

5' **GGTACC** TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGT

Kpn-1 CMV-IE promoter

TACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCCATTTGACGTCAATAATG
ACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAA
CTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAA
ATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTA
TTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACT
CACGGGGATTTCGAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGTGTTTGGCACCAAAATCAACGGGA
CTTTCCAAAATGTCGTAACAACCTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTC
TATATAAGCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGCGCTACCGGT **GCCGCCACCMQ**GPLAVL

Kozak sequence

BoHV-1 gD signal sequence (1-19 aa)

GALLAVAVSL**MEDPHLRNRP**KGHNIDGMTQEDATCKPVTYAGACSSFDVLLLEKKGKFLFQSYAHHRTL

RVFV Gn sequence (1, 154-581 aa)

LEAVHDTIIAKADPPSCDLLSAHGNPCMKELVMKTHCPNDYQSAHYLNNDGKMASVKCPKYELEDN
FCRQMTGASLKKGSYPLQDLFCQSSSEDDGSKLKTMMKGVCEVGVQALKKCDGQLSTAHEVVPFAVFNKSK
KVYLDKLDLKTENLLPDSFVCFEHKGQYKGTMDSGQTKRELKSFDISQCPKIGGHGSKKCTGDAAFCSA
YECTAQYANAYCSHANGSGIVQIQVSGVWKKPLCVGYERVVVKRELSAKPIQRVEPCTTCITKCEPHGLV
VRSTGFKISSAVACASGVCVTGSQSPSTEITLKYPGISQSSGGDIGVHMAHDDQSVSSKIVAHCPPQDPC
LVHDCIVCAHGLINYQCHTRVLKCLKIAPRKVLNPLMWITAFIRWIYKKMVARVADNINQVNREIGWMEG

Gn cytoplasmic tail (605-674 aa)

GQLVLGNPAPIPRHAPIPRMAPTRPNTATRPWQHVDALKEALSLLNHSSDTPDAVMNDTEVVSEKFDSQE

Bovine GMCSF (1, 18-143 aa)

PTCLQTRLKLYKNGLQGSLSLMGSLTMMATHYEKHCPTPETSCGTQFISFKNFKEDLKEFLFIIPFDC
WEPAQK**GACTACAAAGACGATGACGACAAG****GSGATNFSLKQAGDVEENPGPM**CSELIQASSRITTCSTE

Flag Tag GSG + Peptide 2A RVFV Gc with transmembrane and cytoplasmic (1, 691-1197 aa)

GVNTKRLSGTALIRAGSVGAELMLKGVKEDQTKFLKIKTVSSELSCREGQSYWTGSFSPKCLSSRRC
HLVGECHVNRCLSWRDNETSAEFSFVGESTTMRNKCFEQCGWGCGCFNVNPSCLFVHTYQLQSVRKEAL
RVFNCIDVWHKLTLEITDFDGSVSTIDLGASSSRFTNWGSVLSLDAEGISGSNSFSFIESPGKYAIVD
EPFSEIPRQGFLGEIRCNESSVLSAHESCLRAPNLISYKPMIDQLECTTNLIDPFVVFERSLPQTRND
KTFAASKGNRGVQAFSGKSVQADLTLMFDNFVDFVGAAVSCDAAFLNLTGCYSCNAGARVCLSITSTGT
GSLSAHNKDGLHIVLPSENGTKDQCQILHFTVPEVEEEFMYSCDGDERPLLKGTLIAIDPFDDRREAG
GESTVVPKSGSWNFFDWFSGLMSWFGGPLKTIILICLYVALSIGLFFLLIYLGRTGLSKMWLAATKKAS

ggtaagcctatccctaaccctctcctcggtctcgattctacgcgtaccggt**TAA**CTGATCATAATCAGCC

V5 Epitope

Stop codon

SV40 PolyA

ATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAA
AATGAATGCAATTGTTGTTGTTAACTTGTGTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATC
ACAAATTTACAAATAAAGCATTTTTTCTACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTAT
CTTA**AAGCTT**^{-3'}

Hind III

Figure S1: RVFV Gn-FLAG-P2A-Gc-V5 chimera sequence. RVFV Gn and Gc sequence with the KpnI and HindIII sites were designed to include 5'-3' the following: KpnI site, CMV-IE promoter, Kozak sequence, BoHV-1 gD signal peptide, RVFV Gn without the transmembrane domain, Gn cytoplasmic tail, Bovine GMCSF, Flag tag, GSG sequence to improve cleavage efficiency, Peptide 2 A sequence, RVFV Gc region with transmembrane domain, V5 epitope, stop codon, SV polyA tail sequence and HindIII site.

Table S1. Vaccine virus or vector virus isolation from nasal swab samples in MDBK cells.

S no.:	Group	Animal #	Nasal virus shedding following immunization (Plaque forming units/nasal swab) (Days post-vaccination)								
			Day 0	Day 3	Day 5	Day 7	Day 14	Day 21	Day 28	Day 28	Day 33
1	BoHV-1qmv vector control group	868	0	3.0×10^3	2.5×10^2	5.0×10^1	0	0	0	0	0
2		866	0	2.0×10^2	0	5.0×10^1	0	0	0	0	0
3		890	0	2.0×10^2	0	0	0	0	0	0	0
1	BoHV-1qmv Sub-RVSV vaccine group	865	0	4.8×10^3	0	0	0	0	0	0	0
2		860	0	4.0×10^2	0	0	0	0	0	0	0
3		875	0	5.0×10^1	5.0×10^1	0	0	0	0	0	0
4		862	0	2.0×10^2	0	0	0	0	0	0	0
5		867	0	5.0×10^2	0	0	0	0	0	0	0
6		876	0	3.0×10^3	0	0	0	0	0	0	0
7		879	0	9.0×10^2	0	0	0	0	0	0	0
8		828	0	4.3×10^3	0	0	0	0	0	0	0

Table S2. Quantification of bovine herpesvirus type 1 (BoHV-1) genome copies in nasal swab samples collected from the vaccine virus or vector virus inoculated calves by qPCR.

S no.:	Group	Animal #	Nasal virus shedding following immunization (qPCR; viral genome copies/nasal swab) (Days post-vaccination)								
			Day 0	Day 3	Day 5	Day 7	Day 14	Day 21	Day 28	Day 28	Day 33
1	BoHV-1qmv vector control group	868	0	138,585	144,680	259	0	0	0	0	0
2		866	0	1900	1015	0	0	0	0	0	0
3		890	0	17,694	1541	0	0	0	0	0	0
		Average	0	52,726	49,078	86	0	0	0	0	0
1	BoHV-1qmv Sub-RVFV vaccine group	865	0	241,080	10,871	0	0	0	0	0	0
2		860	0	10,032	13,810	0	0	0	0	0	0
3		875	0	5913	111,590	0	0	0	0	0	0
4		862	0	2868	945	0	0	0	0	0	0
5		867	0	5673	200,640	0	0	0	0	0	0
6		876	0	48,049	12,677	57	0	0	0	0	0
7		879	0	22,754	27,315	0	0	0	0	0	0
8		828	0	35,225	5859	77	0	0	0	0	0
		Average	0	46,449	47,963	16	0	0	0	0	0

Table S3. Bovine herpesvirus type 1 (BoHV-1)-specific neutralizing antibody titer in serum.

S no.:	Group	Animal #	BoHV-1-specific neutralizing antibody titer in serum (Days post-vaccination)					
			Day 0	Day 7	Day 14	Day 21	Day 28	Day 33
1	BoHV-1qmv vector control group	868	<4	17.57	46.11	83.05	66.64	*
2		866	<4	11.59	39.27	53.66	20.92	16.40
3		890	<4	15.78	43.39	55.17	26.6	20.97
		Average	<4	14.98	42.92	63.96	38.05	18.66
1	BoHV-1qmv Sub-RVFV vaccine group	865	<4	18.84	61.37	94.35	28.62	*
2		860	<4	22.12	70.45	103.6	41.63	39.62
3		875	<4	<4	5.67	13.12	12.5	*
4		862	<4	7.43	8.80	16.53	10.91	9.94
5		867	<4	11.63	20.96	25.01	15.44	10.19
6		876	<4	7.42	9.03	14.73	11.61	7.93
7		879	<4	4.11	5.26	11.40	8.15	*
8		828	<4	<4	6.02	11.07	10.04	*
		Average	<4	11.93	23.44	36.23	17.36	16.92

* - Sacrificed on day 28 post vaccination

Table S4. Rift Valley fever virus (RVFV)-specific neutralizing antibody titer in serum.

S no.:	Group	Animal #	RVFV-specific neutralizing antibody titer in serum (Days post-vaccination)					
			Day 0	Day 7	Day 14	Day 21	Day 28	Day 33
1	BoHV-1qmv vector control group	868	<4	<4	<4	<4	<4	*
2		866	<4	<4	<4	<4	<4	<4
3		890	<4	<4	<4	<4	<4	<4
		Average	<4	<4	<4	<4	<4	<4
1	BoHV-1qmv Sub-RVFV vaccine group	865	<4	10.02	13.13	54.71	53.98	*
2		860	<4	9.87	11.39	26.45	14.19	9.75
3		875	<4	4.09	5.36	13.02	10.34	*
4		862	<4	4.06	5.64	5.93	9.42	9.00
5		867	<4	5.79	6.49	8.17	7.67	6.29
6		876	<4	5.54	6.3	7.77	12.02	32.72
7		879	<4	5.66	6.48	10.95	15.67	*
8		828	<4	6.64	7.61	10.95	7.57	*
		Average	<4	6.46	7.68	17.24	16.34	14.44

* - Sacrificed on day 28 post vaccination

Table S5. Fold change in interferon (IFN)-gamma mRNA expression in peripheral blood mononuclear cells collected from immunized calves upon stimulation with heat-inactivated Rift Valley fever virus antigen.

S no.:	Group	Animal #	Fold change in IFN-gamma mRNA expression after PBMC stimulation (Days post-vaccination)		
			Day 0	Day 7	Day 14
1	BoHV-1qmv vector control group	868	1	1.01	1
2		866	1	0.87	1.13
3		890	1	1	0.9
		Average	1	0.96	1.01
1	BoHV-1qmv Sub-RVSV vaccine group	865	0.97	15.6	7.5
2		860	1	8.6	2
3		875	1	11.2	3.5
4		862	1	4.6	3.7
5		867	1	16.40	3.31
6		876	1	5.74	3.46
7		879	1	2.51	2.03
8		828	1	2.07	3.55
		Average	1	7.30	3.08