Genetic characterization of 11 porcine reproductive and respiratory syndrome virus isolates in South China from 2014 to 2015

Linyang Yu†, Pandeng Zhao1,2†, Jianguo Dong1,3†, Yanling Liu1, Leyi Zhang1, Pengshuai Liang1, Lei Wang1 and Changxu Song1*

Abstract

Background: Porcine reproductive and respiratory syndrome (PRRS) has led to an enormous loss per year to the swine industry, its etiology porcine reproductive and respiratory syndrome virus (PRRSV) is a highly mutated virus in pigs. To fully understand the genetic characteristics of PRRSV genome in South China, this study collected the lung samples infected with PRRSV in Guangdong and Hainan province from 2014 to 2015 and tried to isolate the PRRSV. Finally, the complete genomes of isolated strains were sequenced and analyzed.

Methods: Virus isolation was performed in MARC-145 cells. The 13 fragments of PRRSV genome were amplified by RT-PCR and the complete PRRSV genome sequence was obtained by SeqMan program of DNASTAR7.0 software. Nucleotide and deduced amino acid (AA) sequences of NSP2 and ORF5 were aligned using the MegAlign program of DNASTAR7.0 software to determine sequence homology. A phylogenetic tree was constructed using MEGA5.2 software with the neighbor-joining method to analyze the evolutionary relationship.

Results: 11 PRRSV strains were isolated in South China from 2014 to 2015. All the isolated strains clustered into subgenotype V along with the HP-PRRSV representative strains JXA1, HuN4 and JXwn06. The subgenotype V was furtherly divided into two groups. AA sequence alignment analysis indicated that all the isolated strains had 1 AA deletion and 29 AA continuous deletion at position 481 and 533-561. Notably, GDHY strain had another 120 AA continuous deletion at position 629-748. All the isolated strains had an A137S mutation in the residue A137 of GP5 which was considered to differentiate vaccine strains. All the isolated strains had a L39I mutation in the primary neutralizing epitope (PNE) of GP5. Except GDHZ had a N34T mutation, all the other isolated strains had conserved N30, N44 and N51 glycosylation sites in the four potential N-glycosylation sites (N30, N34, N44 and N51) of GP5.

Conclusions: Our study showed that the prevalent strains in this region were highly pathogenic PRRS virus-like. Moreover, one new strain having another 120 amino acids continuous deletion except the discontinuous 30 (29+1) amino acids deletion in NSP2 region had emerged. Besides, the isolated strains had extensive amino acids substitutions in the putative signal, extravirion and intravirion regions of GP5. These results showed that PRRSV has undergone extensive variation in South China, providing some theoretical basis for researching effective vaccince to better controlling the PRRSV in this area.

Keywords: PRRSV, Phylogenetic analysis, NSP2, GP5, Mutation

* Correspondence: cxsong2004@163.com; cxsong@scau.edu.cn
† Equal contributors
1 College of Animal Science & National Engineering Center for Swine Breeding Industry, South China Agriculture University, Guangzhou 510642, China
Full list of author information is available at the end of the article

© The Author(s). 2017 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background
Porcine reproductive and respiratory syndrome (PRRS) is an important swine contagious disease across the world, leading to an enormous loss per year to the swine industry [1]. In 1987, PRRSV was first reported in the United States, and later it appeared in Europe [2, 3]. Unfortunately, after a short while, PRRS also outbroke in Asia countries. In 2006, a highly pathogenic strain of porcine reproductive and respiratory syndrome virus (HP-PRRSV) broke out in China and spreaded rapidly to most areas of China and neighboring countries [4–6]. Recently, mang NADC30 like strains had been monitored and isolated in the Middle, North-east and South-east China [7–9].

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, positive-sense single-stranded RNA virus belonging to the family Arteriviridae, order Nidovirales [10]. The PRRSV complete genome is about 15 kb in length, including at least 10 open reading frames (ORFs): ORF1a, ORF1b, ORF2a, ORF2b, ORF3, ORF4, ORF5, ORF5a, ORF6 and ORF7 [11, 12]. ORF1a and ORF1b encode viral replicase polyproteins, which are furtherly cleaved into 16 nonstructural proteins (Nsp’s), including NSP1α, NSP1β, NSP2, NSP2F, NSP2N, NSP3, NSP4, NSP5, NSP6, NSP7α, NSP7β, NSP8, NSP9, NSP10, NSP11 and NSP12 [13–15], whereas other ORFs encode the viral structural proteins GP2a, E, GP3, GP4, GP5, ORF5a, M, and N, respectively [11, 12].

To fully understand the genetic characteristics of PRRSV genome in South China, we collected the lung samples infected with PRRSV in Guangdong and Hainan provinces of most areas of China and neighboring countries [4–6]. In 2014 to 2015, we tried to isolate the samples infected with PRRSV in Guangdong and Hainan and tried to isolate the PRRSV. Finally, 11 PRRSV strains were successfully isolated and the complete genomes were sequenced and analyzed.

Methods
Clinical samples
Lung samples were collected from sick pigs infected with PRRSV in Guangdong and Hainan provinces from 2014 to 2015 and tried to isolate the PRRSV. Finally, 11 PRRSV strains were successfully isolated and the complete genomes were sequenced and analyzed.

Virus isolation
Virus isolation was performed in MARC-145 cells which were maintained in Dulbecco’s Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS; Thermo), 100 mg/mL penicillin, and 100 units/mL of streptomycin. MARC-145 cells seeded in 6-well cell culture plates (Corning Inc., USA) were incubated with the supernatants from the homogenized lung samples for 1 h, then the supernatants were discarded and DMEM was added into the 6-well cells and the cells were maintained at 37 °C with 5% CO2. The cultured cells and supernatants were harvested when cytopathic effect (CPE) appeared in 70% and the recovered strains were passaged twice in MARC-145 cells and the viral cultures of the third passage were used for genomic sequence analysis.

Primers design
To determine the full genome sequence of isolated strains, primers were designed based on the referenced PRRSV sequences available in NCBI. The primers used for complete genome sequencing were given in Table 1.

Table 1 Primers used for PRRSV genome amplification. The primers for amplifying PRRSV genome were listed in Table 1.

| Primers Name | Sequence(5'-3') | Position |
|--------------|----------------|----------|
| PRRSV1F      | ATGACGTATAGGTGTTGCT | 1–20    |
| PRRSV1R      | GTCGCACAGAGCGTGCTT | 1357–1377 |
| PRRSV1F      | CAGAATCAGGGTTGAGCCCAAT | 1255–1276 |
| PRRSV1R      | GAGCTCACCATATGTCAGCAC | 2551–2573 |
| PRRSV2F      | CCACGTGACTTGGCCCGAC | 2451–2471 |
| PRRSV2R      | CTAAGGGCCAAATCATGGA | 3780–3800 |
| PRRSV3F      | CGGCCTGCGCAACACCTGCG | 3680–3700 |
| PRRSV3R      | GAAATAACCCCAAGAGGGGA | 4029–4050 |
| PRRSV4F      | CAACCTTCTCAATCTGACAG | 3921–3942 |
| PRRSV4R      | GCAAGGCCATATTTACGAATACT | 5368–5389 |
| PRRSV5F      | CTCCTACAGTGGCTTTTGCT | 5280–5301 |
| PRRSV5R      | GAACCGTGACCCCTGAGTGC | 6521–6542 |
| PRRSV6F      | GGATGTCTCTTGTGACTTGGC | 6378–6399 |
| PRRSV6R      | CCGGCTACAGTTCATACCTAG | 7684–7706 |
| PRRSV7F      | CAGAGAGGGCGCCCTTGTGAT | 7583–7604 |
| PRRSV7R      | CAAGGCCCTCGCTAATTACCGA | 8844–8866 |
| PRRSV8F      | GGTTGATTGGGTGTTCCGGG | 8756–8777 |
| PRRSV8R      | TTTCCCTCTGAGTAGAGCGG | 10,281–10,302 |
| PRRSV9F      | GGTTTGGGGAGCTGGCCGT | 10,164–10,185 |
| PRRSV9R      | AACTGATTCCTTGGAAAGAAG | 11,400–11,421 |
| PRRSV10F     | CTCAGTCCCCTCCACATGCTT | 11,312–11,333 |
| PRRSV10R     | GAATGATTTATATACATAG | 12,613–12,632 |
| PRRSV11F     | TGAGATGTGTGGCTACATTTC | 12,498–12,518 |
| PRRSV11R     | CCCCACATTTGAGAACATTCA | 13,776–13,797 |
| PRRSV12F     | CCAAATGCTCAGAGGATTTC | 13,646–13,666 |
| PRRSV12R     | CTCCTGCGTCTCCTGGGTGTA | 14,898–14,919 |
| PRRSV13F     | TACGGTTAACGCCACATTGTC | 14,798–14,819 |
| PRRSV13R     | d(T)20AATTTCGGCCGCATGG | 15,395–15,432 |

Numbers represents the nucleotide position within the genome of CH-1a (GenBank accession number: AY032626)
RNA extraction and RT-PCR
Total RNA was extracted using TRIzol reagent (Life Technologies, USA) according to the manufacturer’s instructions. Reverse transcription was performed in a total volume of 20 μL containing 10.5 μL total RNA, 4 μL 5× reverse transcription buffer, 2 μL deoxynucleotide triphosphate (dNTP) mixture (10 mM), 1 μL 9-mer random primers (50 pM), 2 μL reverse transcriptase M-MLV (Takara, Dalian), and 0.5 μL RNase inhibitor (40 U/μL). The reactants were mixed gently, placed in a water bath at 42 °C for 1 h, then incubated on ice for 2 min. The polymerase chain reaction was conducted using PrimeSTAR HS DNA Polymerase (Takara, Dalian).

Nucleotide cloning and sequencing
PCR products were purified using the Wizard SV Gel and PCR Clean-Up system (Promega, USA), and then cloned into pEASY Simple Blunt vector (TransGen tech Co., Beijing, China). Plasmids were submitted to BGI (Guangzhou, China) for sequencing and the complete PRRSV genome sequence was obtained by SeqMan program of DNASTAR7.0 software (DNASTAR Inc., Madison, WI, USA). The complete genome sequence was submitted to GenBank and the accession no. was listed in Table 2.

Sequence alignment and phylogenetic analysis
Nucleotide and deduced amino acid (AA) sequences were aligned using the MegAlign program of DNASTAR7.0 software (DNASTAR Inc., WI, USA) to determine sequence homology. A phylogenetic tree was constructed using MEGA5.2 software with the neighbor-joining method; bootstrap values were calculated for 1000 replicates for alignment with multiple sequences of representative PRRSV sequences available in GenBank (Table 3).

Results

Phylogenetic analysis of the isolated PRRSV genome
To understand the evolution relationships of all the isolated PRRSV strains with the representative strains, phylogenetic trees were constructed using the neighbor-joining method based on the complete genome, NSP2 nucleotide and ORF5 nucleotide sequences, respectively. As shown in Fig. 1a, the isolated strains and the representative strains could be divided into five subgenotypes: Subgenotype I, II, III, IV and V. All the isolated strains clustered into subgenotype V along with the HP-PRRSV representative strains JXA1, HuN4 and JXwn06. The subgenotype V was furtherly divided into two groups. GDHZ, GDHY and GDSG belonged to Group I, sharing a high homology with HP-PRRSV strains HuN4 and GD. The other isolated strains belonged to Group II, sharing a high identity with the HP-PRRSV strains JXA1 and JXwn06. When the phylogenetic trees were constructed based on NSP2 and ORF5 gene sequences of all the isolated strains and the reference strains, they had a similar

Table 2 Information of 11 PRRSV isolated from South China. The designation, isolated year, accession No, sample, area of isolated strains were listed in Table 2.

| No. | Designation | Isolated year | Accession No. | Sample | Area    |
|-----|-------------|---------------|---------------|--------|---------|
| 1   | GDGZ        | 2014          | KY488471      | Lung   | Guangzhou |
| 2   | GDJM        | 2014          | KY488470      | Lung   | Jiangmen |
| 3   | GDMM        | 2014          | KY488472      | Lung   | Maoming |
| 4   | GDST        | 2014          | KY498542      | Lung   | Shantou |
| 5   | GDZQ        | 2014          | KY488473      | Lung   | Zhaoping |
| 6   | HNHK1       | 2014          | KY488474      | Lung   | Haikou  |
| 7   | HNHK2       | 2014          | KY488475      | Lung   | Haikou  |
| 8   | GDHY        | 2015          | KY488476      | Lung   | Heyuan  |
| 9   | GDHZ        | 2015          | KY488477      | Lung   | Huizhou |
| 10  | GDQY        | 2015          | KY488478      | Lung   | Qingyuan |
| 11  | GDSG        | 2015          | KY488479      | Lung   | Shaoguan |

Table 3 Information of the representative strains. The strain designation, area, isolated time, accession No. of representative strains were listed in Table 3.

| No. | Strain        | Area     | Time   | Accession No. |
|-----|---------------|----------|--------|---------------|
| 1   | EDRD-1        | Japan    | 1992   | AB288356      |
| 2   | Leystad virus | Netherlands | 1993 | M96262        |
| 3   | CH-1a         | Beijing, China | 1996 | AY032626      |
| 4   | BJ-4          | Beijing, China | 1996 | AF331831      |
| 5   | PL97–1        | South Korea | 1997 | AY58241J      |
| 6   | HB-1(sh)/2002 | Hebei, China | 2002 | AY150312      |
| 7   | VR-2332       | America  | 2003   | AY150564      |
| 8   | Resp PRRS MLV | America  | 2005   | AF066183      |
| 9   | SHB           | Guangdong, China | 2005 | EU864232      |
| 10  | JXA1          | Jiangxi, China | 2006 | EF112445      |
| 11  | JXwn06        | Jiangxi, China | 2006 | EF641008      |
| 12  | GD            | Guangdong, China | 2006 | EU825724      |
| 13  | HuN4          | Hunan, China | 2007 | EF635006      |
| 14  | CH-1R         | Heilongjiang, China | 2008 | EU807840      |
| 15  | JXA1-P80      | Guangdong, China | 2008 | FJ548853      |
| 16  | GDBY1         | Guangdong, China | 2008 | GQ374442      |
| 17  | NADC30        | America   | 2008   | JN654459      |
| 18  | GD-100        | Guangdong, China | 2009 | GU143913      |
| 19  | QY2010        | Guangdong, China | 2010 | JO743666      |
| 20  | GX1003        | Guangxi, China | 2010 | JX912249      |
| 21  | GM2           | Guangdong, China | 2011 | JN662424      |
| 22  | QYYZ          | Guangdong, China | 2011 | JO308798      |
| 23  | SD16          | Shanxi, China | 2012 | JX087437      |
| 24  | JLSB0         | Jilin, China | 2013   | KJ706343      |
| 25  | CHsx1401      | Beijing, China | 2015 | KP861625      |
Fig. 1 Phylogenetic trees based on the complete genome, NSP2 and ORF5 of PRRSV. (a) Complete genome; (b) NSP2 nucleotide; (c) Open reading frame5 (ORF5). The isolate identified in this study was indicated by black dots.
cluster. GDHZ, GDHY and GDSG belonged to Group I, sharing a high homology with HP-PRRSV strains HuN4 and GD. The other isolated strains belonged to Group II, sharing a high identity with the HP-PRRSV strains JXA1 and JXwn06 (Fig. 1b and c).

Alignment and analysis of NSP2 amino acid sequences
To explore the genetic characteristics of the isolated PRRSV strains, the NSP2 AA sequence of isolated strains were analyzed. The results showed that AA sequence identities among all the isolated strains ranged from 92.3%–97.7%. All the NSP2 AA sequence of isolated strains shared 75.3%–78.0%, 84.8%–88.0%, and 93.7%–98.7% AA identity with the reference strains VR-2332, CH-1a and JXA1, respectively. Compared with the NADC30 and recently isolated NADC30-like strains JL580 and CHsx1401, they shared 66.4%–67.7% AA identity. However, they only shared 11.8%–13.2% AA identity with the European genotypic strain Lelystad virus (LV). These results indicated that all the isolated strains belonged to North American genotype. The AA sequence alignment analysis indicated that all the isolated strains had 1 AA deletion and 29 AA continuous deletion at position 481 and 533. Notably, GDHY strain had another 120 AA continuous deletion at position 629–748, which was similar with the MLV vaccine strain TJM derived from HP-PRRSV TJ strain and shared 99.8% identity with TJM (Fig. 2).

Alignment and analysis of GP5 amino acid sequences
The GP5 AA sequences of all isolated strains were the same size with the representative strains. AA sequence alignments showed that AA sequence identities among all the isolated strains ranged from 95.5%–99.5%, with 86.1–89.1%, 91.0–92.5%, and 96.0–99.0% AA similarity with reference strains VR-2332, CH-1a and JXA1, respectively. They shared 85.2–95.4% AA identity with the NADC30 and recently isolated NADC30-like strains JL580 and CHsx1401. However, they only had 56.9–58.4% AA identity with the European genotypic strain LV. These results indicated that all the isolated strains had more closer relationship with the North American genotype.

As Fig. 3 showed, the AA substitutions mainly focused on the putative signal, extravirion and intravirion region. The three transmembrane region (TM1, TM2 and TM3) were relatively conserved [16]. The residues R13 and R151 of GP5 are relevant to PRRSV virulence [17, 18]. All the isolated strains had the same AA R13 with the reference strain. However, in group I, GDHZ, GDHY and GDSG had a R151K residue mutation, which was identical to the NADC30 and NADC30-like strains (JL580 and CHsx1401). The residue A137 of GP5 was considered to differentiate vaccine strains (18). Compared with VR-2332, all the other reference and isolated strains in this study had an A137S mutation.

It was reported that the primary neutralizing epitope (PNE) of GP5 AA37–44 played a vital role in inducing immune responsiveness [19]. All the isolated strains had a L39I mutation, compared to the VR-2332 strain.

The N30, N34, N44 and N51 of four potential N-glycosylation sites of GP5 are related with viral infection and antigen characteristics [20]. In group I, only GDHZ had a N34 T mutation. All the other isolated strains had conserved N30, N44 and N51 glycosylation sites.

Discussion
PRRSV has been one of the most prevalent diseases in pigs since its emergence in China, recently the NADC30 like strains have been extensively reported in most regions of China and it has caused huge economic loss to pig farmers (7–9). Considerable genetic diversity of PRRSV in field and invalid protection of current commercial vaccine to new emerging strains and in order to providing effect disease control, it is very necessary to execute frequent surveillance of the emerged new strains. In our study, 11 PRRSV strains were isolated from PRRSV positive samples in Guangdong and Hainan provinces of South China and the complete genomes were sequenced and analyzed. The results revealed that all the isolated strains were HP-PRRSV. Moreover, one isolated strain had another 120 AA continuous deletion except the discontinuous 30 (29 + 1) AA deletion in NSP2 region, which was similar with the MLV vaccine strain TJM derived from HP-PRRSV TJ strain [21].

Researchers have showed that recently isolated PRRSV strains belonged to North American strain and some strains had a close identity with NADC30 strain [22]. In our study, phylogenetic analysis indicated that all the isolated strains furtherly formed a subgenotype V with the representative strains. The subgenotype V was furtherly divided into two groups. GDHZ, GDHY and GDSG belonged to Group I, sharing a high homology with HP-PRRSV strains HuN4 and GD. The other isolated strains belonged to Group II, sharing a high identity with the HP-PRRSV strains JXA1 and JXwn06. No NADC30 like strains were isolated. These results indicated that the prevalent strain in Guangdong and Hainan was HP-PRRSV strains. Therefore, using the effective vaccine to resist the HP-PRRSV strain is a primary choise for controlling HP-PRRS outbreak in these areas. Further surveillance should be reinforced to monitor the possibly emerging new strains.

NSP2 is the most variable region in the genome of PRRSV and was used for monitoring the evolution of PRRSV in the viral genome [23–25]. Comparing the NSP2 amino acid sequence of the 11 isolated strains with the reference strains VR-2332 and CH-1a, the
Fig. 2 Alignment of the partial NSP2 amino acid sequences of isolated PRRSV strains. The deleted regions were indicated by a dotted box.
NSP2 region of 10 isolated strains had 30 AA discontinuous deletion, including 1 AA deletion at position 481 and 29 AA continuous deletion at position 532 to 560. The result was identical with other Chinese isolated HP-PRRSV strains since 2006 [26]. Moreover, 1 strain GDHY had another 121 AA continuous deletion at position 630 to 750 in NSP2 region, which was completely different from the previous reports (4–7, 17, 26). Compared with other isolated strains from pig farms, the clinic symptoms of pig infected GDHY was more severe. This result showed that a new extensive deleted PRRSV strain had emerged in Southern China. It is necessary to monitor the positive rate of PRRSV infected samples to determine whether this strain would be an epidemic strain in the future, so farmers can draw up a reasonable plan to control PRRSV outbreak.

GP5 is one of the most variant structural proteins in PRRSV, so it often been used to analyze viral genetic mutation [27, 28]. The PNE epitope and potential glycosylation sites of GP5 are related to the neutralizing activity, immune responsiveness, antigen characteristics and viral susceptibility (19, 20). Comparing to reference strain VR-2332, all the isolated strains had a L39I mutation in the PNE (AA37–44) epitope. In group I, only GDHZ had a N34 T mutation, which was different from the CH-1R and JXA1 P80 vaccine strains. All the other
isolated strains had conserved N30, N44 and N51 glycosylation sites. It was reported that the AA changes of N-glycosylation sites in GP5 could benefit mutant virus escaping the neutralization [29]. At present, the attenuated modified live PRRS vaccine could not provide complete protection. Whether the failure of vaccine protection was relevant to the variation of N-glycosylation sites of GP5 will be further researched.

The residues R13 and R151 of GP5 are related to PRRSV virulence (15, 16). All the isolated strains had the same AA R13. However, in group I, three strains GDHZ, GDHY and GDSG had a R151K residue mutation, which was the same with the NADC30 and NADC30-like strains (JL580 and CHsx1401). It was reported that NADC30 like strain was virulent and extensively epidemic in China. Therefore, it might be that the change of R151 was relevant to the virulence of isolated PRRSV strains. Besides, our researches showed that AA mutation mainly focused on the putative signal, extravirion and intravirion regions of GP5. The changes of AA in these regions might affect the form of normal GP5 in cells and promote mutation of PRRSV [27, 28].

Conclusion
In summary, we analyzed evolution characteristics of 11 PRRSV isolated strains in South China from 2014 to 2015. These results indicated that HP-PRRSV is still the prevalent strains in the region. One strain had another 120 AA continuous deletion except the discontinuous 30 (29 + 1) AA deletion in NSP2 region compared with the other isolated strains and reference strains. The GP5 of all isolated strains had many AA substitutions in the putative signal, extravirion, intravirion and extravirion regions. These results will benefit for vaccine research and disease control in this area.

Abbreviations
AA: Amino acid; dNTP: Deoxynucleoside triphosphate; E: Envelope; HP-PRRSV: Highly pathogenic porcine reproductive and respiratory syndrome virus; M: Membrane; N: Nucleoprotein; NSPs: Nonstructural proteins; ORFs: Open reading frames; PRRS: Porcine reproductive and respiratory syndrome; PRRSV: Porcine reproductive and respiratory syndrome virus; S: Spike

Acknowledgements
We thank Mr. Fu-an Liu from South China Agriculture University for his help with editorial assistance.

Funding
This work was supported by the National Key Technologies R&D Program, (2015BAD12B02–5), Guangzhou City Project (201508020062), Henan Science and Technology Project (172102110198) and Key Subject of Prevention Veterinary Science of Henan University of Animal Husbandry and Economy (MXK2016102).

Availability of data and materials
Genetic data presented in this paper are publicly available via GenBank.

Authors’ contributions
Conceived and designed the experiments: LYY, PDZ, JGD, CXS. Performed the experiments: LYY, PDZ, JGD, YLL, LYZ, PSL, LW. Analyzed the data and wrote the paper: LYY, PDZ, JGD, CXS. All authors read and approved the final manuscript.

Ethics approval and consent to participate
All the samples were collected according to the animal ethical regulation of National Engineering Center for Swine Breeding Industry (NECSBI 2015–16).

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interest.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
1College of Animal Science & National Engineering Center for Swine Breeding Industry, South China Agriculture University, Guangzhou 510642, China. 2Henan University of Animal Husbandry and Economy, Zhengzhou 450046, China. 3College of Animal Husbandry and Veterinary, Xinyang Agriculture and Forestry University, Xinyang 464000, China.

Received: 10 March 2017 Accepted: 14 July 2017

Published online: 24 July 2017

References
1. Neumann EJ, Kliebenstein JB, Johnson CD, Mabry JW, Bush EJ, Setzinger AH, Green AL, Zimmerman JJ. Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. J Am Vet Med Assoc. 2005;227:385–92.
2. Benfield DA, Nelson E, Collins JE, Harris L, Goyal SM, Robison D, Christianson WT, Morrison RB, Gorczyca D, Chladek D. Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). J Vet Diagn Invest. 1992;4:127–33.
3. Wensvoort G, Terpstra C, Pol JM, ter Laak EA, Bloemmaad M, de Kuyver EP, Kragten C, van Buiten L, den Besten A, Wagenaar F, Et A. Mystery swine disease in The Netherlands: the isolation of Leystad virus. Vet Q. 1991;13:121–30.
4. An TQ, Tian ZJ, Leng CL, Peng JM, Tong GZ. Highly pathogenic porcine reproductive and respiratory syndrome virus, Asia. Emerg Infect Dis. 2011;17:1782–8.
5. Ni J, Yang S, Bounlom D, Yu X, Zou H, Song J, Khamphouth V, Vathamha T, Tian K. Emergence and pathogenicity of highly pathogenic porcine reproductive and respiratory syndrome virus in Vitaminae, Lao People’s Democratic Republic. J Vet Diagn Invest. 2012;24:349–54.
6. Li Y, Wang X, Bo K, Wang X, Tang B, Yang B, Jiang W, Jiang P. Emergence of a highly pathogenic porcine reproductive and respiratory virus in the mid-eastern region of China. Vet J. 2007;174:577–84.
7. Zhao K, Ye C, Chang XB, Jiang CG, Wang SJ, Cai XH, Tong GZ, Tian ZJ, Shi M, An TQ. Importation and recombination are responsible for the latest emergence of highly pathogenic porcine reproductive and respiratory syndrome virus in China. J Virol. 2015;89:10712–6.
8. Zhou L, Wang Z, Ding Y, Ge X, Guo X, Yang H. NADC30-like strain of porcine reproductive and respiratory syndrome virus, China. Emerg Infect Dis. 2015;21:2256–7.
9. Li C, Zhuang J, Wang J, Han L, Sun Z, Xiao Y, Ji G, Li Y, Tan F, Li X, Tian K. Outbreak investigation of NADC30-like PRRSV in south-East China. Transbound Emerg Dis. 2016;63:474–9.
10. Cavanagh D. Nidovirales: a new order comprising Coronaviridae and Arteriviridae. Arch Virol. 1997;142:629–47.
11. De Clercq E. Treatment of corneal and conjunctival diseases with vidarabine. Arch Ophthalmol. 1981;83:934–54.
12. Snijder EJ, Posthuma CC. Discovery of a small arterivirus gene that overlaps the arterivirus 5’UTR. Proc Natl Acad Sci U S A. 2002;99:1974–8.
13. Beerens N, Selisko B, Ricagno S, Imbert I, van der Zanden L, Snijder EJ, Canard B. De novo initiation of RNA synthesis by the arterivirus RNA-dependent RNA polymerase. J Virol. 2007;81:8384–95.
14. Fang Y, Trefers EE, Li Y, Tas A, Sun Z, et al. Efficient –2 frameshifting by mammalian ribosomes to synthesize an additional arterivirus protein. Proc Natl Acad Sci U S A. 2012;109:E2920–8.

15. den Boon JA, Faaberg KS, Meulenberg JJ, Wassenaar AL, Plagemann PG, et al. Processing and evolution of the N-terminal region of the arterivirus replicate ORF1a protein: identification of two papain-like cysteine proteases. J Virol. 1995;69:4500–5.

16. Zhou L, Chen S, Zhang J, Zeng J, Guo X, Ge X, Zhang D, Yang H. Molecular variation analysis of porcine reproductive and respiratory syndrome virus in China. Virus Res. 2009;145:97–105.

17. Allende R, Kutish GF, Laegreid W, Lu Z, Lewis TL, Rock DL, Friesen J, Galeota JA, Doster AR, Osorio FA. Mutations in the genome of porcine reproductive and respiratory syndrome virus responsible for the attenuation phenotype. Arch Virol. 2000;145:1149–61.

18. Wesley RD, Mengeling WL, Lager KM, Vorwald AC, Roof MB. Evidence for divergence of restriction fragment length polymorphism patterns following in vivo replication of porcine reproductive and respiratory syndrome virus. Am J Vet Res. 1999;60:463–7.

19. Li B, Xiao S, Wang Y, Xu S, Jiang Y, Chen H, Fang L. Immunogenicity of the highly pathogenic porcine reproductive and respiratory syndrome virus GP5 protein encoded by a synthetic ORF5 gene. Vaccine. 2009;27:1957–63.

20. Ansari IH, Kwon B, Osorio FA, Pattnaik AK. Influence of N-linked glycosylation of porcine reproductive and respiratory syndrome virus GP5 on virus infectivity, antigenicity, and ability to induce neutralizing antibodies. J Virol. 2006;80:3994–4004.

21. Leng X, Li Z, Xia M, Li X, Wang F, Wang W, Zhang X, Wu H. Mutations in the genome of the highly pathogenic porcine reproductive and respiratory syndrome virus potentially related to attenuation. Vet Microbiol. 2012;157(1–2):50–60.

22. Brockmeier SL, Loving CL, Vorwald AC, Kehrli MJ, Baker RB, Nicholson TL, Lager KM, Miller LC, Faaberg KS. Genomic sequence and virulence comparison of four type 2 porcine reproductive and respiratory syndrome virus strains. Virus Res. 2012;169:212–21.

23. Allende R, Lewis TL, Lu Z, Rock DL, Kutish GF, Ali A, Doster AR, Osorio FA. North American and European porcine reproductive and respiratory syndrome viruses differ in non-structural protein coding regions. J Gen Virol. 1999;80(Pt 1):307–15.

24. Nielsen CJ, Murtaugh MP, Faaberg KS. Porcine reproductive and respiratory syndrome virus comparison: divergent evolution on two continents. J Virol. 1999;73:270–80.

25. Han J, Wang Y, Faaberg KS. Complete genome analysis of RFLP 184 isolates of porcine reproductive and respiratory syndrome virus. Virus Res. 2006;122:175–82.

26. Zhang M, Xie J, Sun L, Cao Z, Gu H, Deng S, Chen Y, Cao Z, Tang F, Su S, Zhang G. Phylogenetic analysis and molecular characteristics of 17 porcine reproductive and respiratory syndrome virus isolates in southern China from 2010 to 2011. Microb Pathog. 2013;65:67–72.

27. Cha SH, Chang CC, Yoon KI. Instability of the restriction fragment length polymorphism pattern of open reading frame 5 of porcine reproductive and respiratory syndrome virus during sequential pig-to-pig passages. J Clin Microbiol. 2004;42:4462–7.

28. Murtaugh MP, Elam MR, Kakach LT. Comparison of the structural protein coding sequences of the VR-2332 and Lelystad virus strains of the PRRS virus. Arch Virol. 1995;140:1451–60.

29. Barfoed AM, Blixenkrone-Moller M, Jensen MH, Botner A, Kamstrup S. DNA vaccination of pigs with open reading frame 1-7 of PRRS virus. Vaccine. 2004;22:3626–41.