

Electronic Supplementary Material

Coexistence of multiple genotypes of porcine epidemic diarrhea virus with novel mutant S genes in the Hubei Province of China in 2016

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S1. PEDV-positive samples among those collected from 34 farms in 6 districts in the Hubei Province of China

Location	Farms	Sample types	PEDV positives/total	Total positives
Ezhou	Farm 1	Feces	3/6	8/31(25.8%)
	Farm 2	Feces	0/5	
	Farm 3	Feces	5/5	
	Farm 4	Feces	0/5	
	Farm 5	Feces	0/5	
	Farm 6	Feces	0/5	
Huanggang	Farm 7	Feces	4/5	28/29(96.6%)
	Farm 8	Intestine	2/2	
		Feces	10/10	
	Farm 9	Intestine	2/2	
Feces		10/10		
Xiangyang	Farm 10	Intestine	5/5	14/40(35%)
	Farm 11	Intestine	2/2	
	Farm 12	Intestine	2/2	
	Farm 13	Intestine	3/3	
		Fecal Swab	0/10	
		Intestine	0/1	
	Farm 14	Feces	0/3	
		Intestine	0/2	
		Feces	0/5	
	Farm 15	Intestine	1/1	
		Fecal Swab	1/3	
		Fecal Swab	0/3	
	Jingzhou	Farm 18	Feces	
Farm 19		Feces	3/3	
Farm 20		Feces	0/4	
Farm 21		Feces	4/4	
Farm 22		Feces	2/4	
Farm 23		Feces	2/2	
Farm 24		Feces	2/4	
Farm 25		Feces	3/3	
Farm 26		Feces	1/3	
Yichang		Farm 27	Feces	0/5
	Farm 28	Feces	0/5	
	Farm 29	Feces	0/6	
	Farm 30	Feces	0/6	
	Farm 31	Feces	1/7	
	Jingmen	Farm 32	Feces	0/4
Farm 33		Fecal Swab	0/5	
Farm 34		Fecal Swab	4/5	
Total				74/172(43%)

S2. New amino acid mutations in the spike proteins of field strains in Hubei in 2016

Virus Straines Mutant amino acid sites in spike Mutation aa numbers in S, S1, and S2

	27	59	70	102	104	112	114	117	118	124	129	133	177	190	227	231	270	289	300	305	306	325	336	358	364	386	413	431	479	505	526	567	597	622	636	638	641	655	672	674	686	756	812	830	865	928	982	1000	1021	1047	1060	1090	1110	1254	1377	1387	S1	S2	Total		
HBJM-1	A	Q	Q	P	G	A	N	T	N	H	P	T	V	D	I	P	L	L	I	S	I	D	V	I	S	H	I	G	F	H	D	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	I	L	Q	V	4	1	5
HBJM-2	A	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	S	I	D	V	I	S	H	I	G	F	H	D	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	I	L	Q	V	3	1	4
HBHG-1	S	Q	Q	P	G	A	N	T	N	R	P	V	E	I	P	L	L	M	S	I	D	V	I	S	H	I	V	L	H	A	T	H	S	L	F	S	E	T	T	F	A	V	I	T	A	T	V	D	M	E	Q	H	T	V	L	Q	E	4	2	6	
HBJM-3	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	S	M	D	V	I	S	H	I	V	F	H	V	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	N	V	L	Q	V	2	2	4	
HBZJ-2	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	S	M	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V	0	0	0
HBXY-5	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	S	M	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	I	S	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V	2	0	2
HBHG-6	S	H	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	S	M	D	V	I	S	H	I	V	F	H	A	T	H	S	L	L	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V	2	0	2
HBXY-4	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	S	M	D	V	I	S	H	I	V	F	A	I	H	S	L	F	P	G	T	T	F	A	V	I	K	T	T	V	D	L	E	Q	H	T	V	L	Q	V	4	2	6	
HBZJ-1	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	M	S	I	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V	0	0	0
HBXY-1	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	M	S	I	D	V	I	A	H	I	V	L	H	A	T	H	Y	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V	3	0	3
HBXY-2	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	M	S	I	D	V	I	A	Y	I	V	L	H	A	T	R	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	G	L	E	Q	H	T	V	L	Q	V	4	0	4
HBHG-2	S	Q	Q	P	A	N	T	N	R	H	T	V	D	I	L	L	M	S	V	D	I	I	S	H	L	V	F	H	A	T	H	S	M	F	F	E	K	T	F	S	V	I	T	A	T	V	D	M	E	Q	H	T	V	H	Q	V	9	2	11		
HBHG-3	S	Q	Q	P	A	N	T	N	R	H	T	V	D	I	L	L	M	S	I	D	V	I	S	H	L	V	F	H	A	T	H	S	L	F	P	E	K	T	F	S	V	I	T	A	T	V	D	M	E	Q	H	T	V	L	Q	V	6	1	7		
HBHG-4	S	Q	Q	P	A	N	T	N	R	H	T	V	D	I	P	L	L	M	S	I	D	V	I	S	H	L	V	F	H	A	T	H	S	L	F	P	E	K	T	F	S	V	I	T	A	T	V	D	M	E	Q	H	T	V	L	Q	V	5	1	6	
HBHG-5	S	Q	Q	P	A	N	T	N	R	P	T	V	D	I	P	L	L	M	S	I	D	V	I	S	H	L	V	F	H	A	T	H	S	L	F	P	E	K	T	F	S	V	I	T	A	T	V	D	M	E	Q	H	T	V	L	Q	V	3	1	4	
HBXY-3	S	Q	Q	S	G	A	N	T	N	R	P	T	V	D	I	P	V	L	V	S	M	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	P	I	I	T	A	T	V	D	L	Q	H	T	V	L	Q	V	4	1	5	
HBEZ-1	S	Q	Q	P	G	A	N	T	N	R	P	T	L	D	I	L	L	I	S	I	D	V	V	S	R	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	T	A	N	I	D	L	E	Q	H	T	V	L	Q	V	5	2	7		
HBYC-1	Q	-	S	P	G	P	D	A	G	R	P	T	V	D	I	P	L	L	I	S	M	D	V	I	S	H	I	V	F	H	A	T	Y	S	L	F	P	E	T	T	F	A	V	I	T	S	T	V	D	L	E	Q	H	T	V	L	Q	V	6	0	6
HBEZ-4	Q	-	G	P	G	A	N	H	N	R	P	T	V	D	I	P	L	L	I	L	M	Y	V	I	S	H	I	V	F	H	A	T	R	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	H	H	T	V	L	H	V	2	2	4
HBEZ-3	Q	-	G	P	G	A	N	H	N	R	P	T	V	D	I	P	L	L	I	L	M	Y	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	H	H	T	V	L	Q	V	2	1	3
HBEZ-2	Q	-	G	P	G	A	N	H	N	R	P	T	V	D	I	P	L	L	I	S	M	Y	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	H	H	T	V	L	Q	V	1	1	2
YC2014	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	M	S	I	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V			
CH-QTC-01-2015	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	M	S	I	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V			
HB-HA2015	S	Q	Q	P	G	A	N	T	N	R	P	T	F	D	I	P	L	L	I	S	I	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V			
US/Coloreador/2013	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	S	I	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V			

Continued

Mutant amino acid sites in spike

Mutation
aa
numbers
in S, S1,
and S2

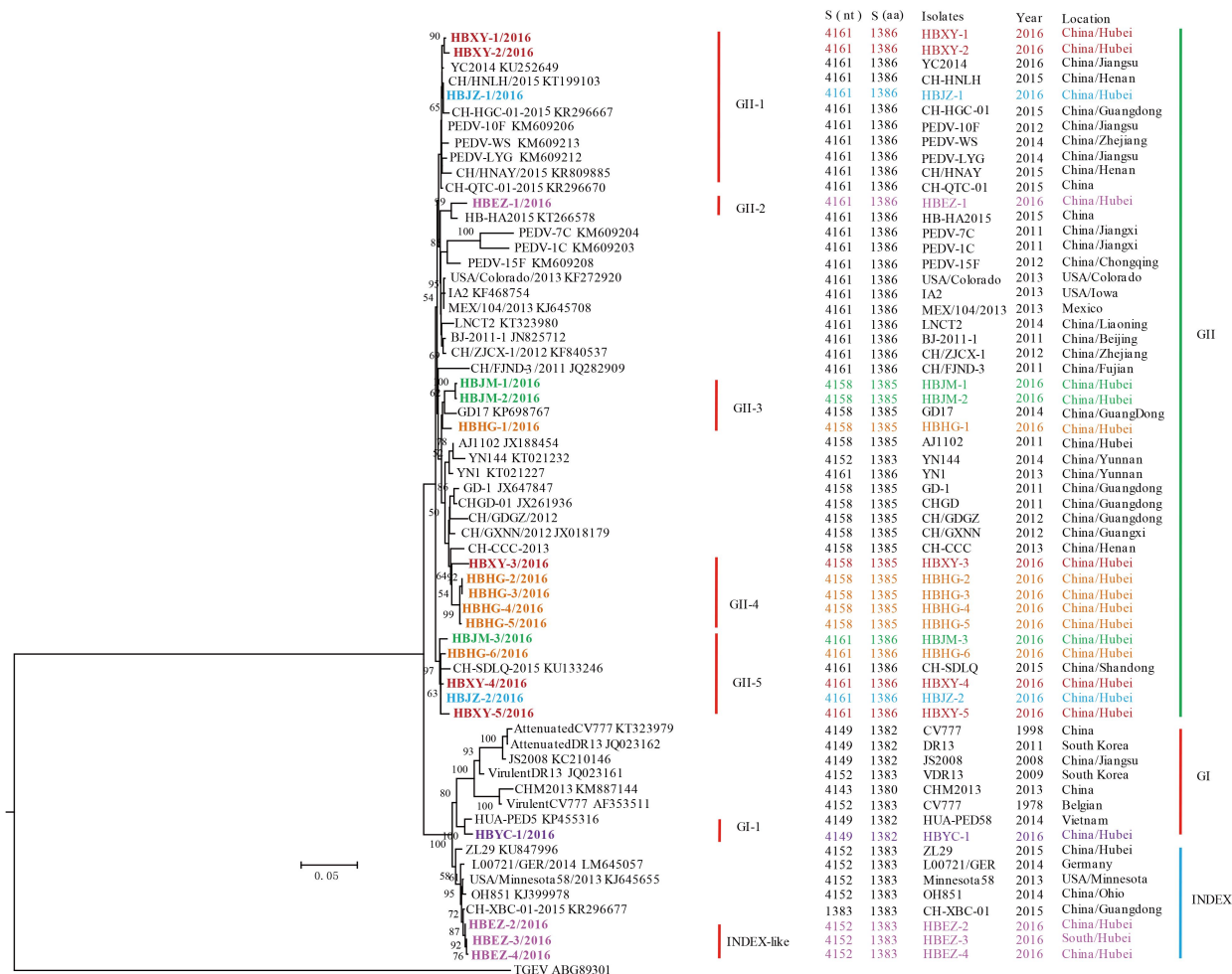
Virus Straines	27	59	70	102	104	112	114	117	118	124	129	133	177	190	227	231	270	287	289	300	305	306	308	325	336	358	364	386	413	431	479	505	526	567	597	622	636	638	641	655	672	674	696	756	812	830	865	928	992	1000	1021	1047	1060	1090	1110	1254	1377	1387	S1	S2	Total
IA2	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	S	I	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V			
GD17	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	S	M	D	V	I	S	L	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V			
CHGD-01	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	S	I	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V			
CH-SDLQ-2015	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	S	M	D	V	I	S	H	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V				
CH-CCC-2013	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	M	S	I	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V		
CH-HGC	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	M	S	I	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V		
CH-XBC-01-2015	Q	-	G	P	G	A	N	H	N	R	P	T	V	D	I	P	L	L	I	S	M	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V			
OH851	Q	-	G	P	G	A	N	H	N	R	P	T	V	D	I	P	L	L	I	S	M	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V			
HUA-PED58	Q	-	G	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	S	M	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V			
ZL29	Q	-	G	P	G	A	N	H	N	R	P	T	V	D	I	P	L	L	I	S	I	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V			
Virulent CV777	Q	-	G	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	S	M	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	I	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V			
AttenuatedDR13	Q	-	G	P	G	A	N	T	S	R	P	T	V	D	I	P	L	W	I	S	M	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	Q	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V			
AttenuatedCV777	Q	-	G	P	G	A	N	T	S	R	P	T	V	D	I	P	L	W	I	S	M	D	V	I	S	H	I	V	F	H	S	T	H	S	L	F	P	Q	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V			
Consensus	S	Q	Q	P	G	A	N	T	N	R	P	T	V	D	I	P	L	L	I	S	I	M	D	V	I	S	H	I	V	F	H	A	T	H	S	L	F	P	E	T	T	F	A	V	I	T	A	T	V	D	L	E	Q	H	T	V	L	Q	V		

Note: Viral strains and new amino acids changes found in spikes of field strains in Hubei 2016 and consensus amino acids were in red.

S3. Nt and aa pairwise identities of S genes and the encoded spike proteins

S4. A fasta format file of the nt pairwise alignment of S genes

S5. A fasta format file of the aa pairwise alignment of spike proteins



S6. Phylogenetic analysis of full-length amino acid sequences of spike proteins of PEDV and transmissible gastroenteritis virus (TGEV). The phylogenetic tree was constructed based on the maximum likelihood method using a Poisson model under 1000 replicates of bootstrap values; for each node, bootstraps $\geq 50\%$ are shown. The scale bar represents 0.005 substitutions per amino acid. The strain names, isolation years and places, and GenBank accession numbers are shown. S (nt) and S (aa) indicate the complete length of the nucleotide and amino acid sequences of the S genes and S proteins, respectively. The results for the GI, GII, and INDEX-like genogroups were inconsistent with those of previous studies; subgroups shown in the figure were proposed in this study for better description of the genetic diversity of spike proteins. Spike sequences detected in this study are colored and in bold. The TGEV was used as an out-group.