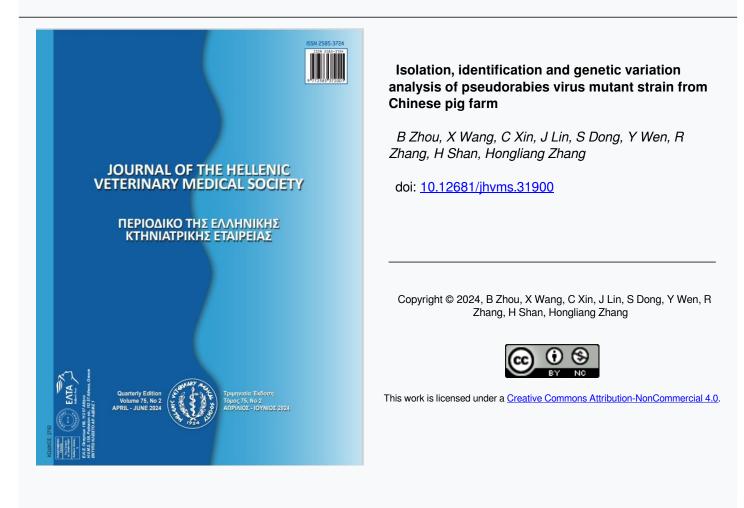




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Isolation, identification and genetic variation analysis of pseudorabies virus mutant strain from Chinese pig farm

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ABSTRACT: Pseudorabies (PR) is an important infectious disease affecting pig farms worldwide. In this study, we reported a pseudorabies virus (PRV) isolated from a Bartha-K61-vaccinated pig farm in Linyi, Shandong Province, China. Evidence from virus isolation, electronic microscope observation \Box laboratory animal infection, and histopathologic examination confirmed that the etiological agent of the disease is PRV SD-2017. Sequence alignment of the gE gene indicated that it belongs to a new mutated PRV strain. The gB sequence alignment showed that compared with the Bartha and Kaplan genotype 1 strains, SD-2017 strain had a deletion of three amino acids at positions 75-78 (SPG) and an insertion of one amino acid at position 94 (G). The gC gene had 7 amino acids (A, A, A, S, T, P and A) inserted at positions 69-75 in comparison with the Bartha-K61 vaccine strain. The median lethal dose (LD50) of PRV SD-2017 strain on rabbits was $3.16 \times 10^{2.0}$ TCID50 by Karber method. Histopathologic examinations showed that multiple lesion sites were observed in brains, lungs, livers and kidneys. PRV SD-2017 is different from other reports and should be paid more attention to avoid economic losses.

Keywords: Pseudorabies virus; Sequence alignment; Amino acid mutations; Mutant strain

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INTRODUCTION

seudorabies virus (PRV), also known as Suid Herpesvirus 1 (SuHV1), belongs to the family Herpesviridae. It is the causative agent of pseudorabies (PR), also known as Aujeszky's disease (AD) (Mettenleiter, 2000). PRV can infect pigs (the only natural host), ruminants such as cattle and sheep, carnivores such as mink, and rodents, with a mortality rate close to 100% (Pensaert, 1989). The disease is an acute, severe, highly contagious infectious disease that causes fever, extreme itching (except in pigs) and encephalomyelitis. PRV is mainly transmitted through the exchange of saliva between animals, nasal secretions and particulate matter in the air (Wozniakowski and Samorek-Salamonowicz, 2015). PRV can be transmitted in multiple species and can infect humans, posing a public health safety risk (Wong et al., 2019). Typical symptoms of PRV infection in humans include weakness, fever, sweating, difficulty swallowing, and neurological disorders. Available case studies reported that PRV infection can cause endophthalmitis and encephalitis in humans (Ai et al., 2018; Yang et al., 2019; Yang et al., 2019).

PR had been popular since the early 1970s and spread almost globally after 1980(Freuling et al., 2017; Müller et al., 2011). At present, PR is still one of the important infectious diseases that need to be controlled in the pig breeding industry worldwide, especially in areas with intensive breeding (Müller et al., 2011). Since the introduction and application of Hungary's Bartha-K61 strain vaccine, the PR epidemic in China had been controlled relatively stable. However, since the emergence of PRV variants in 2011, PR outbreaks had occurred in pig farms that had been immunized with the Bartha-K61 vaccine in many provinces and regions in China (Wu et al., 2017; Yu et al., 2014). Bartha-K61 vaccines showed reduced protection against the variant, although this view was controversial (An et al., 2013; Gu et al., 2015). PRV variants have spread in northern, eastern and southern China, which seriously threatens the healthy and sustainable development of the pig breeding industry (Sun et al., 2018; Zhai et al., 2019).

In this study, PRV detection and virus isolation were performed on specimens collected from a pig farm suspected of having a PR outbreak in Linyi, Shandong, China. The PRV virus was successfully isolated from PK-15 cells, and the isolated SD-2017 strain was proved to be a PRV variant by sequencing analysis. Through the genetic variation analysis of gE, gB and gC virulence genes, it was found that there were different degrees of variation in the main virulence genes of PRV SD-2017 strain. This study lays the foundation for the follow-up study of PRV virus characteristics and the development of new genetically engineered vaccines.

MATERIALS AND METHODS

Clinical samples

Six piglets at 2 weeks of age suspected of porcine pseudorabies infection in a pig farm in Linvi Shandong province, China, were sent for inspection, in Dec.2017. The main symptoms of piglets were fever, anorexia, depression, and then appear shivering, ataxia, hind limb paralysis, intermittent spasms, limb scratching and other neurological symptoms. Some piglets appeared vomiting, diarrhea symptoms. Samples (brain, lung, spleen, kidney, and inguinal lymph nodes) were collected after necropsy. Samples for viral isolation were stored at -80°C until use. 2~3 g of homogenized tissue samples were frozen and thawed three times and were centrifuged at 5000×g for 10 min at 4°C. The resulting supernatant was filtered by a needle type filter (0.22 µm) and stored at -80°C until use (An et al., 2013).

Cell Lines and Virus

The Porcine kidney cell line (PK-15, ATCC CCL-33) was used to culture PRV. PK15 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, USA) supplemented with 10% Fetal Bovine Serum (FBS, Gibco, USA) at 37°C in 5% CO2 a humidified incubator. PRV Bartha-K61 vaccine strain and pMD19-T vector were preserved by the Key Laboratory of Preventive Veterinary Medicine of Shandong Province.

Viral genome extraction and PCR

DNA was extracted from the above treated clinical samples supernatant by extraction kit (TIANGEN, China). Referring to the PRV TJ strain in GenBank (accession No. KJ789182.1) and literature (Fan et al., 2016; Yu et al., 2016; Zhou et al., 2019), specific primers (Table 1) for amplifying the whole genes of PRV gE, gD, TK, gB, gC, gI and gM were designed by Primer Premier5.0 (Premier, Canada). Primers were synthesized by Shanghai Sangon Co., Ltd. The amplification was performed in a 50 μ I reaction mixture containing 2.5U PrimeSTAR HS DNA Polymerase (TaKaRa, Beijing), 2×PrimeSTAR GC Buffer (Mg²⁺plus), 2.5 mM of each dNTP, 10 μ M of each primer, and 200 ng of DNA. The reaction was run in a thermocycler with the following program: denaturation at 98 °C for 1 min; followed by 35 cycles of 98 °C for 10 s, gE,gB,TK and gC 60 °C for 5 s, gM 68 °C for 2min, gI 55 °C for 15 s, and 72 °C for 2 min; and a final extension at 72 °C for 5 min. The PCR products were subjected to electrophoresis on 1% agarose gel. The all target genes was recovered and ligated into the pMD19-T vector, transformed into DH5 α competent cells, and positive clones were selected. The plasmids were extracted by High Pure Maxi Plasmid Kit (TIANGEN, China) from the positive clones and sent to Shanghai Sangon Co., Ltd for sequencing.

Virus isolation and purification

PK-15 cells were subcultured, and when the confluence reached about 70% in T25 cell flasks, 1.0 mL of the processed supernatant of clinical samples was inoculated with PK-15 cells for virus isolation and culture. The cells were incubated at 37 °C and examined daily for cytopathic effect (CPE). The supernatants were harvested and used to infect fresh PK-15 cells again when CPE was observed. The second passage PRV was plaque-purified six times by virus plaque formation (Gu *et al.*, 2015). The virus was designated as PRV SD-2017. PRV Bartha-K61 strain was used as a control for plaque-purification, which was obtained from Shandong Huahong Biological Engineering Co., LTD (Huahong).

Observation of virus morphology

The purified PRV was seeded into PK-15 cells at 70% confluence in T25 cell flasks. Cultured at 5% CO2 and 37° C for $48{\sim}56$ h. The culture supernatant

was harvested, and then the virus was purified use the ultracentrifuge (Beckman, L-80XP, Germany) by sucrose density gradient centrifugation (55%, 45%, 35%, 25%, 15% gradient sucrose solutions were added in sequence). The purified samples were sent to the Central Laboratory of Qingdao Agricultural University for transmission electron microscopy (Hitachi, HT7700, Japan) observation.

One-step growth kinetics of PRV

One-step growth kinetics was conducted as described previously (Smith and Enquist, 1999). PK-15 cells were grown in 96-well plates to 80% confluence and infected with PRV SD-2017 strain at the multiplicity of infection (MOI) of 1.0. At 4 h, 8 h, 12 h, 24 h, 36 h, 48 h, 60 h, and 72 h postinfection (hpi), the supernatants were collected and titrated by a microtitration infectivity assay and recorded as TCID50/ ml. The TCID50 of the virus was calculated by Reed-Muench method. All assays were performed in triplicate, and the resulting titers were averaged.

Phylogenetic analyses of PRV

We analyzed PRV SD-2017 sequence data and compared the gE, gB and gC genes with the sequences available in the GenBank database (Table 2). Lasergene sequence analysis software MegAlign (DNASTAR, Madison, WI, USA) was used to perform multiple sequence alignments and phylogenetic analyses. In detail, sequences were added in MegAlign and aligned using clustal W method. Phylogenetic tree was constructed using the neighbor-joining method with the MEGA program (ver. 5.05) and bootstrap

Table 1 PRV primer seq	uence information	
Primers	Primer sequences (5'-3')	Amplification size(bp)
PRV-gE-for	TCGCACACCCGGGGTTGAG	1734
PRV-gE-rev	GGTGGGCATGTCGGAATG	1/54
PRV-gD-for	ATGCTGCTCGCAGCGCTATT	1220
PRV-gD-rev	TACTGCGGAGGCTACG	1220
PRV-TK-for	ATGCGCATCCTCCGGATCTACCT	963
PRV-TK-rev	TCACACCCCCATCTCCGACGTGAA	905
PRV-gB-for	TGTACCTGACCTACGAGGCGTCATGC	2742
PRV-gB-rev	TATTTCCATCTGCGGGGGGGGGGGGCTA	2772
PRV-gC-for	GGATCCATGGCCTCGCTCGCGCGTGCGAT	1440
PRV-gC-rev	GAATTCTCACAGCGCGGACCGGCGGTAGT	1440
PRV-gI-for	ATGATGATGGTGGCGCG	1101
PRV-gI-rev	TTATTGTTCCTCTGCGATGGT	1101
PRV-gM-for	ACCATGTGCGATCGAAACGAA	1182
PRV-gM-rev	GGTCGCAACCGTATAGCATC	1102

Table 2 Backgro	und information in the NCBI d	atabase of reference	PRV strains		
PRV isolates	GenBank accession No.	Country	Year	Variant (Yes/No)	Species
Bartha	JF797217	Hungary	1960	No	Swine
SC	KT809429	China	1986	No	Swine
Ea	KU315430	China	1990	No	Swine
LA	KU552118	China	1997	No	Swine
Hercules	KT983810	Greece	2010	No	Swine
Kolchis	KT983811	Greece	2010	No	Swine
Kaplan	JF797218	Hungary	2011	No	Swine
BJ/YT	KC981239	China	2012	No	Dog
Fa	KM189913	China	2012	Yes	Swine
HN1201	KP722022	China	2012	Yes	Swine
HB1201	KU057086	China	2012	Yes	Swine
HeN1	KP098534	China	2012	Yes	Swine
TJ	KJ789182	China	2012	Yes	Swine
ZJ01	KM061380	China	2012	Yes	Swine
HNB	KM189914	China	2012	Yes	Swine
HNX	KM189912	China	2012	Yes	Swine
JS-2012	KP257591	China	2012	Yes	Swine
HLJ8	KT824771	China	2013	No	Swine
HLJ-2013	MK080279	China	2013	No	Swine
GD0304	MH582511	China	2015	Yes	Swine
MY-1	AP018925	Japan	2015	Yes	Swine
RC1	LC342744	Japan	2016	No	Racoon
HeNLH/2017	MT775883.1	China	2017	Yes	Swine
HuBXY/2018	MT468549.1	China	2018	Yes	Swine
SY7	MT150583.1	China	2018	Yes	Swine
JSY13	MT157263.1	China	2018	Yes	Swine
hSD-1/2019	MT468550.1	China	2019	Yes	Human
GD1802	MT949535.1	China	2020	Yes	Swine
SD18	MT949536.1	China	2020	Yes	Swine
HuB17	MT949537.1	China	2020	Yes	Swine

Table	2	Backgrou	nđ	info	ormation	in	the	NCBI	database	of	reference	PRV	strains
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analyses were conducted using 1,000 replicates (Fan *et al.*, 1994). Phylogenetic tree was viewed using phenogram mode as described (Fauquet *et al.*, 2008).

Infection of rabbits with PRV

Three-month old healthy SPF rabbits (weight $2\sim2.5$ kg) were randomly divided into six groups each with five. Groups 1-4 were injected intramuscularly with $1\times10^{2.0}$ TCID50, $1\times10^{3.0}$ TCID50, $1\times10^{4.0}$ TCID50 and $1\times10^{5.0}$ TCID50 of PRV SD-2017 strain in 500 µl DMEM, respectively. Group 5 was injected with 500 µl PBS. Clinical signs were checked by the state registered veterinarian daily. After the challenge, rabbits with serious clinical symptoms of depression, pruritus and anorexia at the same time were judged as the humanitarian endpoint. Rabbits were euthanized by intravenous injection of 100 mg/kg sodium pentobarbital and the death was counted as a statistical result. All surviving rabbits were humanely euthanized on day 7 post-challenge. The median lethal

dose (LD50) was calculated and determined using the Karber method (Zhang *et al.*, 2021). The brain, lungs, livers and kidneys tissue samples were collected from each rabbit to detect PRV using the gE-specific PCR as mentioned above. The paraffin imbedding tissue samples were cut into 4 μ m-thick sections, stained with HE, and pathological changes were observed under an optical microscope. All rabbit experiments were performed in the standardized animal room. In accordance with the protocols approved by the Animal Care and Ethics Committee, under the number 201907003, which adhere to the "Guidelines for ethical review of Laboratory Animal Welfare" (GB/T 35892-2018).

RESULTS

PCR amplification of PRV partial genes

We amplified the main PRV virulence genes gE, gD, TK, gB, gC, gI and gM respectively, and ligated

into the pMD19-T vector (Fig.1). The main genomic information of gE, gD, TK, gB, gC, gI and gM were published in GenBank (Acc. No. MW535259-MW535265).

PRV isolation and purification

The filtrate of tissue supernatant identified as positive for PRV gE gene by PCR was inoculated into PK-15 cells cultured in T25 cell flasks. At 36 hours post infection, obvious CPE was observed in SD-2017 infected PK-15 cells (Fig.2A), and similar CPE was seen in positive control cells seeded with PRV Bartha-K61 (Fig.2B), whereas no CPE was observed in control PK-15 cells (Fig.2C). The culture was successively passaged for 6 times, all of which could produce obvious CPE, and the cell cultures of each passage were all positive for PRV gE gene by PCR (data not shown).

Observation of virus morphology

After the PK-15 cell culture of the sixth-generation PRV SD-2017 strain was concentrated by ultracentrifugation, many obvious virus particles were found in transmission electron microscopy (Fig. 3). Virus particles were spherical, with a diameter of about 140-180 nm. There was a thick capsule with radial fibers on the surface of the capsule. And the particle core was dense, with the typical virion structure of herpes virus.

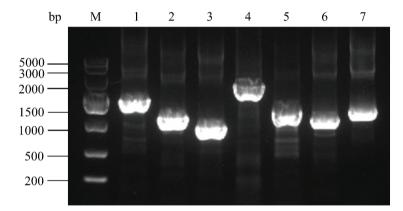


Fig.1. PCR amplification of PRV virulence genes.



Fig.2. Obvious CPE produced in PK-15 cells infected with PRV strains (36h).

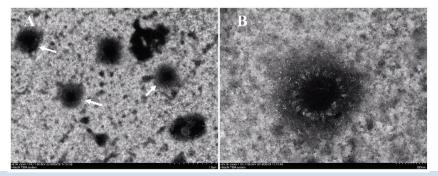


Fig.3. Electron microscopic observation of virus particle morphology (A.8000×, B.40000×).

One-step growth curves of PRV

The virus titer of PRV SD-2017 strain measured by Reed-Muench method at 48h had reached $10^{9.0}$ TCID50/ml (Fig. 4). This indicated that PRV SD-2017 strain had good propagation characteristics in PK-15 cells.

Phylogenetic analyses of PRV

We uploaded the sequencing results of the main virulence genes gB, gE, gC, gD, TK, gI and gM of PRV SD-2017 strain to the GenBank under the accession numbers MW535259, MW535262, MW535260, MW535261, MW535265, MW535263 and MW535264. We compared the gE, gB, and gC gene of PRV SD-2017 with other PRV strains available in the GenBank database (Table 2). Compared with genotype I isolates, SD-2017 strain had insertion and substitution mutations at the gE amino acid site. Compared with the classic strains Kaplan, Hercules and Kolchis, SD-2017 strain had amino acid (D) inserted at positions 48 and 497, which was the typical of PRV popular variants. In addition, gE had amino acid site substitutions at positions 54 (G \rightarrow D), 59 (D \rightarrow N), 63 (N→D), 149 (R→M), 179 (T→S), 181 (Q→L), 503 $(A \rightarrow I)$, 503 $(S \rightarrow A)$, 521 $(V \rightarrow A)$ (Fig. 5).

The gB sequence alignment showed that compared with the Bartha and Kaplan genotype 1 strains, SD-2017 strain had a deletion of three amino acids at positions 75-78 (SPG) and an insertion of one amino acid at position 94 (G). In addition, gB gene had amino acid substitution at positions 53 (A \rightarrow T), 55 (P \rightarrow T), 70 (T \rightarrow A), 72 (V \rightarrow G), 73 (P \rightarrow T), 81 (N \rightarrow D), 82 (D \rightarrow G), 83 (V \rightarrow F), 87 (A \rightarrow E), 93 (E \rightarrow D), 96 (F \rightarrow V), 102 (E \rightarrow D), 454 (R \rightarrow K), 553 (G \rightarrow S), 571 (S \rightarrow G) (Fig.6). Compared with genotype I strains, SD-2017 strain and most Chinese isolates gC had 7 amino acids (A, A, A, S, T, P and A) inserted at positions 69-75. However, this variation was absent in GD1802 and HLJ-2013 isolates. In addition, compared with Bartha, Kaplan, Hercules, Kolchis and SC strains, SD-2017 strain and Chinese isolates after 2011 have amino acid substitutions at positions 16 (A \rightarrow T), 25 (T \rightarrow S), 52 (P \rightarrow S), 61(A \rightarrow E), 63(A \rightarrow V), 82(A \rightarrow V), 93(P \rightarrow Q), 96(N \rightarrow G), 108(A \rightarrow S), 136(F \rightarrow V), 148(Y \rightarrow C), 200(G \rightarrow E), 437(L \rightarrow M), 443(V \rightarrow I), 455(A \rightarrow T), 463(S \rightarrow T), 467(V \rightarrow T) and 473(G \rightarrow A) (Fig. 7).

We further analyzed the relationship of PRV SD-2017 strain with other PRV isolates using a phylogenetic tree based on the gE gene. Phylogenetic analysis of PRV gE gene showed that PRV SD-2017 strain was clustered to an independent branch together with recent PRV isolates in China, such as HeNLH/2017 and HLJ8 strains (Fig. 8).

Infection of rabbits with PRV

Two days after infection, itching and nibble at injected position was observed in all rabbits of groups 1-4. Subsequently, nerve symptom appeared. The rabbits were euthanized according to their symptoms. However, the rabbits of group 5 were normal and healthy during the experiment and humanely euthanized on day 7 post-challenge. The results of PCR detection showed that all rabbits of groups 1-4 were PRV SD-2017 strain positive while all rabbits of group 5 were negative. The median lethal dose (LD50) of PRV SD-2017 strain on rabbits was $3.16 \times 10^{2.0}$ TCID50 by Karber method. Histopathologic examinations showed that multiple lesion sites were observed in brains, lungs, livers and kidneys (Fig. 9).

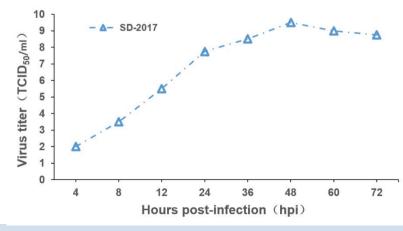


Fig.4. One-step growth curves of PRV SD2017 strain in PK-15 cells.

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201 gE chis_gE wills_gE gE 201 gE 2012_gE 1_gE gE 1_gE f_gE f_gE 1_agE 1_agE 1_agE 1_agE 1_agE 1_agE 1_agE	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.	GDDDDDEE	AGVIRRRP	LPPEVPRLRRE ASPGGDSGY 510	PPIVTPERWS EGPYASLDF 520	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI	JASGPRAVFFV	AVGDRPPAD	AD. AD. AD. AD. AD. AD. AD. AD. AD. AD.	S70	MSR
201_qE chis_gE gE gE 201_qE 2012_qE 1_qE qE qE qE 1_qE gE 1_qE lan_gE 1_qE 1_qE 1_qE 1_qE 1_qE 1_qE 1_qE 1_q	.S. S. S. S. S. S. S. S. VAVTTVCFETACHPD SE.M. TSLPTHEDYYII	GDDDDDEE	AGVIRRRP	ASPGGDSGY 510 A.S	PPIVTPERWS	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI	DASGPRAVFFV EAPRSGFD	AVGDRPPAD	AD AD AD AD AD AD AD AD AD AD AD AD AD A	GVTANRLL	MSR
201_qP chis_qE 0_qE 0_qE 201_qP 201_qP 201_qE 201_qE 1_qE qE qE r_qE 1_q	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.	GDDDDDEE	AGVIRRRP 00 I2	ASPGGDSGY 510 A.S	EGPYASLDF 520 AF	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI	DASGPRAVFFV EAPRSGFD	AVGDRPPAD	AD AD AD AD AD AD AD AD AD AD AD AD AD A	S70	MSR
201_qP chis_qE 201_gE 2012_qP 2012_qP 2012_qP qE qE qE 1_qE 1_qE 1_qE 1_qE 1_qE 1_qE 1_qE 1_qE 1_qE 1_qE 2012_qP 1_qE 2012_qP	.S. S. S. S. S. S. S. S. S. S. S. S. S.))))))))))))))	AGVIRRRP 00 	ASPGGDSGY 510 A. S A. S	EGPYASLDF 520 AF AF AF	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI DDGLYVRPE 540	EAPRSGFD 55	//////////////////////////////////////	AD AD AD AD AD AD AD AD AD AD AD AD AD A	2GVTANRLL 570	MSR MS. MS.
201_gP chis_gE g_g 201_gF 2012_gE 1_gE g_g g_g g_g 1_gE 1_gE 1_gE 1_gE	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.	0GDDDDDEE2 0 5(D. D. D.	AGVIRRRP 00 I I	ASPGGDSGY 510 A.S A.S A.S	EGPYASLDF 520 AF A.F A.F	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI DDGLYVRPE 540	DASGPRAVFFV EAPRSGFD 55(AD AD AD AD AD AD AD AD AD AD AD AD AD A	2GVTANRLL 570 	MSR MS. MS. MS.
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201_gE chis_gE cules_gE 8_gE 201_gE 2012_gE 1_gE gE gE gE 1_gE	.S. S. S. S. S. S. S. S. S. S. S. S. S.))))))))))))))	AGVIRRRP 00 	ASPGGDSGY 510 A.S A.S A.S A.S A.S A.S A.S	EGPYASLDF 520 A.F A.F A.F A.F A.F A.F A.F	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI DDGLYVRPE 540	DASOPRAVPFV EAPRSGFD 55	// / / / / / / / / / / / / / / / / / /	AD AD AD AD AD AD AD AD AD AD AD AD AD A	2GVTANRLLN 570 	MSR MS. MS. MS.
201_gE chis_gE b_gE 201_gE 2012_gE 1_gE gE gE gE 1_gE gE 1_gE gE 1_gE 1_	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.))))))))))))))	AGVIRRRP 00 	ASPGGDSGY 510 A.S A.S A.S A.S A.S A.S A.S A.S	EGPYASLDE 520 A. F A. F A. F A. F A. F A. F A. F A. F A. F	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI DDGLYVRPE 540	EAPRSGFD 55(7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	AD AD AD AD AD AD AD AD AD AD AD AD AD A	(GVTANRLL) 570 	MS. MS. MS. MS. MS.
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201_qP chis_gE 201_qE 201_qP 201_qP 201_qP 201_qP qE qF qF qF qF qF qF qF qF qF qF	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.	0GDDDDDEE2) 5(, D. , D.	M. AGVIRRRP 00 	ASPGGDSGY 510 A.S A.S A.S A.S A.S A.S A.S A.S A.S A.S A.S	EGPYASLDE 520 A. F A. F	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI DDGLYVRPE 540	EAPRSGFD 55(AVGDRPPAPI	AD AD AD AD AD AD AD AD AD AD AD AD AD A	(GVTANRLL) 570 	MS. MS. MS. MS. MS. MS. MS. MS. MS.
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201_qP chis_gE 0_gP 0_gP 201_qP 2012_qP 1_gP gF 1_gP gF 1_gP 1_gP 1_gP 1_gP 1_gP 0_gP 1_gP 0_gP 1_gP 0_gP 1_gP 0_gP 1_gP 0_gO 0	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.))))))))))))))	M. AGVIRRP) 00 	ASPGGDSGY 510 A. S A. S A. S A. S A. S A. A. A. A. A. A. A. A. A. A. A. A. A. A. A	EGPYASLDE 520 A. E A. E	.S.L. .S.S.L. .S.L.L. .S.L.L. .S.L.L. .S.L.L. .S.L.L. .S.L.L. .S.L.L.L. .S.L.L. .S.L.L.L.L	NDTGLYTLHI DDGLYVRPE 540	EAPRSGFD 55	//////////////////////////////////////	AD AD AD AD AD AD AD AD AD AD AD AD AD A	(GVTANRLL) 570 	MSR MS. MS. MS. MS. MS. MS. MS. MS. MS. MS.
201_qE chis_gE cules_gE 8_gE 201_qE 201_qE 201_qE gE 1_gE gE 1_gE gE 1_gE 1_gE 1_gE 1_gE	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.	0 <u>GDDDDDEE5</u> 0 50 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	M	ASPGGDSGY 510 A.S	EGPYASLDF 520 A. F A. FA. F A. F A. F A. F A. F A. F	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI DDGLYVRPF 540	EAPRSGFD 55(AD AD AD AD AD AD AD AD AD AD AD AD AD A	(GVTANRLL) 570 	MS. MS. MS. MS. MS. MS. MS. MS. MS. MS.
201_qE chis_qE chis_qE %_qE %_qE 201_qE 201_qE 201_qE 201_qE qE _qE _qE _qE _qE 1_gE gE _qE 1_gE 1_gE 1_gE 1_gE 000000000000000000000000000000000000	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.	0GDDDDDEE2) 5(, D. , D.	M	ASPGGDSGY 510 A.S.A.S. A.S.A.S. A.S. A.S. A.S. A.A. A. A. A. A. A. A. A. A. A. A. A.	EGPYASLDF 520 	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI DDGLYVRPE 540	PASGPRAVPFV EAPRSGFD 55	//////////////////////////////////////	AD AD AD AD AD AD AD AD AD AD AD AD AD A	(GVTANRLLM 570 	MS. MS. MS. MS. MS. MS. MS. MS. MS. MS.
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201_qE chis_qE cules_gE 8_gE 201_qE 201_qE 201_qE 201_qE qE _qE _qE 1_gE qE 1_gE 1	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.	0 <u>GDDDDDEE5</u> 0 50 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	MM.	ASPGGDSGY 510 A.S	EGPYASLDF 520 AF A.F A.F A.F A.F A.F A.F A.F A.F A.F A.F A.F A.F	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI DDGLYVRPF 540	EAPRSGFD 55(AD AD AD AD AD AD AD AD AD AD AD AD AD A	(GVTANRLL) 570 	MSR MS. MS. MS. MS. MS. MS. MS. MS. MS. MS.
201_qE chis_qE cules_qE 8_gE 201_qE 201_qE 201_qE 201_qE qE _qE _qE _qE _qE 1_gE gE _qE 1_gE 1_gE 1_gE 1_gE 1_gE 1_gE 1_qE 2012_qE 1_qE 1_qE 2012_qE 1_qE 2012_qE 1_qE 2012_qE 1_qE 2012_qE 1_qE 2012_qE 1_qE 2012_qE 1_qE 2012_qE 1_qE 2012_qE 1_qE 2012_qE 1_qE 2012_qE 2012_qE 1_qE 2012_qE 2	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.))))))))))))))	M	ASPGGDSGY 510 A.S.A.S. A.S. A.S. A.S. A.S. A.S. A.S.	EGPYASLDF 520 	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI DDGLYVRPE 540	PASGPRAVFFV EAPRSGFD 55	// / / / / / / / / / / / / / / / / / /	AD AD AD AD AD AD AD AD AD AD AD AD AD A	(GVTANRLLN 570 	MSR MS. MS. MS. MS. MS. MS. MS. MS. MS. MS.
201_gE chis_gE % _gE 201_gE 2012_gE _gE _gE _gE _gE _gE 1_gE gE 1_gE gE 1_gE gE 1_gE 1_	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.	0GDDDDDEE2 0 50 	M. RGVIRRPJ 00 	ASPGGDSGY 510 A. S A. S A. S A. S A	EGPYASLDF 520 A. F A. F	. S. L. . S	NDTGLYTLHI DDGLYVRPE 540	EAPRSGFD 55	//////////////////////////////////////	AD AD AD AD AD AD AD AD AD AD AD AD AD A	(GVTANRLL) 570 	MSR MS. MS. MS. MS. MS. MS. MS. MS. MS. MS.
201_gE chis_gE cules_gE 8_gE 201_gE 2012_gE 1_gE gE 1_gE gE 1_gE 1_gE 1_gE 1_gE	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.	0 <u>GDDDDDDEE</u> D D D D D D D D D D D D D	M	A.S.A.S.A.S.A.S.A.S.A.S.A.S.A.S.A.S.A.S	EGPYASLDE 520 A. F A. F	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI DDGLYVRPF 540	EAPRSGFD 55(//////////////////////////////////////	AD AD AD AD AD AD AD AD AD AD AD AD AD A	2GVTANRLLN 570 N. N. P N. P N. P N. P N. P N. P N. P N	MSR MS. MS. MS. MS. MS. MS. MS. MS. MS. MS.
201_gE chis_gE chis_gE %_gE 201_gE 201_gE 201_gE 201_gE 1_gE _gE _gE _gE 1_gE	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.))))))))))))))	M	ASPGGDSGY 510 A.S	EGPYASLDF 520 	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI DDGLYVRPE 540	ASGPRAVEFV EAPRSGED 55(//////////////////////////////////////	AD AD AD AD AD AD AD AD AD AD AD AD AD A	2GVTANRLLN 570 	MSR MS. MS. MS. MS. MS. MS. MS. MS. MS. MS.
201_gE chis_gE cules_gE 8_gE 201_gE 2012_gE 1_gE gE de de de de de de de de de de de de de	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.))))))))))))))	M. AGVIRRPP 00 I	ASPGGDSGY 510 A.S	EGPYASLDF 520 A. F A. F	.S.L. .S.L.L. .S.L.L. .S.L.L. .S.L.L. .S.L.L. .S.L.L. .S.L.L. .S.L.L. .S.L.L. .S.L.L. .S.L.L.L.L	NDTGLYTLHI DDGLYVRPE 540	EAPRSGFD 55	// / / / / / / / / / / / / / / / / / /	AD AD AD AD AD AD AD AD AD AD AD AD AD A	(GVTANRLL) 570 	MSR MS. MS. MS. MS. MS. MS. MS. MS. MS. MS.
201_gE chis_gE cules_gE 8_gE 201_gE 201_gE 201_gE 201_gE 1_gE _gE 1_	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.) D D D D D D D D D D D D D	M	ASPGGDSGY 510 A.S	EGPYASLDE 520 A. F A. F	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI DDGLYVRPE 540	EAPRSGFD 55(//////////////////////////////////////	AD AD AD AD AD AD AD AD AD AD AD AD AD A	2GVTANRLLN 570 N. N. P N. P N. P N. P N. P N. P N. P N	MSR MSR. MS. MS. MS. MS. MS. MS. MS. MS. MS. MS
2017_gE J-2013_g1 0304_gE 1_gE 1201_gE 1chis_gE rcules_g1 18_gE _gE 1201_gE 1201_gE N1_gE N1_gE 8_gE _gE X_gE	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.))))))))))))))	M	ASPGGDSGY 510 A.S.A.S.A.S.A.S.A.S.A.S.A.S.A.S.A.A.S.S.A.S.	EGPYASLDF 520 	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI DDGLYVRPE 540	ASGPRAVEFV EAPRSGED 55	//////////////////////////////////////	AD AD AD AD AD AD AD AD AD AD AD AD AD A	2GVTANRLLN 570 	MSR MS. MS. MS. MS. MS. MS. MS. MS. MS. MS.
201_gE chis_gE chis_gE 10_gE 2012_gE 10	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.))))))))))))))	M	ASPGGDSGY 510 A.S.A.S.A.S.A.S.A.S.A.S.A.S.A.S.A.A.A.A	EGPYASLDF 520 A. F A. F	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI DDGLYVRPE 540	DASOPRAVEFV EAPRSGFD 55	// / / / / / / / / / / / / / / / / / /	AD AD AD AD AD AD AD AD AD AD AD AD AD A	(GVTANRLLN 570 	MSR MS. MS. MS. MS. MS. MS. MS. MS. MS. MS.
201 gE chis gE chis gE chis gE colles gE 8 gE 201 gE gE gE gE gE 1 gE 1 201	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.	>GDDDDDDEE2) 50	M. AGVIRRP) 00 	ASPGGDSGY 510 A.S A.S A.S A.S A.S A.S A.S A.A. A.A. A.A. A.A. A.A. A.A. A.A. A.S	EGPYASLDE 520 A. F A. F	. S. L. . S	NDTGLYTLHI DDGLYVRPE 540	EAPRSGFD 55	AVGDRPPAPI	AD AD AD AD AD AD AD AD AD AD AD AD AD A	(GVTANRLLM 570 	MSR MS. MS. MS. MS. MS. MS. MS. MS. MS. MS.
201_gE chis_gE chis_gE 10_gE 2012_gE 10	.S. .S. .S. .S. .S. .S. .S. .S. .S. .S.))))))))))))))	M	ASPGGDSGY 510 A.S.A.S.A.S.A.S.A.S.A.S.A.S.A.S.A.S.A.S	EGPYASLDF 520 520 A. F A. F	S.L. S.L. S.L. S.L. S.L. S.L. S.L. S.L.	NDTGLYTLHI DDGLYVRPE 540	ASGPRAVEFV EAPRSGED 55(//////////////////////////////////////	AD AD AD AD AD AD AD AD AD AD AD AD AD A	2GVTANRLLN 570 	MSRI MS. MS. MS. MS. MS. MS. MS. MS. MS. MS.

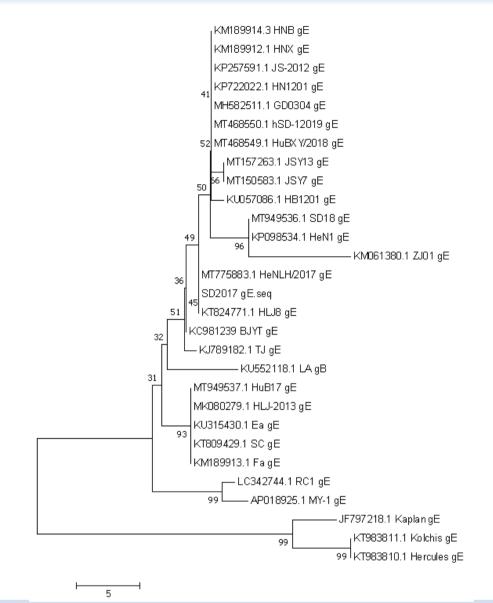
Fig.5. Comparison of PRV SD-2017 gE amino acid sequence with other PRV strains.

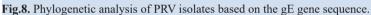
Majority	MPAGGGLWRGPRGHR	RPGHHGGAGLGE			LLLALAAT'P'	FCGAAAVTRA	ASASPAPGTG-	ATPDGFSA	EESLEEIDGA	VSPGPSDAP	DGEYGDLDAR	TAVRAA
	10	20	30	40	50	60	70	80	90	100	110	120
SY13_gB					T.S	F	A.GT		.EDG.			
JSY7_gB HuBXY2018 gB					T.	Г Р			.EDG.			
SD-12019 gB					T.S	F			.EDG.			
D18_gB					····.T.	F	A.GT		.EDG.			
uB17_gB D1802_gB	••••••				T.	r	A.GT	ADGF.T	.EDG.	VSD		
D1802_gB					т г	 г	A GT -	A DGF	F DG	VS D		•••••
LJ-2013_gB												
D0304_gB					T.S	r	A.GT	ADGF	.EDG.			
C1_gB							A.GT	ADGF				
LA_gB Ea_gB					T.S	F	A.GT	ADGF ADGF.T				
HB1201 gB						F	A.GT	ADGF				
Kolchis_gB												
Hercules_gB												
HLJ8_gB SC gB					T.	r	A.GT	ADGF ADGF.T				
N1201 gB					т.r	г	A.GT	ADGF				
S-2012 gB					T.S	Γ	A.GT	ADGF				
eN1_gB					T.	F	A.GT	ADGF				
NB_gB					T.S	F	A.GT	A DGF				
a_gB NX_gB					T.		A.GT	ADGF.T ADGF				
J01_gB					т. ^с		A.GT		.EDG.			
J_gB					T.S	r	A.GT		.EDG.			
JYT_gB					T.S	F	A.GT	ADGF	.EDG.	VSD		
aplan_gB	MDACCCIMPCDDCUD	Deutecherer						DOTEDNOVON			DCEVCDIDAD	
Bartha_gB MY-1_gB	MPAGGGLWRGPRGHR	PGHHGGAGLG	RLWPAPHHAA	AAARGAVALAL	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	PCGAAAVTRAZ	ASASPTPVPGS	PGLTPNDVSA	EASLEEIE-A		DGEYGDLDAR	TAVRAA
95												
ajority	TPHFTVAWDWAPKTR	RVCSLAKWREA	AEEMIRDETH	RDGSFRFTSRA	LGASFVSDV	TOLDLORVHLO	GDCVLREASEA	IDAIYRRRYN	NTHVLAGDKPI	EVYLARGGE	VVAFRPLISN	ELAQLY
	370	380	390	400	410	420	430	440	450	460	470	480
		300	390	400	410	420	430	440	450	400	470	400
ISY13_gB ISY7_gB		•••••				• • • • • • • • • • • •	• • • • • • • • • • • • •	R	К. к	• • • • • • • • • • •		•••••
uBXY2018 gB								R	K.			
SD-12019 gB								R	K.			
D18_gB								R	K.			
uB17_gB						••••••		R				
GD1802_gB SD2017_gB						• • • • • • • • • • • •		R	к			
HLJ-2013_gB								R				
GD0304_gB								R	K.			
RC1_gB								R				
LA_gB Ea_gB								VR				
HB1201_gB												
Kolchis gB								R	K.			
								R	K.	· · · · · · · · · · · · ·		
Hercules_gB								R R R	K.			·····
HLJ8_gB								R R R R R 				
HLJ8_gB SC_gB								R R R R R R				
HLJ8_gB SC_gB HN1201_gB JS-2012_gB								RR RR RR RR RR				
LJ8_gB SC_gB IN1201_gB IS-2012_gB SeN1_gB								RR RR RR RR RR RR				
HLJ8_gB SC_gB MN1201_gB JS-2012_gB MeN1_gB MNB_gB								RRRRRRRR				
ILJ8_gB GC_gB IN1201_gB GS-2012_gB GEN1_gB INB_gB 'a gB								RRRRRRRR				
HLJ8_gB SC_gB IN1201_gB JS-2012_gB HeN1_gB HINB_gB Ya_gB INX_gB								R				
HLJ8_gB SC_gB HN1201_gB JS-2012_gB HeN1_gB HeN1_gB Fa_gB HNX_gB JJ01_gB NJ_gB								R				
HLJ8_gB SC_gB IN1201_gB JS-2012_gB HeN1_gB HNB_gB Fa_gB INX_gB JJ01_gB JJ01_gB JJ71_gB								R. R	К. К. К. К. К. К. К. К. К. К. К.			
HLJ8_gB SC_gB HN1201_gB JS-2012_gB HeN1_gB Fa_gB INN_gB ZJ01_gB ZJ01_gB ZJ01_gB ZJ71_gB AJYT_gB Aaplan_gB	TPHFTVANDWAPKTR	RVCSLAKWREJ	AEEMIRDETT	NDGSFRFTSRA	LIGASEVSDV	TOLDLORVHL	DCVLREASEA	R. R. R. R. R. R. R. R. R. R. R. R. R. R	K. K	EVYLARGGE	VVAFRPLISN	ELAQLY
HJJ8_gB SC_gB NN1201_gB JS-2012_gB HeN1_gB NNB_gB Fa_gB NJX gB ZJ01_gB NJ_gB SJYT_gB Kaplan_gB Bartha_gB	TPHFTVAMDWAPKTF	RVCSLAKWREJ	AEEMIRDETF	RDGSFRFTSRA	LGASFVSDV:	FQLDLORVHLG	SDCVLREASEA	R R R R R R R R R R R R R R R R R R R	K. K	EVYLARGGF	VVAFRPLISN	ELAQLY
HJJ8_gB SC_gB NN1201_gB JS-2012_gB HeN1_gB NNB_gB Fa_gB NJX gB ZJ01_gB NJ_gB SJYT_gB Kaplan_gB Bartha_gB	TPHFTVAWDWAPKTR	RVCSLARWREZ	REEMIRDETE	RDGSFRFTSRA	LIGASEVSDV	LÖTDTÖKAHTY	SDCVLREASEA	R R R R R R R R R R R R R R R R R R R	K. K. K. K. K. K. K. K. K. K. K.	EVYLARGGE	VVAFRPLISN	ELAQLY
HJB gB GC_gB NN1201_gB FS-2012_gB HeN1_gB NN5_gB Fa_gB NJG B JJ01_gB JJ01_gB JJ01_gB SJ01_gB SJ01_gB SJ01_gB SJ01_gB SJ01_gB SJ01_gB SJ01_gB SJ01_gB	TPHFTVAWDWAPKTR	RVCSLARWRE	AEEMIRDETE	RDGSFRFTSRA	LIGASFVSDV	FQLDLORVHLG	SDCVLREASEA	R R R R R R R R R R R R R R R R R R R	K. K. K. K. K. K. K. K. K. K. K. NTHVLAGDRPI	EVYLARGGF	VVAFRPLISN	ELAQLY
ILJ8 gB ILJ8 gB IC_gB IC_gB IC_l201_gB IC_l201_gB ISS-2012_gB INB_gB a_gB INB_gB JJgB JJgB JJT_gB IJT_gB IAJTA_gB IAJTA_gB IY-1_gB	TPHFTVAMDWAPKTF ARELERLGLAGVVGF 490	ARVCSLARWRE PASPAARRARI 500	AEEMIRDETE RSPGPAGTPH 510	RDGSFRFTSRA 2004 520	LIGASFVSDV RITTGSAEF/ 530	TOLDLORVHLG ARLOFTYDHIG 540	SDCVLREASEA	R R R R R R R R R R R R R R R R R R R	K. K. K. K. K. K. K. K. K. K. K. K. K. K	EVYLARGGF LNPSAVATA 580	VVAFRPLISN ALGORVSARM 590	
LIJE gB C: gB N1201_gB (eN1_gB (eN1_gB a_gB (JJI_gB (JJI_gB (JJI_gB (JJI_gB (JJI_gB (JTI_gB (Aplan_gB (Artha_gB (Y-1_gB (Aplan_gB (Y-1_gB (Aplan_gB (Y-1_gB) (Y-1_gB (Aplan_gB) (Y-1_gB)			510									ELAQLY LGDVMA 600
LLJ8 gB LLJ8 gB N1201_gB N1201_gB N2-2012_gB NNB_gB NNX_gB JJ01_gB JJ01_gB JJ71_gB JJ71_gB JJ71_gB JJ71_gB LJ71_gB LJ71_gB LJ71_gB LJ71_gB LJ71_gB			510 .SP.P		530	540	550					
HJ8 gB HJ8 gB NT201_gB NT2012_gB NT2012_gB NTB gB NTB gB NTT gB Laplan_gB JJ7T_gB Laplan_gB JJ7T_gB Laplan_gB Laplan_gB Laplartha_gB HY-1_gB Laplority JSY13_gB JSY7_gB LabY2018_gB	490	500	510 .SP.P .SP.P	520	530	540	550	560	570 G G	580	590	600
L158 gB L158 gB L1201 gB leN1_gB rs-2012_gB leN1_gB ra_gB ra_gB ry ry ry ry ry ry ry ry ry ry ry ry ry r	490	500	510 .SP.P .SP.P .SP.P .SP.P	520	530	540	550 s. s. s. s.	560	570 G G	580	590	600
H.J8 gB ILJ8 gB ILJ8 gB IN1201 gB IN201 gB IN201 gB IN3	490	500	510 .SP.P .SP.P .SP.P .SP.P	520	530	540	550 	560	570 G G G G	580	590	600
H.J5_gB GC_gB SC_gB SC_gB SC_gB SF_2012_gB HNN_gB Fa_gB SJU1_gB SJU1_gB SJU1_gB SJU1_gB SJU1_gB SJU1_gB Majority JSY13_gB Majority JSY13_gB SSU1_gB SD15_gB HDB-12019_gB SD16_gB HBJ7_gB	490	500	510 .SP.P .SP.P .SP.P .SP.P .SP.P	520	530	540	550 	560 	570 G G G G G G G	580	590	600
H.J6 gB SC gB INI201_gB INI201_gB INI201_gB INI201_gB INI2 gB INI2 gB	490	500	510 .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P.	520	530	540	550 	560	570 G. G. G. G. G.	580	590	600
HJ8 gB HJ8 gB (C gB (N1201 gB (N1201 gB (N1201 gB (N1201 gB (N12 gB) (N12 gB)	490	500	510 .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P.	520	530	540	550 	560 	570 G G G G G G	580	590	600
H.J5_gB SC_gB N1201_gB SS-2012_gB HN1201_gB HNN_gB Fa_gB NNN_gB JJ01_gB HNX_gB JJ01_gB HNX_gB JJ01_gB HNX_gB JJ01_gB H121_gB H121_gB H121_gB H121_gB H121_121_gB H121_121_gB SD2117_gB SD2107_gB SD2017_gB SD2017_gB SD2017_gB SD2017_gB SD2017_gB SD2017_gB SD2017_gB SD2017_gB SD2017_gB	490	500	510 .SP.P .SP.P .SP.P .SP.P .SP.P .SP.P .SP.P	520	530	540	550 	560 	570 G. G. G. G. G. G. G. G.	580	590	600
HJ8 gB HJ8 gB (S gB IN1201 gB (H01.gB) (H01.	490	500	510 .SP.P .SP.P .SP.P .SP.P .SP.P .SP.P .SP.P .SP.P .SP.P .SP.P	520	530	540	550 8. 8. 8. 8. 8. 8. 8. 8. 8. 8. 8. 8. 8.	560	570 	580	590	600
H.J8 gB H.J8 gB KS -2012 gB ieN1_gB isN1_gB isO1_gB iJ01_gB	490	500	510 SP.P. SP.P. SP.P. SP.P. SP.P. SP.P. SP.P. SP.P. SP.P. S.SP. S.SP.	520	530	540	550 550 888888 58	560 	570 	580	590	600
H.J8 gB H.J8 gB INI 201 gB INI 201 gB INI 201 gB INI GB	490	500	510 .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .S.P.S. .SP.P. .S.S.P. .S.S.P. .S.S.P. .S.S.P.	520	530	540	550 550 	560 	570 	580	590	600
H.J8 gB H.J8 gB S = 2012 gB HN1201 gB HN1201 gB HN1201 gB HN1 gB	490	500	510 .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .S.P.P.P. .S.P.P.P.P. .S.P.P.P.P. .S.P.P.P.P.P.P.P. .S.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	520	530	540	550 	560 	570 	580	590	600
HJ8 gB HJ8 gB (C gB (C gB (N1201 gB (H01 gB (F 2012 gB (H01 gB (F 2012 gB (H01 gB (F 2012 gB	490	500	510 .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P.	520	530	540	550 550 	560 	570 G G G G G G G G.	580	590	600
HJ8 gB HJ8 gB HJ201_gB HS-2012_gB HS-2012_gB HS-2012_gB HS-2012_gB HJ301_gB	490	500	510 .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P.	520	530	540	550 550 88.	560 	570 G G G G G G G G G G G G G	580	590	600
HJ8 gB HJ8 gB (C gB (C gB (N1201 gB (H01.gB) (H01.gB (H01.gB (H01.gB (H01.gB (H01.gB) (H01.gB (H01.gB (H01.gB) (H01.g	490	500	510 SP.P. SP.P. SP.P. SP.P. SP.P. SP.P. SP.P. S.SP. S.SP. S.SP. S.SP.P. SP	520	530	540	550 550 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	560 	570 G G G G G G G G G G G G G	580	590	600
H.J5_gB SC_gB HN1201_gB HN1201_gB HN1_gB Fs_2012_gB HNN_gB Fs_gB HNN_gB JJJT_gB JJTT_JJTT_JJTT_JJTT_JJTT_JJTT_JJTT_JJT	490	500	510 SP.P. SP.P. SP.P. SP.P. SP.P. SP.P. SP.P. SP.P. S.S.P. S.S.P.P. S.S.P.P. S.S.P.P. S.S.P.P. SP.P.	520	530	540	550 550 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	560 	570 G G G G G G G G G G G G G	580	590	600
LJ8 gB LJ8 gB LJ8 gB N1201 gB (s-2012 gB (eN1 gB r3 gB r3 gB NN gB r3 gB NN gB r3 gB NN gB r3 gB r3 gB r4 gB r4 gB r5 201 gB	490	500	510 .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .SP.P. .S.S.P. .S.S.P. .S.S.P. .S.S.P. .S.S.P. .S.S.P. .S.S.P. .S.P.P. .S.S.P.P. .S.P	520	530	540	550 550 	560 	570 	590	590	600
H.J6 gB H.J6 gB SC gB IN1201 gB SC-2012 gB HN1201 gB FS-2012 gB HNN gB JJ01 gB	490	500	510 .SP.P.	520	530	540	550 550 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	560 	570 G G G G G G G G G G G G G	590	590	600
H.J6 gB H.J6 gB SC gB INI201 gB Holl gB FS-2012 gB Holl gB FS-2012 gB Holl gB FS-2012 gB INN gB TS-2012 gB TS-2012 gB HOLL gB HOLL gB FS-12019 gB FS-2012 gB HS-2012 gB	490	500	510 SP.P. SP.P	520	530	540	550 550 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	560 	570 G. G. G. G. G. G. G. G. G. G.	590	590	600
H.J5 gB SC gB HN1201 gB J5-2012 gB HN1201 gB HN1201 gB J5-2012 gB HNX gB ZJ01 gB J7T gB Bartha gB Bartha gB HNX gB Bartha gB HAUBY J3 Majority J5Y13 gB J5Y7 gB SD102 gB HSD-12019 gB SD10 gB SD2017 gB GD1034 gB SD2017 gB GD034 gB Ea gB Herculs gB Herculs gB Herculs gB HHJ20 gB SC gB HN1201 gB HSC1 gB HSC2 gB H	490	500	510 .SP.P.	520	530	540	550 550 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	560 	570 G G G G G G G G G G G G G	590	590	600
H.J5 gB H.J5 gB SC gB HI201 gB FS-2012 gB HN201 gB FS-2012 gB HNX gB ZJ01 gB FJ gB HIX gB ZJ01 gB FJ gB HIX gB Asptan gB HIT gB	490	500	510 SP.P. SP.P	520	530	540	550 550 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	560 	570 G G G G G G G G G G G G G	590	590	600
Hercules_gB HJJ2_gB SC_gB SC_gB SC_gB HeN1_01_gB HeN1_gB HHX_gB ZJ01_gB TJ_gB ASTYT_gB Asplan_gB Bartha_gB Majority JSY13_gB Majority JSY13_gB Majority JSY13_gB HUB17_gB SD18_gB HUB17_gB SD18_gB HUB17_gB SD18_gB EALA_gB EALA_gB HB1201_gB HB1201_gB HC12_g	490	500	510 SP.P. SP.P	520	530	540	550 550 	560 	570 G G G G G G G G G G G G G	590	590	600
HJJ8 gB HJ3 gB SC gB HN1201 gB HN1201 gB HN1201 gB HN1 gB F8_gB JJT gB JJT gB Bartha gB HJJ gB Bartha gB HJJ gB JSY1 gB HABYY2018 gB HSD 12019 gB SD1201 gB SD1201 gB SD201 gB SD201 gB SD201 gB HJ2 gB	490	500	510 SP.P. SP.P	520	530	540	550 550 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	560 	570 G. G. G. G. G. G. G. G. G. G.	590	590	600
HJJ8 gB HJ201 gB HN1201 gB J5-2012 gB HN1201 gB J5-2012 gB HNX gB J5-2012 gB HNX gB J75 gB HNX gB J77 gB HNX gB HNX J7 gB HNST J7 gB HNST J7 gB HNST J7 gB HNST J7 gB HNST J7 gB HJ2013 gB J500304 gB HJ2013 gB HJ2013 gB HJ2013 gB HJ2013 gB HJ2012 gB HS12012 gB HS12012 gB HS12012 gB HS12012 gB HS12012 gB HS12012 gB HS12012 gB HS12012 gB HS12012 gB J72 gB HS12012 gB J72 gB J73 gB J73 gB J73 gB J73 gB J74 gB	490	500	510 .SP.P.	520	530 RITTGSAEF2	540 RRLQFTYDHIG	550 550 	560 	570 G G G G G G G G G G G G G	590	590 L	600

Fig.6. Comparison of PRV SD-2017 gB amino acid sequence with other PRV strains.

Majority	MAGEADAMEATEATY	TAATAADCC	TTAT COTTONC	CCCCNERACE	T CDCDDC	TOF DUCCOT	CAASCTDAA	VCTDDVDDD	TUCDDEDODN	CNDTDUUCI	KATSHGRKRI	VCPEPT
Majoricy	10	20	30	40	50	60	70	80	90	100	110	120
JSY13_gC												
JSY7_gC	L.	TS			S		AAASTPA.	VI	?Q	G	S	
HuBXY2018_gC	L.	TS			S		AAASTPA.	v		G	S	
hSD-12019_gC	L.	TS			·····S		AAASTPA.	· · · · · · · · · · · · · · · · · · ·		G	s	
SD18_gC	L.	TS			S	E.V.GT1	AAASTPA.	v		G	S	
HuB17_gC	L.	TS	T.		·····s	E.V.GTI	AAASTPA.	· · · · · · · · · · · · · · · · · · ·		G	.ES	
HeNLH2017_gC	·····b·	TS			·····S	E.V.GT1	AAASTPA.	· · · · · · · · · · · · · · · · · · ·		G	s	
GD1802_gC						E N. C.						
SD2017_gC HLJ-2013 gC	·····.	T				E.V.GT1	AAASTPA.	· · · · · · · · · · · · · · · ·	Q	G	S	
GD0304_gC	тт	m c			·····	E V Cmg	AAAGTDA.				e	
RC1_gC		Τ			s	A.ETG.GTT	AAASTPA.	v		G	s	
LA_gC		Τ					AASTPA.	v		G		
Ea gC	L.	TS	T.		s	E.V.GTT	AAASTPA.	v		G	ES	
HB1201 gC	L.	TS			s	E.V.GTT	AAASTPA.	v		G	s	
Kolchis gC	L.			E								
Hercules_gC	L.			E								
HLJ8_gC	L.	TS			S	E.V.GT1	AAASTPA.	V	· · · · · · Q · ·	G	S	
SC_gC											E	
HN1201_gC	L.	TS			S	E.V.GT1	AAASTPA.	· · · · · V · · · ·	· · · · · · · Q. ·	G	s	
JS-2012_gC	L.	TS	• • • • • • • • • • •		·····S	E.V.GT1	AAASTPA.	· · · · · · · · · · · · · · · · · · ·		G	s	
HeN1_gC	·····b·	TS			·····S	E.V.GTI	AAASTPA.	· · · · · · · · · · · · · · · · · · ·		G	· · · · S · · · · · ·	
HNB_gC	·····b·	TS				E.V.GT	AAASTPA.			6	S	
Fa_gC HNX gC	т.	Ψ 9			s	E V GTT	AAASTPA.	v		G	g	
ZJ01 gC		Τ				E.V.GTT	AAASTPA	v		G	s	
TJ gC	L.	TS			s	E.V.GTT	AAASTPA.	v		G	s	
BJYT gC	L.	TS			s	E.V.GTT	AAASTPA.	v		G	s	
Kaplan gC	L.			E								
Bartha_gC	MASLARAMLALLAPY	AAAIAAAPST	TTALGTTPNG	GGGGGNSSAGE	LSPSPPP	TPAPASPEA	GA	VSTPRAPPPS	VSRRKPPRN	NNRTRVHGI	KATAHGRKRI	VCRERL
MY-1_gC	L.	т	T.		SPPS	STPSETG.GTT	VAASTPA.	v		G	s	
Majority	FSARVGDAVSFGCAV	VPRAGETFEV	RFCRRGRFRS	PDADPEYFDE	PPRPELPREF	LLFSSANASLAH	ADALASAVV	VEGERATVAN	VSGEVSVRV	AAADAETEG	VYTWRVLSAN	GTEVRS
	130	140	150	160	170	180	190	200	210	220	230	240
	130	140	150	-	1		1	200	210	220	230	240
JSY13_gC												
JSY7_gC		V			P		ASA. V	VE				
HuBXY2018_gC		V	·		· · · · P · · · · · ·		ASA.V	VE				
hSD-12019_gC SD18_gC		v			P		ACA UN	VE				
HuB17_gC		v			p		ASA VI	7				
HeNLH2017 gC		v			P		ASA.V	V				
GD1802_gC												
SD2017_gC		v			P		ASA. V	VE				
HLJ-2013_gC												
GD0304_gC		v	c		P		ASA. V	V				
RC1_gC		v			P		ASA. V	V				
LA_gC		v			P		ASA.V	V				
Ea_gC		v			P		ASA. V	7				
HB1201_gC		v			P		ASA V					
Kolchis_gC								· E				
Henry les mil					P		A.ALD	.D				
Hercules_gC			·····		P P		A.ALD	.D				
HLJ8_gC		v	c		P		A.ALD A.ALD ASA.V	.D D VE				
HLJ8_gC SC_gC		v	c		P PP PP		A.ALD A.ALD ASA.V	.D				
HLJ8_gC SC_gC HN1201_gC		v vv.	c				A.ALD A.ALD ASA.V	D D V.E V.E V.E.				
HLJ8_gC SC_gC		v v vv.	c				A.ALD A.ALD ASA.V ASA.V ASA.V	D D VE VE VE VE.				
HLJ8_gC SC_gC HN1201_gC JS-2012_gC HeN1_gC HNB_gC		v v v v v	c. c. c. c. c.				A.ALD A.ALD ASA.V ASA.V ASA.V ASA.V ASA.V	.D. .D. V.E. V.E. V.E. V.E. V.E.				
HLJ8_gC SC_gC HN1201_gC JS-2012_gC HeN1_gC HNB_gC Fa_gC		v vv. vv. vv. vv.					A.ALD A.ALD ASA.V ASA.V ASA.V ASA.V ASA.V ASA.V	.D. .D. V.E. V.E. V.E. V.E. V.E. V.E.				
HLJ8_gC SC_gC HN1201_gC JS-2012_gC HeN1_gC HNB_gC Fa_gC HNX_gC		V V V V V V V					A. ALD A. ALD ASA. V ASA. V ASA. V ASA. V ASA. V ASA. V ASA. V	D				
HLJ8_gC SC_gC HN1201_gC JS-2012_gC HeN1_gC HNB_gC Fa_gC HNX_gC 2J01_gC		V V V V V V V			P. P. P. P. P. P. P. P. P. P. P. P. P. P		A. ALD A. ALD ASA. V ASA. V ASA. V ASA. V ASA. V ASA. V ASA. V ASA. V ASA. V	D. D. V. E. V. E. V. E. V. E. V. E. V. E. V. E.				
HLJ8_gC SC_gC HN1201_gC JS-2012_gC HeN1_gC HNB_gC Fa_gC HNX_gC ZJ01_gC TJ gC		V V						D D V.E V.E V.E V.E V.E V.E V.E V.E V.E V.E V.E V.E				
HLJ8_gC SC_gC HN1201_gC JS-2012_gC HeN1_gC HNB_gC Fa_gC HNX_gC ZJ01_gC TJ_gC BJYT_gC		VV VVVV VV VV VV VV VV VV V VV VV V VV V VV V V VV V V V V V V V V V V V V V V V V V V V	c. .c. .c. .c. .c. .c. .c. .c. .c					D. D. V. E. V. E.				
HLJ9_gC SC_gC HN1201_gC JS-2012_gC HN1_gC Fa_gC Fa_gC ZJ01_gC TJ_gC BJYT_gC Kaplan_gC	FSARVGDAVSFGCCAV	VV. VV. VV. VV. VV. VV. VV. VV. VV.	C. 	PDADPEYPDE	PP. PP. PP. PP. PP. PP. PP. PP. PP. PP. PP. PP.	LLFSSANASLAH	A. ALD A. ALD A. ALD A.SA. V' A.SA. V'	D. D. V. E. V. E. V. V. E. V. E. V. E. V. V. E. V. E. V. V. E. V. E. V. V. E. V. E. V. E. V. V. E. V. E. V. V. E. V. E. V. E. V.	IVSGEVSVRV	AAADAFTEG		GTEVRS
HLJ0_gC SC_gC HNI201_gC JS=2012_gC HNB_gC HNB_gC HNX_gC ZJ01_gC BJYT_gC BJYT_gC Bartha_gC	FSARVGDAVSFGCAV	V. V. V. V. V. V. V. V. V. V. V. V. V. V		PDADPEYFDE		LLFSSANASLAH	A. ALD A. ALD A. AL V ASA. V	D. D. V. E. V. E. V	IVSGEVSVRV	AAADAETEG		GTEVRS
HLJ9_gC SC_gC HN1201_gC JS-2012_gC HN1_gC Fa_gC Fa_gC ZJ01_gC TJ_gC BJYT_gC Kaplan_gC	FSARVGDAVSFGCAV	V. V. V. V. V. V. V. V. V. V. V. V. V. V		PDADPEYFDE	PP. PP. PP. PP. PP. PP. PP. PP. PP. PP. PP. PP. PP. PP. PP.	LLFSSANASLAH	A. ALD A. ALD ASA. V' ASA. V'	D. D. V. E. V. E. V. E. V. E. V. E. V. E. V. E. V. E. DEGGRATVAN V. E.	IVSGEVSVRV.	AADAETEC		GTEVRS
HLJ0_gC SC_gC HNI201_gC JS=2012_gC HNB_gC HNB_gC HNX_gC ZJ01_gC BJYT_gC BJYT_gC Bartha_gC	FSARVGDAVSFGCAV	V V V V V V V V V V V V V V V V V V V	C. 	:PDADPEYFDE MADTVYHLGA	P. P. P. P. P. P. P. P. P. P.	KLLFSSANASLAH				NAADAETEG		GTEVRS
HLJ8_gC SC_gC HN1201_gC HeN1_gC HeN1_gC HNM_gC Fa_gC HNX_gC ZJ01_gC TJ_gC BJ7T_gC Bartha_gC MY-1_gC							VDYTCRLEG	MPSQLPIFEI				
HLJ0_gC SC_gC HNI201_gC J=2012_gC HNM_gC HNM_gC Fa_gC HNX_gC ZJ01_gC TJ_gC BJTT_gC BJTT_gC Batha_gC MY-1_gC Majority	370	380	390	400	410	420	VDYTCRLEG	MPSQLPIFEI 440	450	460	470	480
HLJ8_gC SC_gC HN1201_gC HeN1_gC HeN1_gC HNB_gC Fa_gC HNX_gC ZJ01_gC TJ_gC BJ7T_gC Bartha_gC Mr1_gC Majority JSY13_gC	370	380	390	400	410	420	VDYTCRLEG 430	MPSQLPIFEI 440	450	460	470	480
HLJ0_gC SC_gC HNI201_gC Js-2012_gC HeN1_gC HNB_gC Fa_gC HNX_gC ZJ01_gC TJ_gC BJTT_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC	370	380	390	400	410	420	PVDYTCRLEG	MPSQLPIFEI 440 MI.	450	460	470 	480
HLJ8_gC SC_gC HN1201_gC Js=2012_gC HeN1_gC HNB_gC Fa_gC TJ_gC BJYT_gC BJYT_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY13_gC JSY13_gC HuBXY2018_gC	370	380	390	400	410	420	A30	MPSQLPIFEI 440 MI	450	460	470 TA. 	480
HLJ8_gC SC_gC HN1201_gC HeN1_gC HeN1_gC HNB_gC Fa_gC HNX_gC ZJ01_gC TJ_gC BJ7T_gC BJ7T_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC HBXY2016_gC HSD-12019_gC	370	380	390	400	410	420	VDYTCRLEG	MPSQLPIFEI 440 MI MI	450 T T	460	470 TA. TA. A.	480
HLJ0_gC SC_gC HNI201_gC Js-2012_gC HeN1_gC HNM_gC Fa_gC HNX_gC ZJ01_gC ZJ01_gC BJTT_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC HuBXY2018_gC hsD-12019_gC SD18_gC	370	380	390	400	410	420	A30	MPSQLPIFEI 440 MI MI MI	450 T T T	460	470 	480
HLJ8_gC SC_gC HN1201_gC HeN1_gC HeN1_gC HNB_gC Fa_gC HNX_gC ZJ01_gC TJ_gC BJ7T_gC BJ7T_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC HBXY2016_gC HSD-12019_gC	370	380	390	400	410	420	430	MPSQLPIFEI 440 MI. MI. MI. MI.	450 	460 	470 	480
HL36_gC SC_gC HN1201_gC H=N1_gC H=N1_gC H=N1_gC H=N1_gC H=N1_gC H=N2_gC H=N2_gC H=N2_gC H=N2_gC H=N2_gC MY-1_gC MY-1_gC MY-1_gC MY-1_gC MY-1_gC MY-1_gC SD18_gC H=D12019_gC H=D12019_gC H=D12_gC	370	380	390	400	410	420	430	MPSQLPIFEI 440 M. I. M. I. M. I. M. I. M. I. M. I.	450 T T T	460	470 	480
HLJ8_gC KC_gC HN1201_gC H=N1_gC H=N1_gC H=N1_gC H=N1_gC H=N2_gC H=N2_gC H=N2_gC H=N2_gC H=N2_gC H=N2_gC H=N2_gC M=1_gC	370	380	390	400	410	420	430	MPSQLPIFEI 440 440 M. I. M. I. M. I. M. I. M. I. M. I. M. I.	450 	460 T. T. T. T.	470 	480
HLJ8_gC SC_gC HN1201_gC Js-2012_gC HNM_gC HNM_gC Fa_gC ZJ01_gC ZJ01_gC ZJ01_gC BJTT_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC HuBXY2018_gC hSD-12019_gC SD18_gC HuB17_gC HeMLH2017_gC GD1802_gC SD2017_gC	370	380	390	400	410	420	VDYTCRLEG	MPSQLPIFEI 440 M I. M I. M I. M I. M I. M I. M I. M I.	450 	460 	470 	480
HLJ8_gC SC_gC HN1201_gC HeN1_gC HeN1_gC HeN1_gC HNF_gC Fa_gC HNX_gC ZJ01_gC TJ_gC BYTT_gC BYTT_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC HUBXY2018_gC HUBXY2018_gC HUB17_gC	370	380	390	400	410	420	VDYTCRLEG	MPSQLPIFEI 440 M. I. M. I. M. I. M. I. M. I. M. I. M. I. M. I. M. I.	450 	460	470 	480
HLJ8_gC KL_GC HN1201_gC HN1201_gC HN1_gC HNM_gC Fa_gC HNX_gC ZJ01_gC TJ_gC BJTT_gC BJTT_gC Majority JSY13_gC JSY7_gC Mbp.12018_gC SD18_gC HuBL7_gC HUBL7_gC HUBL7_gC HUJ2013_gC SD2017_gC HLJ2013_gC SD2017_gC HLJ2013_gC SD2017_gC	370	380	390	400	410	420	430	MPSQLPIFEI 440 MI.	450 T T T T T T T T T	460 T. T. T. T. T. T. T.	470 	480
HLJ8_gC SC_gC HN1201_gC HeN1_gC HeN1_gC HHE_gC Fa_gC HNX_gC ZJ01_gC TJ_gC BJYT_gC BJYT_gC BATTA_gC MY-1_gC MY-1_gC MSD-12019_gC SD18_gC HLB17_gC HLB2017_gC HLD2013_gC GD1804_gC RC1_gC LA_gC	370	380	390	400	410	420	A30	MPSQLPIFEI 440 M. I. M. I. M. I. M. I. M. I. M. I. M. I. M. I. M. I. M. M. M.	450 T T T T T T T T T T T T	460 T. T. T. T. T. T. T. T.	470 T	480
HL36_gC HL201_gC JS-2012_gC HeN1_gC HeN1_gC HNM_gC Fa_gC HNX_gC Z001_gC TJ_gC BJYT_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC HuBXY2018_gC HuB17_gC HUB17_gC HUB17_gC HUB17_gC HUB17_	370	380	390	400	410	420	A30	MPSQLPIFEI 440 M. I.	450 T T T T T T T T T T T T T	460 T. T. T. T. T. T. T. T. T. T.	470 T. A. T. A.	480
HLJ8_gC HN1201_gC HN1201_gC HN1201_gC HNM_gC HNM_gC ZJ01_gC TJ_gC BJ7T_gC BJ7T_gC MY-1_gC MY-1_gC MY-1_gC MY-1_gC MSP-12019_gC SD18_gC HUB17_gC GD0304_gC RC1_gC EA_gC HD12_gC EA_gC HD12_gC HD201_gC	370	380	390	400	410	420	VDYTCRLEG 430	MPSQLPIFEI 440 M. I.	450 T T T T T T T T T T T T T	460 T. T. T. T. T. T. T. T. T. T.	470 	480
HLJ8_gC HL201_gC HN1201_gC HeN1_gC HeN1_gC HNN_gC Fa_gC HNX gC ZJ01_gC TJ_gC BYTT_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC HLB17_gC H	370	380	390	400	410	420	VDYTCRLEG 430	MPSQLPIFEI 440 M. I.	450 T T T T T T T T T T T T T	460 	470 T. A. T. A.	480
HLJ0_gC SC_gC HN1201_gC Js-2012_gC HNB_gC Fa_gC HNM_gC TJ_gC BJTT_gC BJTT_gC Bartha_gC MY-1_gC My-1_gC	370	380	390	400	410	420	VDYTCRLEG	MPSQLPTFEI 440 M. M. <tr< th=""><th>450 T T T T T T T T T T T T T T T T</th><th>460 </th><th>470 T. A. T. A.</th><th>480</th></tr<>	450 T T T T T T T T T T T T T T T T	460 	470 T. A. T. A.	480
HLJ8_gC HN1201_gC JS-2012_gC HeN1_gC HNN_gC Fa_gC HNX_gC ZJ01_gC TJ_gC BYTT_gC BYTT_gC Bartha_gC MY-1_gC MY-1_gC Majority JSY13_gC JSY7_gC HuBX72018_gC HuB17_gC HuB17_gC HuB17_gC HuB17_gC HuB17_gC HuB17_gC HLJ2013_gC SD2017_gC HLJ201_gC Kolchis_gC HuB1201_gC Kolchis_gC HuS2_gC	370	380	390	400	410	420	VDYTCRLEG	MPSQLPTFEI 440 MI.	450 T T T T T T T T T T T T T T	460 	470 T. A. T. A.	480
HLJ8_gC HN1201_gC JJ=2012_gC HeN1_gC HeN1_gC HNN_gC ZJ01_gC TJ_gC DYT_gC Bartha_gC MY-1_gC My-1_gC Majority JSY13_gC JSY7_gC HuBX72018_gC HuB17_gC HuB17_gC HuB17_gC HuB17_gC HuB17_gC HLJ2013_gC GD0304_gC RC1_gC HL32_gC Ea_gC HL32_gC HL32_gC HL38	370	380	390	400	410	420	VDYTCRLEG 430	MPSQLPTFEI 440 MI.	450 T T T T T T T T T T T T T T T T T	460 	470 T. A. T. A.	480
HLJ8_gC SC_gC HN1201_gC Js-2012_gC HNA_gC HNA_gC Fa_gC HNX_gC ZJ01_gC TJ_gC BJTT_gC BJTT_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC HuBXY2018_gC HSD-12019_gC SD18_gC HND7_gC HND7_gC HND7_gC HLJ-2013_gC GD0304_gC SD2017_gC HLJ-2013_gC R01_gC LA_gC Ea_gC HBI201_gC HCCLES_gC HCCLES_gC HCCLES_gC HLJ8_gC	370	380	390	400	410	420	VDYTCRLEG	MPSQLPTFEI 440 MI.	450 T T T T T T T T T T T T T T T	460 	470 T	
HLJ8_gC SC_gC HN1201_gC HN1201_gC HN1_gC HNM_gC ZJ01_gC TJ_gC BJ7T_gC BJ7T_gC BJ7T_gC MY-1_gC MY-1_gC MY-1_gC MY-1_gC MSD-12019_gC SD18_gC HLJ2017_gC GD1804_gC R0304_gC R0304_gC R0304_gC R1_gC Ea_gC H22017_gC H22017_gC R10_gC R10_gC R10_gC R10_gC R10_gC R10_gC R10_gC H20034_gC R10_gC H20034_gC R10_gC H2003_gC H20034_gC R10_gC H2003_gC	370	380	390	400	410	420	VDYTCRLEG	MPSQLPIFEI 440 M.	450 T T T T T T T T T T T T T T T T T T	460 	470 T	
HLJ8_gC HN1201_gC JS-2012_gC HeN1_gC HeN1_gC HNN_gC Fa_gC JJ01_gC TJ_gC BJ7T_gC BJ7T_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC HUBY2018_gC HUB17_gC HU30_gC HU30_gC HU30_gC HU1201_gC HU30_gC HU1201_gC HU30_gC HU30_gC HU1201_gC	370	380	390	400	410	420	VDYTCRLEG 430	MPSQLPIFEI 440 MI.	450 T T T T T T T T T T T T T T T T T T T	460 	470 T. A. T. A.	480
HLJ8_gC KL_201_gC HN1201_gC HN1201_gC HN1_gC HNM_gC Fa_gC HNX_gC ZJ01_gC TJ_gC BJTT_gC BJTT_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC HuBY2018_gC HND7_gC HND7_gC HND7_gC HND7_gC HND7_gC HLJ-2013_gC GD0304_gC K01_gC HLJ-2013_gC HLJ-2013_gC HLJ-2013_gC HLJ-2013_gC HLJ-2013_gC HLJ-2013_gC HLJ201	370	380	390	400	410	420	VDYTCRLEG 430	MPSQLPTFEI 440 MI.	450 T T T T T T T T T T T T T T T T T T T	460 	470 T.A.	
HLJ8_gC HN1201_gC JS-2012_gC HeN1_gC HNN_gC Fa_gC HNX_gC ZJ01_gC TJ_gC BYTT_gC BYTT_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC HUBXY2018_gC HUBY2018_gC HUB12017_gC HUB12017_gC HUB1201_gC GD1802_gC SD2017_gC HLJ201_gC HL30_gC HSD-1201_gC HL30_gC HSD-1201_gC HL30_gC HSD-1201_gC HL30_gC HSD-1201_gC HL30_gC HSD-1201_gC HL30_gC HSD-1201_gC HL30_gC HSD-1201_gC HL30_gC HSD-1201_gC HSD-1201_gC HSD-1201_gC HL30_gC HSD-1201_gC HN1_gC HNN_gC Fa_gC HNN_gC	370	380	390	400	410	420	VDYTCRLEG	MPSQLPTFEI 440 MI.	450 T T T T T T T T T T T T T T T T T T T	460 	470 T.A.	
HLJ8_gC HLJ8_gC HN1201_gC HN1201_gC HN1_gC HNN_gC Fa_gC HNX_gC ZJ01_gC TJ_gC BYTT_gC BYTT_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC HUBY2018_gC HSD-12019_gC SD18_gC HSD-12019_gC SD2017_gC HLJ8_gC RC1_gC HLJ8_gC HLJ8_gC HLJ8_gC HSC_gC HN1_gC HNS_gC HSC_gC HN1_gC HNS_gC HSC_gC HN1_gC HNS_gC HN1_gC HNS_gC HN1_gC HNS_gC HN1_gC HNS_gC HN1_gC HNS_gC HN1_gC HN1_gC HN1_gC HN1_gC HN3_gC HN1	370	380	390	400	410	420	VDYTCRLEG 430	MPSQLPIFEI 440 MI.	450 T T T T T T T T T T T T T T T T T T T	460 	470 T. A. T. A.	480
HLJ8_gC KC_gC HN1201_gC HN1201_gC HN1_gC HNM_gC ZJ01_gC TJ_gC BYT_gC BYT_gC BYT_gC BYT_gC MY-1_gC MY-1_gC MY-1_gC MSD-12019_gC SD18_gC HLJ2017_gC HLJ2017_gC HLJ2017_gC HLJ2017_gC HLJ2017_gC HLJ201_gC Kolchis_gC HLJ201_gC Kolchis_gC HLJ201_gC SC_gC HN1201_gC HN1201_gC HLJ201_gC	370	380	390	400	410	420	VDYTCRLEG	MPSQLPTFEI 440 M.	450 T T T T T T T T T T T T T T T T T T T	460 	470 T.A.	
HLJ8_gC HN1201_gC HN1201_gC HN1201_gC HN1_201_gC HNN_gC Fa_gC HNX gC ZJ01_gC TJ_gC BYT_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC HNEY2018_gC HNEY2018_gC HNE12017_gC HNE12017_gC HLJ8_gC HLJ8_gC HLJ8_gC HLJ8_gC HLJ8_gC HLJ8_gC HN201_gC HLJ8_gC HN201_gC HN201_gC HLJ8_gC HN201_gC HN	370	380	390	400	410	420	VDYTCRLEG 430	MPSQLPIFEI 440 MI.	450 T T T T T T T T T T T T T T T T T T T	460 	470 T. A. T. A.	
HLJ8_gC KLJ8_gC HN1201_gC HN1201_gC HN1_gC HN1_gC HN1_gC HNX_gC ZJ01_gC TJ_gC BJTT_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC HLSY2018_gC HSD-12019_gC SD18_gC HLJ7_gC HSD12019_gC SD18_gC HLJ7_gC HNX_gC ZJ01_gC TJ_gC HJ7_gC HXT_gC Kaplan_gC	370	380	390	400	410	420	VDYTCRLEG	MPSQLPTFEI 440 MI.	450 T T T T T T T T T T T T T T T T T T T	460 	470 	
HLJ8_gC HN1201_gC HN1201_gC HN1201_gC HN1_201_gC HNN_gC Fa_gC HNX gC ZJ01_gC TJ_gC BYT_gC Bartha_gC MY-1_gC Majority JSY13_gC JSY7_gC HNEY2018_gC HNEY2018_gC HNE12017_gC HNE12017_gC HLJ8_gC HLJ8_gC HLJ8_gC HLJ8_gC HLJ8_gC HLJ8_gC HN201_gC HLJ8_gC HN201_gC HN201_gC HLJ8_gC HN201_gC HN	370	380 FAVCDGLCVP	390 PEARLAWSDH	400	410 CAEHPGLLM	420 VRSARPLSDLDGF	VDYTCRLEG	MPSQLPTFEI 440 MI.	450 T T T T T T T T T T T T T	460 	470 T.A. T	

Fig.7. Comparison of PRV SD-2017 gC amino acid sequence with other PRV strains.





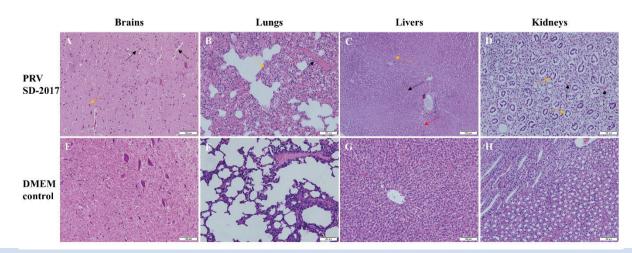


Fig.9. Histopathologic examinations of the tissue samples of brains, lungs, livers and kidneys.

DISCUSSION

Pseudorabies was one of the important infectious diseases that threatens the pig industry. Newborn piglets infected with virulent strains of PRV often exhibit high fever, depression, diarrhea, tremors, anorexia, respiratory distress, cough, and high mortality (An et al., 2013; Wang et al., 2014; Yu et al., 2014). Affected by viral immune evasion and natural genetic evolution, PRV variant outbreaks had occurred in pig farms vaccinated with Bartha-K61 vaccine in China since 2011, causing serious damage to pig herds (Tang et al., 2016; Ye et al., 2016; Ye et al., 2015). Newborn piglets infected with PRV mutants develop sudden onset with significant clinical symptoms, which lasts only about 5 hours from onset to death, and the mortality rate was as high as 50% (An et al., 2013; Wang et al., 2014; Yu et al., 2014). The Bartha-K61 vaccine was effective against lethal challenge with PRV SC classic strains, but did not provide comprehensive protection against PRV variant strains. It indicated that the PRV variant had antigenic changes and had different immunogenicity compared with the Bartha-K61 strain. Therefore, the generation of vaccines that antigenically more closely match emerging variant PRV strains may represent an added value to control these infections.

In this study, we successfully isolated a highly pathogenic PRV strain named SD-2017 from PRV-infected piglets in a Bartha-K61 vaccinated pig farm in Linyi, Shandong, China. Through virulence gene sequencing and genetic evolution analysis, the isolated SD-2017 strain was identified as a PRV epidemic variant. Homology comparison and genetic evolution analysis of the main virulence genes gE, gB and gC of PRV SD-2017 strain were carried out. The nucleotide homology of gE genes of 2017, HuB17, MY-1 and other mutant strains is between 99.4% and 100%, and there is an aspartic acid at positions 48 and 497 of the gE protein amino acid sequence (D), which is consistent with the mutation characteristics of the PRV variant strains prevalent in China. The gE protein is a major virulence protein of PRV (Wang et al., 2015). It has been reported that changes in only a few gE amino acids can alter the virulence of PRV isolates (Mettenleiter et al., 1994). The gB protein is the most conserved glycoprotein among herpesviruses and is essential for PRV to invade target cells and facilitate cell-to-cell spread (Mettenleiter 2003). Moreover, gB protein is the main immunogen of PRV and can stimulate the host to produce complement-dependent and complement-independent neutralizing antibodies (Okazaki, 2007). Compared with the Bartha-K61 strain, the SD-2017 strain and other mutant strains have three mutant forms of amino acid insertion. deletion and substitution through gB protein comparison analysis. These variants may lead to changes in the neutralizing epitope of the gB protein, thereby reducing the protective efficacy of the Bartha-K61 vaccine. According to the genetic evolution analysis of gC gene, SD-2017 strain belongs to the PRV genotype II strain circulating in China. It is on the same evolutionary branch as the Chinese PRV isolates that appeared after 2011. gC protein is another important neutralizing antigen involved in the adsorption process between PRV and target cells (Karger et al., 1998). The amino acid sequence alignment of gC protein found that compared with Bartha-K61, SD-2017 strain and most domestic isolates had seven consecutive amino acid (AAAASTPA) insertions and multiple amino acid substitutions at positions 69-75. These changes may affect the gC glycoprotein structure of the variant strains, thereby affecting the adhesion of PRV to host cells.

Rabbits are extremely susceptible to PRV infection and the clinical symptoms are very typical, so they are often used to observe the efficacy of viral challenge in quality standards for various virus strains (Pomeranz *et al.*, 2005). PRV SD-2017 strain can kill rabbits with an LD50 of $3.16 \times 10^{2.0}$ TCID50 in 500 µl DMEM. Typical symptoms observed in PRV-infected rabbits are itching, convulsions, biting at the injection site, and difficulty breathing. In this study, we isolated and identified SD-2017 strain can be used for the development of new vaccines against PRV variants, as the vaccine parent strain, or the vaccine challenge strain. In the future research, we can try to develop new live attenuated vaccines and subunit vaccines using SD-2017 genome sequence information.

CONCLUSION

In conclusion, we reported a PRV variant named SD-2017, which was the causative agent of piglet disease on a farm in Linyi, Shandong Province, China. The genetic variation analysis of the main virulence genes showed that the SD-2017 strain had multiple positions of variation, and caused important harm to piglets in the farm, which needed to be paid enough attention. At the same time, the results of this study provided a new candidate strain for the construction of vaccines against PRV variant strains.

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CONFLICT OF INTEREST

None declared.

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