VIROLOGICA SINICA

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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Supporting information to DOI: 10.1007/s12250-017-4021-8

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	
	Farm 14	Fecal Swab	0/10	
		Intestine	0/1	
		Feces	0/3	
	Farm 15	Intestine	0/2	
		Feces	0/5	
	Farm 16	Intestine	1/1	
		Fecal Swab	1/3	
	Farm 17	Fecal Swab	0/3	
Jingzhou	Farm 18	Feces	2/2	19/29(65.5%)
	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	1/29(3.4%)
	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	4/14(28.6%)
	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	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
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	**R**	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	**F**	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	**Q**	**N**	V	L	Q	V	2	2	4
HBJZ-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	**F**	I	S	M	D	V	I	S	H	I	V	F	**Y**	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
HBJZ-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**	A	N	T	N	R	**H**	T	V	D	I	**L**	L	L	M	S	**V**	D	**I**	I	S	H	**L**	V	F	H	A	T	H	S	**M**	F	P	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**	A	N	T	N	R	**H**	T	V	D	I	**L**	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**	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**	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	**D**	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	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	**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/Colorado/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

To be continued

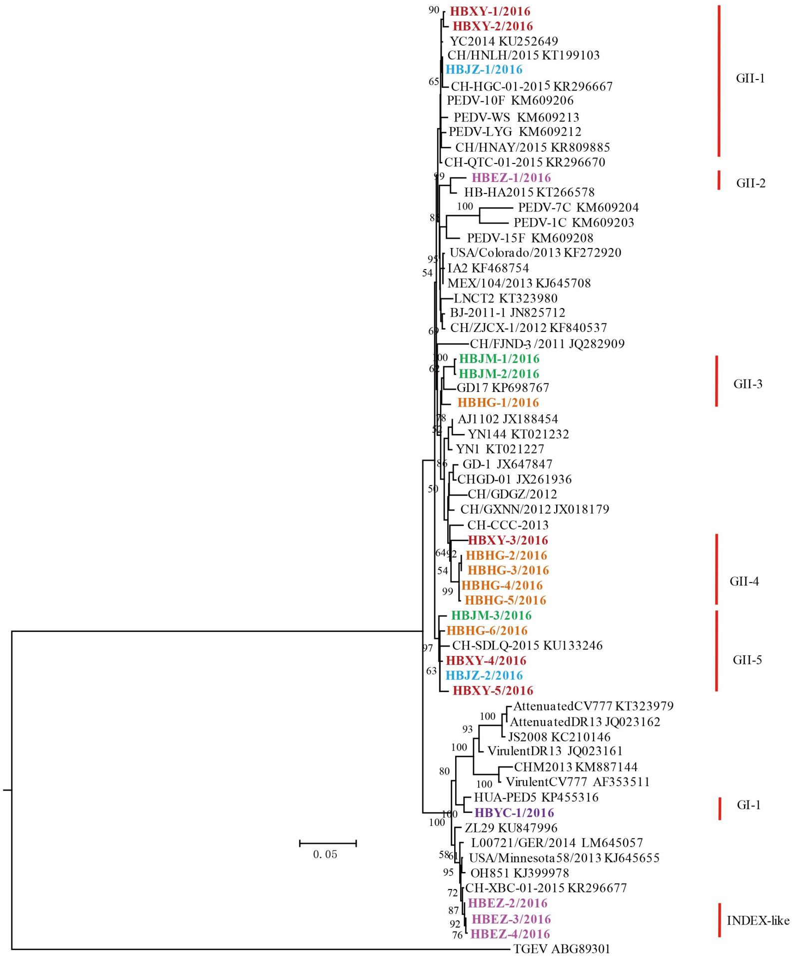
Virus Straines	Mutant amino acid sites in spike																																																									Mutation aa numbers in S, S1, and S2		
	27	59	70	102	104	112	114	117	118	124	129	133	177	190	227	231	270	287	289	300	305	306	308	325	336	358	364	386	413	431	479	505	526	567	597	622	636	638	641	655	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	V	F	H	A	T	P	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	M	S	I	D	V	I	S	H	I	V	F	H	A	T	P	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	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	L	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



S (nt)	S (aa)	Isolates	Year	Location	
4161	1386	HBXY-1	2016	China/Hubei	GII
4161	1386	HBXY-2	2016	China/Hubei	
4161	1386	YC2014	2016	China/Jiangsu	
4161	1386	CH-HNLH	2015	China/Henan	
4161	1386	HBJZ-1	2016	China/Hubei	
4161	1386	CH-HGC-01	2015	China/Guangdong	
4161	1386	PEDV-10F	2012	China/Jiangsu	
4161	1386	PEDV-WS	2014	China/Zhejiang	
4161	1386	PEDV-LYG	2014	China/Jiangsu	
4161	1386	CH/HNAY	2015	China/Henan	
4161	1386	CH-QTC-01	2015	China	
4161	1386	HBEZ-1	2016	China/Hubei	
4161	1386	HB-HA2015	2015	China	
4161	1386	PEDV-7C	2011	China/Jiangxi	
4161	1386	PEDV-1C	2011	China/Jiangxi	
4161	1386	PEDV-15F	2012	China/Chongqing	
4161	1386	USA/Colorado	2013	USA/Colorado	
4161	1386	IA2	2013	USA/Iowa	
4161	1386	MEX/104/2013	2013	Mexico	
4161	1386	LNCT2	2014	China/Liaoning	
4161	1386	BJ-2011-1	2011	China/Beijing	
4161	1386	CH/ZJCX-1	2012	China/Zhejiang	
4161	1386	CH/FJND-3	2011	China/Fujian	
4158	1385	HBJM-1	2016	China/Hubei	
4158	1385	HBJM-2	2016	China/Hubei	
4158	1385	GD17	2014	China/GuangDong	
4158	1385	HBHG-1	2016	China/Hubei	
4158	1385	AJ1102	2011	China/Hubei	
4152	1383	YN144	2014	China/Yunnan	
4161	1386	YN1	2013	China/Yunnan	
4158	1385	GD-1	2011	China/Guangdong	
4158	1385	CHGD	2011	China/Guangdong	
4158	1385	CH/GDGZ	2012	China/Guangdong	
4158	1385	CH/GXNN	2012	China/Guangxi	
4158	1385	CH-CCC	2013	China/Henan	
4158	1385	HBXY-3	2016	China/Hubei	
4158	1385	HBHG-2	2016	China/Hubei	
4158	1385	HBHG-3	2016	China/Hubei	
4158	1385	HBHG-4	2016	China/Hubei	
4158	1385	HBHG-5	2016	China/Hubei	
4161	1386	HBJM-3	2016	China/Hubei	
4161	1386	HBHG-6	2016	China/Hubei	
4161	1386	CH-SDLQ	2015	China/Shandong	
4161	1386	HBXY-4	2016	China/Hubei	
4161	1386	HBJZ-2	2016	China/Hubei	
4161	1386	HBXY-5	2016	China/Hubei	
4149	1382	CV777	1998	China	GI
4149	1382	DR13	2011	South Korea	
4149	1382	JS2008	2008	China/Jiangsu	
4152	1383	VDR13	2009	South Korea	
4143	1380	CHM2013	2013	China	
4152	1383	CV777	1978	Belgian	
4149	1382	HUA-PED58	2014	Vietnam	
4149	1382	HBYC-1	2016	China/Hubei	
4152	1383	ZL29	2015	China/Hubei	INDEX
4152	1383	L00721/GER	2014	Germany	
4152	1383	Minnesota58	2013	USA/Minnesota	
4152	1383	OH851	2014	China/Ohio	
1383	1383	CH-XBC-01	2015	China/Guangdong	
4152	1383	HBEZ-2	2016	China/Hubei	
4152	1383	HBEZ-3	2016	South/Hubei	
4152	1383	HBEZ-4	2016	China/Hubei	

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 ≥ 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.