

Supplemental Table S1. Clinical Scoring Criteria

Clinical score	Rectal temperature	Respiratory symptoms	Mental state	Nasal secretions	Diarrhea symptoms
Normal	≤39.5℃	Normal breathing	Normal status	none	Normal stool
Mild	39.6~40.0℃	slight difficulty breathing	Head down, moving infrequently	Serous nasal fluid	Stools are soft and slightly runny
Moderate	40.1~40.5℃	Dyspnea, obvious abdominal breathing, with cough	Lying down, reluctance to move	Purulent nasal fluid	Watery diarrhea
Severe	40.6~41.5℃	Grunting or harsh breathing	Difficulty standing and unresponsive to stimuli from people and the environment	Massive mucopurulent nasal fluid	Watery bloody diarrhea

Supplemental Table S2. BoHV-1 shedding of nasal swabs in guinea pigs after challenge

[illegible]

[illegible]

guinea pigs after challenge

[illegible]

Supplemental Table S4. White blood cell count in guinea pigs
after challenge

Group and guinea pigs number	Challenge days																
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
BVDV-1 immunization group	1	7.4	6.1	7.3	7.6	6.3	6.8	6.8	6.8	7.0	7.4	6.5	7.1	7.9	8.8	8.8	8.3
	2	5.2	7.8	6.6	5.8	7.2	4.9	5.8	7.2	8.6	6.5	6.0	7.2	9.0	6.5	8.8	10.0
	3	8.0	7.7	7.3	7.0	7.1	6.7	6.8	6.9	6.7	7.1	7.5	7.3	6.9	7.9	8.0	6.6
	4	7.6	6.5	7.2	8.0	7.2	6.9	6.7	7.2	7.6	8.2	6.3	6.4	7.0	6.7	6.2	7.3
	5	6.8	7.2	7.0	7.3	6.8	6.0	6.4	6.8	7.1	6.7	7.5	6.9	6.3	6.4	6.2	6.0
BoHV-1 gE/E2-Linker-E2 ⁺ immunization group	6	8.3	7.9	6.9	6.9	7.2	8.2	7.6	6.9	7.9	7.4	7.6	8.6	8.4	8.2	7.2	7.5
	7	7.6	8.0	8.5	7.7	8.3	7.0	7.6	8.6	8.4	7.9	7.6	10.9	8.1	8.5	7.8	8.4
	8	8.5	8.6	8.6	7.6	7.3	6.4	7.2	7.1	8.6	8.1	7.8	7.8	7.6	8.2	7.9	8.6
	9	9.1	8.2	10.6	10.1	9.2	9.3	8.5	9.0	9.0	7.7	8.6	8.2	9.6	8.8	8.3	8.7
	10	7.6	7.2	6.9	6.9	7.1	7.2	8.1	7.7	7.4	7.3	6.9	8.2	7.7	7.9	7.5	7.4
Challenge control group	11	8.2	7.4	7.1	8.2	8.0	7.5	7.6	7.8	7.4	6.9	6.8	7.0	7.0	7.2	7.5	7.7
	12	8.1	7.6	6.9	8.0	6.9	6.8	7.2	7.5	7.9	6.9	7.4	7.0	6.8	6.7	7.1	8.0
	13	7.5	6.9	8.2	8.5	8.3	8.2	6.0	6.2	6.4	5.8	8.5	9.7	6.6	6.7	7.1	7.6
	14	6.3	7.2	7.5	7.6	10.3	7.7	5.8	5.7	5.5	5.5	8.2	6.8	7.5	6.4	6.9	8.0
	15	8.2	6.5	7.2	6.3	9.7	8.0	6.1	4.5	7.2	6.5	8.3	7.8	9.4	7.9	8.1	6.6
Blank control group	16	7.7	7.4	7.0	7.7	7.5	8.1	6.2	6.2	6.2	6.0	6.9	7.1	7.7	7.4	7.3	7.8
	17	7.6	8.0	6.8	7.2	7.4	6.9	6.2	6.4	6.4	6.5	6.8	6.8	7.2	7.4	7.4	7.4
	18	6.9	7.5	7.3	8.0	8.1	7.6	6.0	6.1	6.1	7.3	6.5	6.9	7.0	7.0	7.2	7.2
	19	8.1	8.5	8.1	9.6	8.3	7.4	7.2	8.2	7.3	7.2	9.3	7.5	10.1	10.1	10.5	10.5
	20	5.6	5.1	6.4	6.2	6.5	5.3	6.1	7.5	6.6	7.2	5.9	5.8	6.2	8.3	8.5	6.6
	21	9.7	8.7	9.0	9.2	9.1	7.8	9.0	6.6	6.9	6.1	6.7	5.4	9.9	9.1	10.0	9.2

Supplemental Table S5. clinical score in calves after challenge

Group and calves number		Challenge days																	
			-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	Blank control group	101	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
		102	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
		103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Challenge control group	104	0	0	0	0	3	2	2	2	2	3	2	2	2	2	2	2	2
		105	0	0	0	0	0	0	2	2	2	2	4	3	3	3	3	2	0
		106	0	0	0	0	2	0	0	0	3	3	3	4	4	3	3	3	2
BoHV-1	BoHV-1 immunization group	107	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		108	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0
		109	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BoHV-1 Δ gE immunization group	110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		111	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
		112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BVDV-1	BoHV-1 immunization group	113	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
		114	0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0	0
		115	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BVDV-1 immunization group	116	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		117	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
		118	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
	BoHV-1 immunization group	119	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		120	0	0	0	0	0	2	0	1	0	1	0	0	1	0	0	0	0
		121	0	0	0	0	0	0	0	0	0	0	0	2	2	1	0	0	0
	Challenge control group	122	0	0	0	0	0	0	2	2	3	3	3	3	2	3	2	2	2
		123	0	0	0	0	0	0	2	3	3	3	4	4	2	2	3	2	2
		124	0	0	0	0	0	0	2	2	3	3	3	2	3	2	2	0	0

Supplemental Table S6. BoHV-1 shedding of nasal swabs in
calves after challenge

Group and calves number		Challenge days														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Challenge control group	104	-	-	+	+	+	-	+	-	+	+	+	+	-	-	-
	105	-	-	+	+	+	-	+	+	+	+	+	+	-	-	-
	106	-	-	+	+	+	-	+	+	+	-	+	+	-	-	-
BoHV-1 immunization group	107	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-
	108	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
	109	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-
BoHV-1 Δ gE immunization group	110	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-
	111	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
	112	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-
BoHV-1 gE/E2-Linker-E2 ⁺ immunization group	113	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-
	114	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-
	115	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-

Supplemental Table S7. BVDV-1 shedding of nasal swabs in
calves after challenge

Group and calves number		Challenge days														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
BVDV-1 immunization group	116	-	+	-	-	-	-	-	-	+	+	-	+	-	-	-
	117	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-
	118	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-
BoHV-1 gE/E2-Linker-E2 ⁺ immunization group	119	-	+	-	-	-	+	+	+	+	+	-	-	-	-	-
	120	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-
	121	-	+	-	-	-	+	+	+	+	+	-	-	-	-	-
Challenge control group	122	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-
	123	-	-	-	-	+	+	+	+	+	+	-	+	-	+	+
	124	-	+	-	+	-	+	+	+	+	-	+	+	-	-	-

Supplemental Table S8. White blood cell count in calves after challenge

Group and calves number		Challenge days																
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
BVDV-1 immunization group	116	10.1	10.1	10.5	11.5	10.2	8.2	4.7	4.9	5.1	12.0	8.3	7.4	7.2	7.5	8.5	8.1	9.6
	117	6.2	8.3	8.5	13.6	10.3	7.7	6.0	4.5	4.4	5.4	6.5	5.3	4.9	5.6	5.1	6.4	6.2
	118	9.9	9.1	10.0	12.1	9.7	8.0	6.1	6.9	6.1	7.2	7.0	7.8	9.0	9.7	8.7	9.0	9.2
BoHV-1 gE/E2-Linker-E2 ⁺ immunization group	119	7.4	6.1	7.3	5.6	6.3	6.8	6.8	6.8	7.0	7.4	6.5	7.1	7.9	8.8	8.8	8.3	9.2
	120	10.2	9.8	10.2	10.0	10.5	10.2	10.1	10.1	10.5	11.8	10.6	8.5	10.2	11.0	9.6	9.7	9.6
	121	8.0	7.7	7.3	7.0	7.1	6.7	6.3	6.2	6.7	7.1	7.5	6.3	6.9	7.9	8.0	6.3	6.9
Challenge control group	122	10.5	10.6	10.2	11.5	9.7	12.1	10.5	9.2	9.2	7.3	7.0	9.7	10.5	8.6	10.6	9.8	9.7
	123	12.3	10.2	11.5	10.5	9.8	11.4	9.8	10.8	9.2	8.9	9.0	9.8	11.5	10.4	9.5	11.2	9.6
	124	9.8	11.5	10.2	12.1	11.3	10.6	10.0	9.2	9.3	7.9	8.0	11.3	9.2	12.6	8.7	10.6	10.0
Blank control group	101	10.2	8.3	8.6	9.5	8.5	8.6	10.6	8.7	9.2	9.1	8.8	9.3	9.9	10.6	12.6	12.5	10.2
	102	8.9	8.4	8.6	8.4	9.0	9.2	8.6	8.9	9.2	9.1	8.8	9.3	9.1	12.3	14.5	10.3	11.3
	103	9.2	9.8	10.5	10.5	12.3	11.5	10.2	9.8	9.3	11.5	12.6	13.4	10.5	9.8	9.8	8.9	10.5

Supplemental Table S9. Lung Histology score in guinea pigs
after challenge BoHV-1

Pathological changes		Bronchi		
Group		Percentage of	Degree of infiltration	Interstitial pneumonia
and guinea pig number		infiltrated sites		
Challenge control group	25	2	2	3
	26	3	3	3
	27	2	1	2
	28	2	2	2
	29	2	2	2
	30	1	1	1
	31	1	0	1
	32	1	0	0
BoHV-1 immunization group	33	0	0	0
	34	1	1	0
	35	0	0	0
	36	0	0	0
	37	0	0	1
	38	0	0	1
	39	0	0	0
	40	0	0	0
BoHV-1 Δ gE immunization group	41	1	2	1
	42	0	1	1
	43	0	0	0
	44	0	0	0
	45	0	0	0
	46	1	0	1
	47	1	0	1
	48	1	0	0
Blank control group	19	1	0	0
	20	0	0	0

21	1	0	0
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Supplemental Table S10. Lung Histology score in calves after
challenge BoHV-1

Group and calf number	Pathological changes	Bronchi		Interstitial pneumonia
		Percentage of infiltrated sites	Degree of infiltration	
Challenge control group	104	3	2	2
	105	2	2	2
	106	1	2	1
BoHV-1 immunization group	107	0	0	0
	108	1	1	0
	109	0	0	0
BoHV-1 Δ gE immunization group	110	0	1	0
	111	0	0	0
	112	0	0	0
BoHV-1 gE/E2-Linker-E2 ⁺ immunization group	113	0	0	0
	114	0	0	0
	115	0	0	0

Supplemental Table S11. Small intestine Histology score in calves after challenge BVDV-1

Group and calf number	Pathological changes	
	Small intestine	
Challenge control group	122	2
	123	3
	124	3
BVDV-1 immunization group	116	0
	117	0
	118	1
BoHV-1	119	0
gE/E2-Linker-E2 ⁺	120	1
immunization group	121	1