

BVDV-2 E2

5' - KpnI

hEF1- α promoter

GGTACCCTCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTG
GGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTTCG
TGTAAGTGCAGTAGTCGCCCGTGAACGT
TCTTTTTCGCAACGGGTTTTCGCCCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTC
TTTACGGGTTATGGCCCTTGCCTTGAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCCG
AGCTTCGGGTTTGAAGTGGGTGGGAGAGTTTCGAGGCCTTGCCTTAAGGAGCCCTTCGCCTCGTGCTTG
AGTTGAGGCCTGGCCTGGGCGCTGGGGCCCGCGTGCGAATCTGGTGGCACCTTCGCCTGTCTCGCT
GCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATA
GTCTTGTAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTTGGGGCCCGGGCGGCGACGGGGC
CCGTGCGTCCCAGCGCACATGTTTCGGCGAGGCGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGG
TAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCCGCTGTATCGCCCCGCCCTGGGCGG
CAAGGCTGGCCCGGTGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAG
CTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGGAGTCAACCCACACAAAGGAAAAGGGCCTTT
CCGTCTCAGCCGTGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCT
CGAGCTTTTGGAGTACGTCGTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTTCCCCACACTGA
GTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTTCTCCTTGGAAATTTGCCCTTTTTGAG
TTTTGGATCTTGGTTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTTCTTCCATTTTCAGGTGTCGT
GAGGAATTAGCTTGGTACTAATACGACTCACTATAGGGAGACCCAAGCTGGCTAGGTAAGTGTACGAGCT
CGATCACTAGTCCAGTGTGG**atcgat**CGCCGCCACCATGCAGGGACCAACACTGGCCGTGCTGGGGGCTC

ClaI

Kozak

BHV-1 gD signal sequence

TGCTGGCTGTGGCTGTCTCCCTGTTCCCGAGTGCAAGGAAGGATTT**CAGTACGCCATCAGCAAGGACCG**
GAAAATTGGACCACTGGGACCAGAGTCCCTGACCACAACCTGGCACCTGCCACCAAGAAAATCGTGGAC
TCTATGGTGCAGGTCTGGTGCATGGCAAGAACCTGAAAATTTCTGGAGACATGTACTAAGGAGGAGAGAT
ACCTGGTGGCTGTCCACGAGCGCGCTCTGTCTACCAGTCCGAGTTCATGCAGATCAGCTCCGGAACAAA
GGGCCCTGAAGTGATCGACATGCACGACGATTTTCAATTTGGCCTGTGCCCTGTGATAGTAAGCCTGTG
ATGCGCGGAAAATTCACGCTTCACTGCTGAATGGCCCTGCCTTTCAGATGGTGTGCCACAGGGGTGGA
CCGGAACAATCGAGTGTATTCTGGCTAACCAGGACACACTGGATAACCACAGTGGTCCGGACTTACCGGAG
GACTACCCCTTTTCAGCGCAGAAAGTGGTGCACCTATGAGAAAATCATTGGCGAGGACATCCACGAGTGC
ATCCTGGGCGGGAATTGGACCTGTATCACAGGCGACCATTCTAAGCTGAAAGATGGGCCAATTAAGAAAT
GCAAGTGGTGTGGCTACGACTTCTTTGATAGTGAGGGACTGCCTCATTATCCAATCGGCAAATGTATGCT
GTCAAACGAAAGCGGGTACAGATATGTGGACGATACTAGCTGCGATCGAGGAGGAGTGGCTATCGTCCCA
ACTGGGACCCTGAAGTGTAGGATCGGAAAAGCTACCGTGCAGGTCATTGCCACAAATACTGACCTGGGAC
CAATGCCTTGCTCCCCAGATGAAGTGATCGCTTCTGAGGGACCTGTCGAAAAGACTGCCTGTACCTTCAA
CTACTCCAAGACACTGCCAAACAAGTACTATGAGCCCCGAGACCGGTACTTCCAGCAGTATATGCTGAAG
GGGAATGGCAGTACTGGTTTGACCTGGATAACCGTGGACCACCATAAGGATTACTTCTCAGAGTTTATCG
TGATTGCCGTGGTCTGCTGCTGGGGGAAAGTACGTGCTGTGGCTGCTGGTACCTATATGATCCTGAG
TGAACAGATGGCCATGGGC**atgcat**ggtaagcctatccctaaccctctcctcgggtctcgattctacgcgt

NsiI

V5 epitope

accggt**CATCATCACCATCACCAT**TGA****GTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCC

6xHis

Stop

BGH Poly A

AGCCATCTGTTGTTTGGCCCTCCCCGTGCCTTCTTGACCCTGGAAGGTGCCACTCCCCTGTCCTTTC
CTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGG
CAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTT

GGTACC-3'

KpnI

Supplementary Fig. 1. Nucleotide sequence of chimeric BVDV-2 E2 gene cassette. BVDV-2 E2 chimeric ORF sequence contained within ClaI and NsiI sites were designed to include 5'-3' the following: Kozak sequence, codon optimized for Bos Taurus (Genscript) nucleotide sequence of predicted BHV-1 gD signal sequence (GenBank accession # AFV53430.1, aa 1-19), BVDV E2-2 coding

nucleotide (GenBank accession # AAC72814.1). The 1.195 bp ClaI/NsiI fragment was cloned into the corresponding ClaI/NsiI sites of the pPreBVDV-E2 chimeric vector (sequence file 1) resulting in BVDV-2 E2 chimeric gene expression cassette. BVDV-2 E2 sequence is indicated by uppercase underlined and with green letters.

5'- NotI

CGGGCCGCaggcaacggggcctgctcccgcgcaggagcacgtggtgctcaagatcggggcctcggcctct

BHV-1 gG flanking upstream

acgctggccgaggctatgctactgcaaaccttggaccacgccaacgtggtcaagctgaaggccgtgctct
tccacggggagctggtgtgctggtgctggcgcgtaccgcgaggacctgcacacgcacctctggagaat
caaccgcccgtggcgtccccgcggcgtggcggtagcgcgggcccgtgctgcgggcctcgcgctacctg
cactcccgcggatcgctcaccgggacgtcaaaacggaaaacgtcttctcaacggcccaggcgacgtgt
gcctgggcgactttggcgcggcacacgggcccgtcaccgagccccgctactacggcctggccggcacct
ggagacgaactcgccagagctgctggcgcgcgcgctacgactgccgcacggacgtgtggagcgcgggc
gtcgtcgcgtaacgagatgctggcatacccgcgcgcgtgttgcacagccccgcgggcccgcagggag
acgccgagcatcgggcccgcgcacgatcttgggcgaccgcgactgcccggcagctgctcccgctgat
tcgccggtggcgtgcacgcgaagagtttccacccagccccactgaccggtgacccgcaacttcaag
cgccacgcgagcagcgcgcgagagccgcacagcccgtaccgctgctggcggtgctccggctgccctgcg
acgccgaccgcctcctacaccagatgctgacctttgactttcgcgcgcgccccaccgcccgcggagctgct
ggagcaccctctctcggtgcccctcgggtagccccgggggttcccgcaaaactgaggcataataagg
cgcgggacaccggaagtttggcatccacacttcgcgctgtggacacgagagcgaacgcgagcgaacgcga
gcgcaagcgcgagcacacgactgcgatc **tacaataaacagGGTACC** TAGTTATTAATAGTAATCAATTAC

Chimeric Us3/Poly A KpnI CMV promoter

GGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGC
TGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGA
CTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCA
TATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATG
ACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGT
TTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGGATTTCCAAGTCTCCACCCCATTGA
CGTCAATGGGAGTTTGTTTTGGCACCAAATCAACGGGACTTTCCAAATGTTCGTAACAACCTCCGCCCA
TTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGTC
AGATCCGCTAGCGCTACCGGTGCCGCCACCATGCAGGGACCTACTCTGGCTGTGCTGGGGCTCTGCTGG

Kozak

BHV-1 gD signal

CTGTGGCTGTCTCTCTGGAAAACATTACTCAGTGGAAATCTGATGGACAATGGCACCGAAGGGATCCAGCA

BVDV 2 E^{ms}

GGCCATGTTTCTGCGAGGCGTGAACCGGTCTCTGCACGGGATCTGGCCTGAGAAGATTTGCACCGGCGTG
CCAACACATCTGGCCACTGACTACGAGCTGAAGGAAATTGTCCGGATGATGGATGCTAGTGAGAAAACTA
ATTATACCTGCTGTCGCCTGCAGAGACACGAATGGAACAAGCATGGGTGGTGTAAATGGTTCCACATCGA
GCCATGGATTTGGCTGATGAACAAAACACAGAACAATCTGACTGAGGGACAGCCACTGAGGGAATGCGCT
GTGACCTGTCGCTACGACAAGGAGACCGAACTGAATATCGTCACACAGGCTCGGGATAGGCCTACCACAC
TGACCGGCTGCAAGAAAGGGAATACTCAGCTTTGCCGGCGTGATCCTGGACGGACCATGCAACTTCAA
GGTGAGCGTCGAGGACGTGCTGTTCAAGGAACACGATTGTGGAAACATGCTGCAGGAAACAGCCATCCAG
CTGCTGGATGGCGCTACAAATACTATTGAGGGAGCTCGAGTGGGAACCGCTAAGCTGACTACCTGGCTGG
GCAAACAGCTGGGAATCCTGGGCAAGAAACTGGAGAACAAGTCTAAAGCCTGGTTTGGGGCCCATGCTAT

GM-CSF

GGCCCCACTAGACCCCCTAACACCGCTACACGACCTTGGCAGCACGTGGACGCCATTAAGGAGGCTCTG
AGCCTGCTGAACCATAGCTCCGACACTGATGCCGTGATGAATGACACCGAGGTGGTCTCCGAAAAGTTTG
ATTCTCAGGAGCCCACCTGTCTGCAGACACGGCTGAAGCTGTACAAAACGGGCTGCAGGGATCACTGAC
CAGCCTGATGGGATCCCTGACTATGATGGCTACCCACTATGAGAAGCATTGCCACCCACACCTGAAACT

AGTTGTGGCACCCAGTTCATCAGCTTCAAGAACTTCAAAGAAGACCTGAAAGAGTTCCTGTTTATTATTC
CATTTGACTGTTGGGAGCCTGCCAGAAG*gactacaagacgatgacgacaag***TAACTGATCATAATCAG**
Flag epitope **Stop** SV40 terminator
CCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACAT
AAAAATGAATGCAATTGTTGTTGTTAACTTGTATTATGCAGCTTATAATGGTTACAAATAAAGCAATAGCA
TCACAAATTTACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGT
ATCTTAAAGCTTggacgcttcgcgcggtgcgccctggccctcttgacatggcggagacggtggtgccc
HindIII BHV-1 gG flanking downstream
ggcggaccgcgagccgcccagcgtcgtcgcgctcggctgggcttaccaagacggggactgcatggtgcctc
tggcatatcgccagtaactttaaactgcacggggggcgcgctgcccggccaaaacgtctgcgcggggtctc
tgagaccgcgcatccgcggtggctttggaacctccgactacgcgctctacgggacgctcgctagtagtgcgc
cccggcctgtaacgaccgcccgaacctacatctacttcttgatacggcccagacgacatctacgtgggca
gsgtcacgctcatggtgggcccgcgacatccacaaatacccctgcgggctggaccgagggctcggtgtggc
cctgcaccacaagagcggaccggcccgaacctctgacagaggacgacgccaccggcgactgggctgcggc
tgcttccccgccttgttgaggttgacgcggtgtggggcaacgtaagcgcgcagagctgggctggccg
accgatcgactacgcccagcgaagggggtgaggtcgaagtgcgcgaggacgaagccgggagcgcagcgg
aaacctgccgcaggacgacccccgaccccgacctcgcagattgccggaccgtcgggctctttagcgaagc
gacatgttccggaccgcccagcgggcccgaatcgctgctgatcggcgccgcttgccaaggacgtcctgacgg
tgcccctcaatctgcccggccgctcttacgaggccctgcgaaacgcatcgctggagtgcaactcccg
cccgcgcgagaccggcgacgcagcgggtggtggtgatgtctctccaggagcccgctcgccctgagcgcgc
cccgatgcccgcgccaccgatccggagtttgggctctttggcctgcccgatgaccccgcgctgcggcgcg
gcattctcatcggcctcgcgatcgcctctgctg**ATGCAT**
NsiI – 3'

Supplementary Fig. 2: Nucleotide sequence of chimeric BVDV-2 E^{ns} GM-CSF-Flag gene insertion plasmid (pBVD2-E2.INS). The 4028 bp long nucleotide sequence for the BHV-1 gΔ BVDV2 E^{ns} GM-CSF Flag insertion was assembled using the plasmid pBHV-1 gΔ and pBVDV2 E^{ns} GM-CSF chimeric gene cassette (p). The BHV-1 gΔ BVDV-2 E^{ns} GM-CSF insertion plasmid consisted of the following (5' to 3'): i) NotI restriction site (bold, underlined); ii) BHV-1 gG flanking upstream (lower case); iii) Us3/Us4 polyadenylation signal (lower case, bold, italic); iv) KpnI restriction site (bold, underlined); v) CMV promoter sequence (italic) vi) Kozak sequence (bold); vii) BHV-1 gD signal (highlighted); viii) BVDV-2 E^{ns} coding sequence; ix) GM-CSF coding sequence (underlined, highlighted); x) Flag epitope coding sequence (lower case, italic); xi) Stop codon (TAA) (bold, italic); xii) SV40 terminator (italic, underlined); xiii) HindIII restriction site (bold, underlined); xiv) BHV-1 gG flanking downstream (lower case); NsiI restriction site (bold, underlined).

Supplementary Table 1. **Clinical scoring scheme**

Clinical Scoring criteria	Rectal Temp [°C]	Nasal/Ocular discharge	Lethargy	Diarrhea	Dyspnea	Mucosal or oral lesions	Anorexia	Cough
Normal (0)	< 39.5	None	Normal in attitude and activities	Normal feces	Normal breathing	No nasal or oral lesions	Normal appetite	None
Mild (1)	39.5-39.9	Serous discharge	Moves slowly, head down	Soft, slightly loose feces	Slight difficulty breathing, short and rapid	White colored lesions	Slightly off feed	<3 episodes occasional cough
Moderate (2)	40-40.4	Mucopurulent discharge	Tends to lie down, moves reluctantly	Watery diarrhea	Labored breathing, noticeable abdominal	Red lesions	Moderately off feed	>3 episodes repeated cough
Severe (3)	40.5-40.9	Copious mucopurulent discharge	Stands with difficulty or not at all, little or no interest in surroundings or response to stimuli	Watery and bloody diarrhea	Very labored, grunting or raspy breathing	Bloody, ulcerated lesions	Not eating	NA
(4)	>40.9	NA	NA	NA	NA	NA	NA	NA

Group	Animal #	Serum neutralizing antibody response against BVDV2 125																	
		Days post vaccination/ Days post challenge																	
		0	Mean	7	Mean	14	Mean	21	Mean	28	Mean	34/0	Mean	40/6	Mean	48/14	Mean	54/20	Mean
Control	626	4		4		4		4		4		4		4		512		1,024	
	636	16		8		4		4		4		4		4		1,024		16,384	
	637	4	31	4	30	4	16	4	16	4	10	4	10	4	6	512	1,638	2,048	5,939
	638	4		4		4		4		4		4		4		2,048		2,048	
	647	128		128		64		64		32		32		16		4,096		8,192	
Bovi	627	4		4		4		4		4		8		8		1,024		4,096	
	628	4		4		4		4		64		256		256		32,768		32,768	
	629	4	4	4	4	4	4	4	118	4	91	32	878	64	885	32,768	16,589	16,384	15,565
	632	4		4		4		512		256		2,048		2,048		8,192		8,192	
	635	4		4		4		64		128		2,048		2,048		8,192		16,384	
QMV – BVD2*	630	4		4		4		4		4		4		64		262,144		65,536	
	633	4		4		4		4		4		16		64		65,536		65,536	
	639	4	4	4	4	4	4	4	4	8	5	16	17	64	64	32,768	94,208	16,384	45,056
	640	4		4		4		4		4		32		64		16,384		32,768	

Supplementary Table 2: BVDV-2-specific serum neutralizing antibody response.

		Leukocyte count [10 ³ /uL]													
Group	Animal #	Days post-vaccination/ Days post-challenge													
		0 dpv	Mean	34/0	Mean	38/4	Mean	40/6	Mean	42/8	Mean	45/11	Mean	48/14	Mean
Control	626	14.00		10.10		7.00		5.50		8.4		7.0		7.2	
	636	21.10		10.70		8.50		6.4		5.20		6.1		5.4	
	637	13.50	15.5	13.40	11.58	8.60	8.1	6.60	6.2	7.2	7.74	6.5	7.26	8.9	8.6
	638	19.10		8.50		7.50		6.00		9.9		7.2		9.2	
	647	9.80		15.20		8.90		6.70		8.0		9.5		12.3	
Bovi	627	18.60		13.30		10.00		13.7		14.6		16.7		16.9	
	628	15.70		11.10		10.70		14.2		11.3		11.2		8.8	
	629	17.60	15.94	10.60	13.02	10.50	11.9	14.3	13.54	14.5	13.04	14.6	14.02	13.6	13.18
	632	13.20		15.60		14.70		13.9		13.10		13.2		13.8	
	635	14.60		14.50		13.60		11.60		11.7		14.4		12.8	
QMV-BVD2*	630	14.60		6.90		4.80		4.20		10.9		8.2		8.1	
	633	11.70	13.35	9.20	10.37	6.50	7.42	7.90	8.92	7.8	10.78	8.8	9.82	8.5	9.4
	639	14.70		11.70		8.50		10.7		11.8		11.0		10.5	
	640	12.40		13.70		9.90		12.9		12.6		11.3		10.5	

Supplementary Table 3: Leukocyte counts in animals for each treatment group at indicated days post –vaccination and post-challenge.

Group	Animal #	Nasal shedding of BVDV-2 challenge virus - Days post-challenge (virus titer; PFUs/ml).									
		0	Mean	4	Mean	6	Mean	8	Mean	11	Mean
Control	626	0		5		90		25		0	
	636	0		40		45		115		5	
	637	0	0	0	48	5	140	45	66	0	1
	638	0		185		555		125		0	
	647	0		10		5		20		0	
Bovi	627	0		0		0		0		0	
	628	0		0		0		0		0	
	629	0	0	0	0	0	0	0	0	0	0
	632	0		0		0		0		0	
	635	0		0		0		0		0	
QMV – BVD2*	630	0		5		35		0		0	
	633	0		0		0		0		0	
	639	0	0	0	1.25	0	8.75	0	0	0	0
	640	0		0		0		0		0	

Supplementary Table 4: BVDV-2 nasal virus shedding following challenge

Group	Animal #	BVDV copies/ng of total RNA (log10) - Days post challenge									
		0	Mean	4	Mean	6	Mean	8	Mean	11	Mean
Control	626	1.443		5.109		150.985		18.140		4.298	
	636	1.064		15.965		865.052		2660.792		136.072	
	637	1.570	1.362	10.307	13.302	186.098	372.260	551.371	677.602	78.710	47.184
	638	1.370		18.677		486.931		108.324		10.519	
	647	1.367		16.454		172.237		49.386		6.324	
Bovi	627	0.703		2.098		2.334		1.806		1.713	
	628	1.958		1.793		2.645		2.597		1.790	
	629	0	1.158	1.156	1.712	0.672	2.294	1.617	2.276	1.154	1.739
	632	1.698		1.826		3.000		3.602		2.134	
	635	1.434		1.690		2.820		1.762		1.907	
QMV – BVD2*	630	1.0337		13.926		222.848		20.044		2.171	
	633	0.819		2.534		2.288		1.753		1.520	
	639	1.104	1.023	2.982	5.881	2.103	57.971	1.613	6.523	1.675	1.726
	640	1.138		4.082		4.645		2.685		1.541	

Table 5: BVDV-2 viremia after challenge.

Group	Animal number	Rectal temperature (°C)																					
		Days post vaccination / Days post challenge																					
		0	2	4	6	9	14	21	28	34/0	36/2	37/3	38/4	39/5	40/6	41/7	42/8	43/9	44/10	45/11	46/12	47/13	48/14
Control	#626	38.9	39.6	38.4	38.8	39.3	39.2	38.8	38.7	39.4	39.3	39.8	39.3	39.6	39.2	39.5	40.5	39.8	39.7	39.6	39.2	39.0	38.8
	#636	39.7	39.2	39.3	39.6	39.2	39.6	38.8	39.3	40.1	39.9	40.0	40.1	39.6	40.0	39.7	40.7	40.7	41.3	39.9	39.7	39.2	39.0
	#637	39.1	38.4	38.7	38.5	39.4	39.1	38.6	39.2	39.5	39.1	39.5	39.4	39.1	39.7	40.0	40.9	39.7	40.2	39.0	38.7	38.7	38.8
	#638	39.4	39.1	38.6	38.6	38.7	38.6	38.1	38.1	40.3	39.8	40.0	39.9	39.7	39.4	40.5	40.8	39.8	39.7	38.8	38.6	38.9	38.7
	#647	39.4	39.0	38.8	39.1	38.8	39.2	39.2	38.4	39.0	39.8	39.7	39.7	39.2	39.8	39.7	40.1	39.7	39.9	39.3	38.9	38.9	38.9
Bovi	#627	39.8	39.3	39.7	39.3	39.2	38.7	38.9	38.5	39.2	39.1	39.4	39.3	39.2	39.1	40.3	39.1	39.2	39.6	39.2	38.4	38.7	38.9
	#628	40.2	39.6	40.0	39.7	40.2	39.3	38.8	39.2	39.8	39.6	40.0	40.1	39.7	39.6	39.0	39.1	39.4	39.6	39.4	38.9	39.1	38.9
	#629	38.6	39.1	39.4	39.3	39.4	38.9	38.9	39.2	39.8	39.8	39.7	39.8	39.2	39.2	38.8	39.0	38.9	39.4	38.8	38.8	38.7	38.9
	#632	40.5	39.0	39.4	39.6	39.1	39.2	36.7	39.0	39.6	39.7	39.1	39.2	39.4	39.0	38.8	38.8	39.1	39.3	39.2	38.7	38.8	38.6
	#635	39.9	39.7	39.1	39.2	39.6	39.3	39.1	38.9	38.5	39.4	39.2	39.2	39.2	39.2	38.9	38.8	39.2	39.1	38.9	38.9	39.2	38.9
QMV-BVD2*	#630	38.9	39.0	39.1	37.6	38.5	38.8	38.8	39.2	39.1	38.9	39.3	39.6	39.8	39.6	38.9	40.0	38.9	39.1	38.7	38.6	38.6	38.6
	#633	39.4	39.4	38.8	38.9	38.9	39.3	38.6	38.6	39.6	39.4	39.3	39.3	39.2	39.4	39.2	38.8	39.1	39.0	38.7	38.7	38.7	38.6
	#639	39.7	39.6	39.1	39.0	39.2	38.8	38.4	39.0	39.3	38.9	39.1	39.2	39.2	39.2	38.8	38.8	38.9	39.2	38.8	38.7	38.7	38.9
	#640	39.5	39.2	39.0	39.1	39.1	39.1	38.7	38.7	39.5	39.2	39.7	39.2	39.0	40.0	39.2	38.6	38.7	39.2	38.8	38.6	38.7	38.8

Supplementary Table 6. Rectal temperatures recorded after the challenge.

	Score	Rectal temperature (°C)
Normal	0	<39.5
Mild	1	39.5 – 39.9
Moderate	2	40 – 40.4
Severe	3	40.5 – 40.9
	4	>40.9

Group	Animal number	Clinical signs score																					
		Days post vaccination / Days post challenge																					
		0	2	4	6	9	14	21	28	34/0	36/2	37/3	38/4	39/5	40/6	41/7	42/8	43/9	44/10	45/11	46/12	47/13	48/14
Control	#626	0	1	0	0	0	0	0	0	0	0	1	0	1	0	1	7	3	3	2	1	1	1
	#636	1	0	0	1	0	0	0	0	2	1	2	2	1	2	1	5	6	9	4	7	5	1
	#637	0	0	0	0	0	0	0	0	1	0	1	0	0	1	2	5	3	5	1	2	1	0
	#638	0	0	0	0	0	0	0	0	2	1	2	1	1	0	3	6	7	4	3	2	1	0
	#647	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	4	4	5	2	0	2	0
Bovi	#627	1	0	1	0	0	0	0	0	0	0	1	0	0	0	2	0	0	1	0	0	0	0
	#628	2	1	2	1	2	0	0	0	1	1	2	2	1	1	0	0	0	1	0	0	0	0
	#629	0	0	0	0	0	0	0	0	1	1	3	1	0	0	0	0	0	0	0	0	0	0
	#632	3	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	#635	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
QMV-BVD2*	#630	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	2	0	0	0	0	0	0
	#633	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	#639	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	#640	1	0	0	0	0	0	0	0	1	0	1	0	0	2	0	0	0	0	0	0	0	1

Supplementary Table 7. Clinical scores recorded after the challenge.

Supplementary Table 8. Lung lesion scores

Treatment	Calf Number	Section	Bronchi	Parenchyma	Septa + Pleura
Control	636	Aa	3	2	2
		Ab	2	1	1
		Ac	0	0	1
Control	647	Ga	3	1	2
		Gb	2	0	2
		Gc	3	1	1
Bovi	635	Ma	1	0	1
		Mb	3	1	2
		Mc	1	0	0
		Md	1	1	1
Control	637	Sa	2	1	2
		Sb	2	0	1
QMV-BVD2*	630	Ya	2	0	0
		Yb	1	0	1
Control	638	AE a	0	0	1
		AE b	2	0	1
		AE c	2	0	1
Bovi	628	AK a	1	0	0
		AK b	1	2	1
		AK c	1	1	1
		AP	3	1	2
QMV-BVD2*	639	AQ a	0	1	0
		AQ b	1	0	2
		AQ c	1	0	1
Bovi	632	AW a	1	1	1
		AW b	3	1	2
Bovi	629	BI a	2	0	1
		BI b	2	1	1
Control	626	BO a	0	0	0
		BO b	3	3	3
		BO c	1	0	0
QMV-BVD2*	640	BT a	2	0	2
		BT b	1	0	1
Bovi	627	BZ a	2	0	2
		BZ b	2	1	1
QMV-BVD2*	633	CF a	0	0	1
		CF b	1	1	1
		CF c	1	1	1