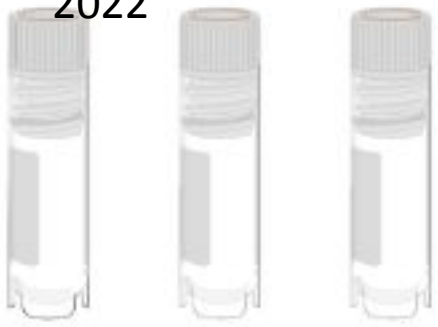
Vaccine production

BCA (Protein quantification)

Concentration	242,2 µg/m	873,5 µg/m	241,3 µg/m
Amount	2,4 mg	1,75 mg	0,48 mg
Volume	10ml	2ml	2ml

Vaccine production

Quintero et al., 2022



Zhu et al., 1997
gD BoHV-1 *P. pastoris*

Zhu et al., 1999
gD + IL-6 Bovina BoHV-1 *P.pastoris*

Araujo et al., 2018
gD BoHV-1/5 *P. pastoris*

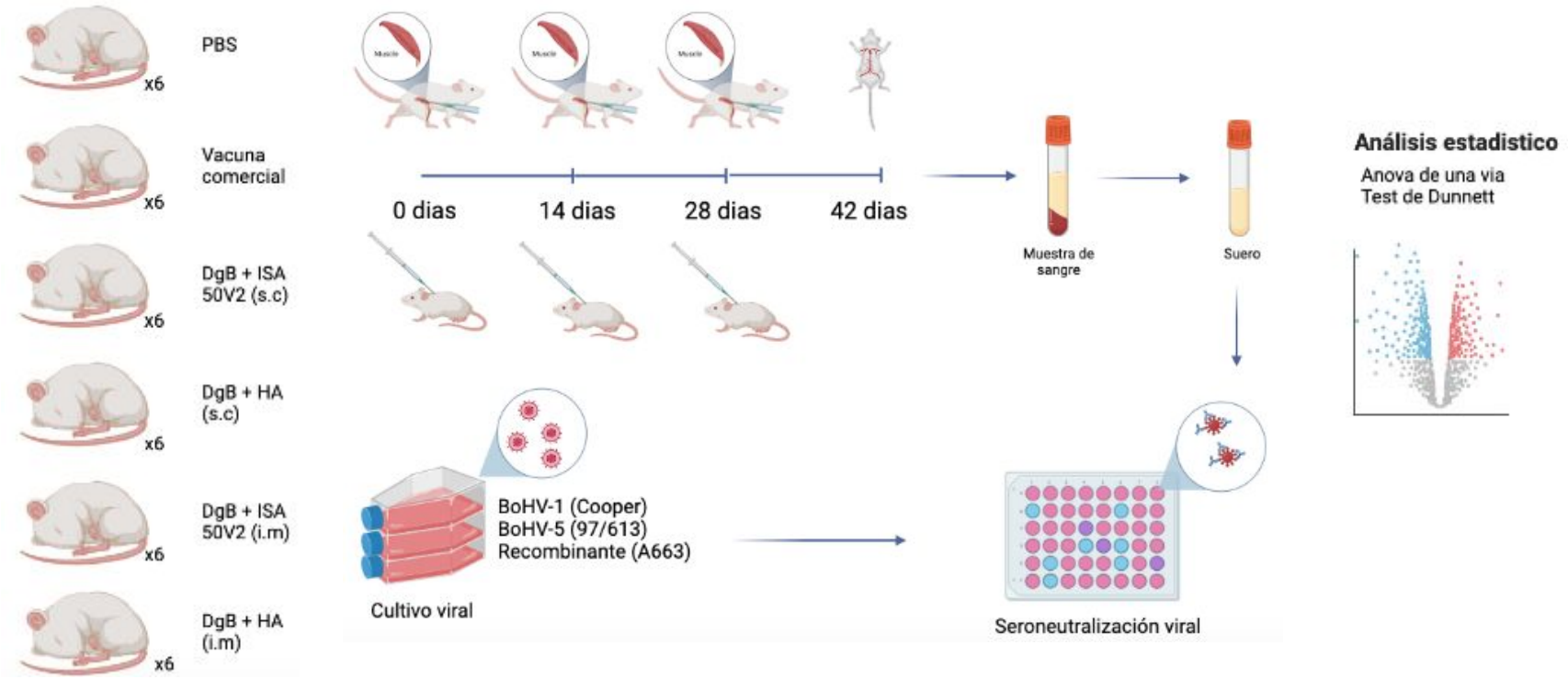
Concentration	242,5 µg/mL
Amount	2,4 mg
Volume	10mL

Dummer et al., 2014
gD BoHV-5 *P. pastoris*

Araujo et al., 2022
gD BoHV-5 *P. pastoris*

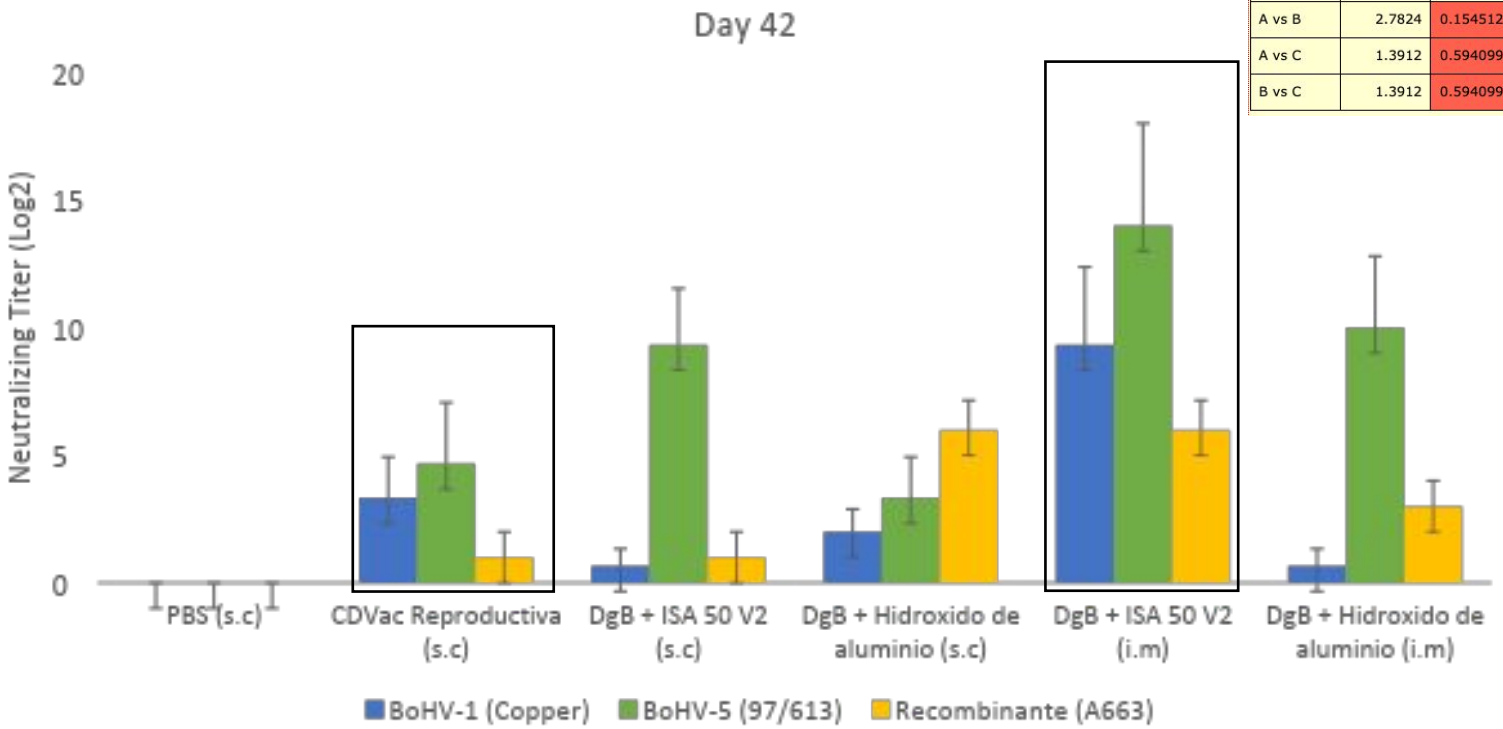
The improved performance of our candidate is attributed to its optimal size, reduced complexity, and absence of post-translational rearrangements, which eliminates the need for bioreactor production, unlike glycoprotein D

Vaccine immune response



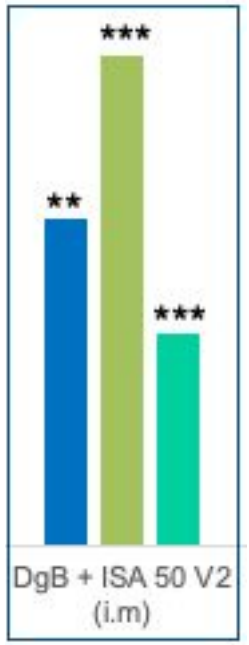
Vaccine immune response

treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
A vs B	2.7824	0.1545120	insignificant
A vs C	1.3912	0.5940999	insignificant
B vs C	1.3912	0.5940999	insignificant



Data represent the mean ± S.E.M of log2 transformed data and are expressed as the reciprocal of the highest dilution that completely inhibited virus-induced CPE. Statistical analysis was performed by one-way ANOVA followed by Dunnett's multiple comparisons test. *P < 0.05, **P < 0.01, and ***P < 0.001. s.c.: subcutaneously; i.m: intramuscularly.

Vaccine immune response



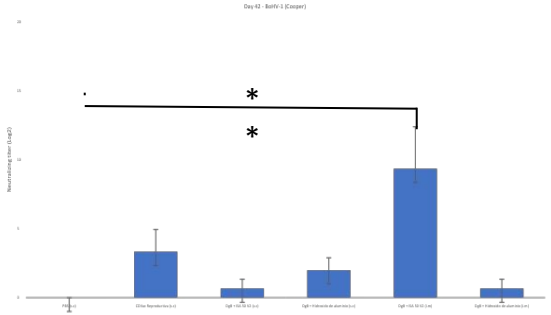
The exposure time to the antigen in intramuscular tissues is longer than that via the subcutaneous route

Oil adjuvants prolong antigen retention in tissues, leading to greater recruitment of antigen-presenting cells

No significant differences were found between the antibody titers against the different viruses, indicating that our candidate is effective against all three viruses

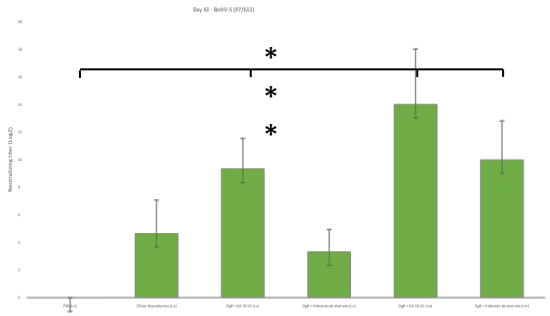
Henriksen-Lacey et al., 2010; Tacken et al., 2011; Coffman et al., 2010; Smith et al. al., 2007; Batista-Duharte et al., 2014; cook et al., 2007; Schynder et al., 2021

Vaccine immune response



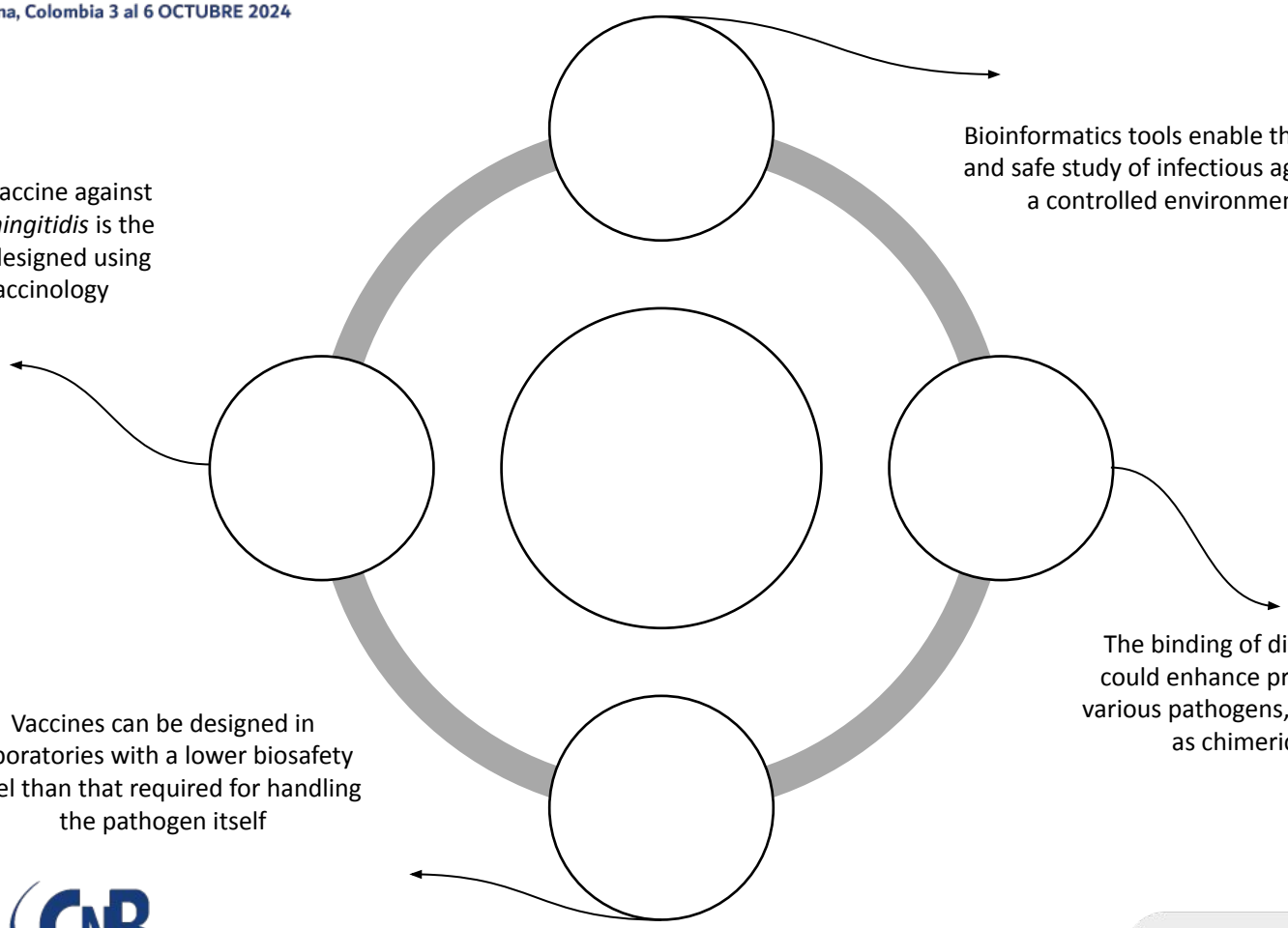
Our obtained neutralizing antibody titers against BoHV-1 are comparable or higher (Zhu et al., 1997; Zhu et al., 1999; Ioannou, et al., 2002; Araujo et al., 2018; Wen et al., 2020; Hou et al., 2022)

Our neutralizing antibody titers were higher against BoHV-5 (Fisher et al., 2007; Dummer et al., 2014; Araujo et al., 2018; Santos et al., 2021; Araujo et al., 2022)



To the best of our knowledge, this is the first vaccine candidate tested for its ability to neutralize a naturally occurring recombinant strain.

Advantages reverse vaccinology



Bioinformatics tools enable the rapid and safe study of infectious agents in a controlled environment

The binding of different antigens could enhance protection against various pathogens, a concept known as chimeric vaccines

Vaccines can be designed in laboratories with a lower biosafety level than that required for handling the pathogen itself

The subunit vaccine against *Neisseria meningitidis* is the first vaccine designed using reverse vaccinology

Articles produced

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RESEARCH Open Access

Characterization and expression of domains of Alphaherpesvirus bovine 1/5 envelope glycoproteins B in *Komagataella phaffi*

Juan Sebastián Quintero Barbosa[1*], Heidy Yohana Triana Rojas[2], Janneth Gonzalez[3], Angela Johana Espejo-Mojica[2], Carlos Javier Alméciga Díaz[2] and María Fernanda Gutierrez[1]

Abstract

Background *Bovine herpes virus* (BoHV 1 and BoHV-5) are the causative agents of infectious bovine rhinotracheitis (IBR). IBR is responsible for important economic losses in the cattle industry. The envelope glycoprotein B (gB) is essential for BoHV infection of cattle's upper respiratory and genital tract. gB is one of the main candidate antigens for a potential recombinant vaccine since it induces a strong and persistent immune response.

Results In this study, gB of BoHV-1 and BoHV-5 was characterized in terms of function, structure, and antigenicity through bioinformatics tools. gB showed conserved sequence and structure, so, both domains named PH Like 1 and 2 domains of each virus were selected for the design of a bivalent vaccine candidate. The immunoinformatic study showed that these two domains have epitopes recognizable by B and T lymphocytes, followed by this, the cDNA domains from BoHV-1/5 gB (Domains-gB) were transformed into the yeast *Komagataella phaffii* GS115 (previously known as *Pichia pastoris*). A recombinant protein with molecular weight of about 110 kDa was obtained from the culture media. The vaccine candidate protein (Domains-gB) was recognized by a monoclonal antibody from a commercial ELISA kit used for IBR diagnostic, which may suggest that the epitopes are conserved of the entire infectious virus.

Conclusion Overall, it was shown that the recombinant domains of BoHV-1/5 gB have antigenic and immunogenic properties similar to the native gB. This vaccine candidate is promising to be used in future studies to assess its immunogenicity in an animal model.

Keywords Alphaherpesvirus bovine, Vaccine, Recombinant protein, IBR

vaccines MDPI

Article

Humoral Immune Response of Mice against a Vaccine Candidate Composed of a Chimera of gB of Bovine Alphaherpesviruses 1 and 5

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Abstract: Infectious bovine rhinotracheitis (IBR) and bovine meningoencephalitis are caused by bovine alphaherpesvirus (BoHV) types 1 and 5, which seriously threaten the global cattle industry. Vaccination to improve immunity is the most direct and effective means to prevent these conditions. Glycoprotein B (gB) is essential for the attachment of both viruses to permissive cells, and is a major target of the host immune system, inducing a strong humoral response. The aim of this study was to evaluate, in a murine model, the immune response of a candidate vaccine formulation composed of a chimeric BoHV-1 and BoHV-5 gB (DgB), expressed in *Komagataella phaffii*. The chimeric DgB vaccine adjuvanted with Montanide 50 ISA V2 or aluminum hydroxide was administered intramuscularly or subcutaneously. A control group and a group that received a commercial vaccine were inoculated subcutaneously. Higher titers of neutralizing antibodies against BoHV-1, BoHV-5, and a natural BoHV-1/5 recombinant strain were obtained with the oil-based candidate vaccine formulation administered intramuscularly. The results demonstrated that the chimeric DgB conserved important epitopes that were able to stimulate a humoral immune response capable of neutralizing BoHV-1, BoHV-5, and the recombinant strain, suggesting that the vaccine antigen is a promising candidate to be further evaluated in cattle.

Citation: Quintero Barbosa, J.S.; Alméciga-Díaz, C.J.; Pérez, S.E.; Gutierrez, M.F. Humoral Immune Response of Mice against a Vaccine Candidate Composed of a Chimera of gB of Bovine Alphaherpesviruses 1 and 5. *Vaccines* **2023**, *11*, x. https://doi.org/10.3390/xxxxx

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