BRIEF REPORT

Epidemiological and genetic characteristics of porcine circovirus 3 in 15 provinces and municipalities of China between 2016 and 2020

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Abstract

Porcine circovirus 3 (PCV3) is a newly emerging virus and has been found associated with porcine dermatitis and nephropathy syndrome in pigs. Compared with PCV2, research into PCV3 cap gene sequencing is deficient. To investigate the prevalence and genotype distribution of PCV3, we collected 1291 samples from 211 pig farms throughout 15 provinces and municipalities. 312 out of 1291 samples were tested positive by PCR. We further sequenced and analyzed 164 PCR-positive samples. The majority (61.8%) of isolates we sequenced belong to genotype PCV3c. PCV3c is also the dominant genotype in Hubei, Hunan, Hebei province and Chongqing city. We found 3 sites under positive selection and located in predicted epitope peptide, revealing that the pig’s immunity may be a reason those sites are undergoing highly positive selection.

Keywords: Porcine circovirus 3, Phylogenetic analysis, Sequence alignment, PCR detection

Introduction

Porcine circovirus 3 (PCV3) is the third circovirus identified in pigs which was first reported in 2016 in the United States using metagenomics [1].

PCV3 was first detected in pigs with cardiac and multi-systemic inflammation and was subsequently proven to have a relationship with Porcine Dermatitis and Nephropathy Syndrome (PDNS), as well as reproductive failure [2]. By challenged with infectious clone of PCV3, specific pathogen-free (SPF) pigs displayed PDNS-like clinical symptoms and subsequently suffered from lung, kidney, and lymph system damage [3]. Another experiment showed that PCV3-challenged mice presented symptoms of pneumonia [4]. PCV3 showed relatively high co-infection rates with porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), and torque teno sus virus (TTSV) [5–7], which are associated with reproductive disorders.

Since its discovery, PCV3 has been detected in locations worldwide and has been found in samples tracing back to 1996 [8]. It has also been identified in wild boar, ticks, mosquitoes, and dogs [9–11]. These animals could act as reservoirs for PCV3, demonstrating this virus’s complicated transmission circulation and origin. Therefore, PCV3 has already attracted a great deal of attention since it was discovered.

We previously conducted the first report in China on PCV3 [12], indicating the primary early infection information of PCV3 in the intensive pig farms, followed by whole genomic sequencing. The genome of PCV3 is...
circular, covalently closed, and consists of 2000 nucleotides. It has two major open reading frames: ORF1 encodes replication-associated proteins, while ORF2 encodes the Cap protein, which forms the capsid of the virus [2]. Compared with Porcine circovirus 2 (PCV2), research into PCV3 cap gene sequencing is deficient. To investigate the prevalence and genotype distribution of PCV3, we collected samples from 15 provinces and municipalities. We also sequenced 164 PCR-positive PCV3 samples and analyzed the epidemiological and genetic characteristics of those sequences.

**Material and method**

**Sample collection and DNA extraction**

1291 samples including hearts, spleens, kidneys, lungs, lymph nodes and anti-agglutinated blood were collected from 211 pig farms throughout 15 provinces and municipalities. Samples were resuspended in PBS (Phosphate-buffered saline) and homogenized by Qiagen TissueLyser II. Three Cycles of freezing and thawing were done to further rupture tissues and release viruses. After centrifuging under 13,400 x g for 10 min, 200 μL supernatant was used for DNA extraction by Tiangen.

**Polymerase chain reaction (PCR) detection and sequencing**

A pair of primers was used to amplify the complete ORF2 gene of PCV3: PCV3-F: TTA CTT AGA GAA CGG ACT TGT AAC G, PCV3-R: AAA TGA GAC ACA GAG CTA TATTCA. The reaction conditions were as follows: pre-denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. PCV3-FS: ACA TGC GAG GGC GTT TAC CTGTG and PCV3- FR: CGG AGC CATCCAATGGGATAACCAC were used to amplify an 840 bp amplicon (annealing at 57 °C, with the same reaction conditions as above PCR) contain the complete cap CDS gene and send to Sangon shanghai for Sanger sequencing. If more than one sample contain the complete cap CDS gene and send to Sangon.

**Data analyses and prediction**

Maxlikehood tree was constructed by IQtree2 [13]. Nucleotide and similarities were analyzed by MEGA [14]. Hyphy [15] was used to calculate selection pressure for each epitope site. Immune Epitope Database (IEDB) prediction tools [16] was used to predict potential epitope site.

**Results and discussion**

In this study, 312 out of 1291 samples were tested positive, and we further collected and sequenced 164 PCR-positive samples which were collected 15 provinces and municipalities across China from 2016 to 2020. The positive rate is 24.17%, compared with existing researches, 12.2% of 616 samples in 21 Provinces of China during 2015–2017 [17]. 5% of 4040 tonsil samples which were collected from 89 farms in 25 provinces [18]. 86.7% of 491 Lung tissue samples from 19 pig slaughterhouses across 11 cities throughout Shanxi Province [19]. 63.14% of 36 large-scale pig farms were collected from 17 provinces [20]. 13.3% of 472 samples from domestic pigs were collected in Northeast China from 2015 to 2018 [21]. 28.4% of 2125 porcine samples from 910 cases in the Midwest of the USA were collected during 2016–2018 [22]. 36.70% of 79 tissue and serum samples from commercial farms in Brazil [6]. 20.5% of 49 tissue samples from Swedish pig herds. 44.2% of 360 samples from 73 pig farms in Korea [23]. Because of the sample collection location and sample type, the positive rate in different researches are very different. Our results only represent the positive rate of the sample we used.

We further analyze the positive rate in pigs at different age, and the results show that the positive rate is higher compared with the healthy pig in the nursery and grow stage, reveal that pcv3 should be tested when the nursery and grow pig have emaciation, dermatitis or respiratory symptoms. In 222 aborted fetus samples we tested, 34.7% tested positive. A closely positive rate of Aborted fetuses also occurred in another research which was detected in 18/53 (33.9%) reproductive failure cases [24]. PCV3 should be considered and have further study when dealing with the abortion problem. The positive rate in healthy sows is 47%, higher than in healthy nursery and healthy grow pigs. More details are in Table 1.

ORF2 sequence-based Maxlikelyhood tree was constructed by IQtree2, HKY + F + R2 were chosen as the models according to BIC, Testing tree branches by SH-like aLRT with 1000 replicates. Based on the phylogenetic analysis tree shown in Additional file 1: Fig. S1, all isolates can be divided into three branches [25], and the geographic distributions of the different genotypes are

| Table 1 The PCV3 DNA positive rates in different age and healthy groups |
|-----------------|-------------|-----------|-------------|
| Group           | Positive sample | Total sample number | Positive rate (%) |
| Nursery pigs with emaciation dermatitis or respiratory symptoms | 28 | 176 | 15.9 |
| Healthy Nursery pigs | 10 | 183 | 5.4 |
| Growing pigs with PDNS like symptom | 32 | 198 | 16.2 |
| Healthy Growing pigs | 8 | 178 | 4.5 |
| Aborted fetus | 77 | 222 | 34.7 |
| Healthy sows | 157 | 334 | 47.0 |
Fig. 1 Geographical distribution and phylogenetic tree of different genotypes. **a** Distribution of different genotypes of PCV3 in different provinces. Provinces in which we have sequenced PCV3 isolates were marked as green. **b** Phylogenetic tree of our isolates with reference isolates of different genotypes. The reference strains was marked with blue dot.
Antibody may be the reason why those sites are undergoing positive selection. These results reveal that the immunity system may correspond with increasing virus fitness of hosts to make PCV3 more adaptive to the host immunity system.

So far, there is no commercial vaccine against PCV3. Since PCV3 has a relatively high positive rate and is under immune selective pressure, PCV3 may increase its immune evasion capacity through mutation. Therefore, vaccine and anti-viral drug development should be put on the agenda, and the site under positive selection should be considered when developing a vaccine.

Compared with PCV2, research into PCV3 cap gene sequencing is deficient. Our sequencing data enriches the PCV3 ORF2 data, and all the sequencing data have been uploaded to the NCBI GenBank database and can be used by other researchers for further analysis. However, a more detailed molecular characterization of PCV3 is required to monitor and study its evolution pattern to develop more specific vaccines like subunits, peptides, DNA, and RNA vaccines.

Biosecurity statement
All the virus detection procedure was performed in BSL-2 laboratory. The rest tissue sample and amplified product was heat-inactivated before disposal.

Supplementary information
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Author contributions
XK conceptualized this research and finished data curation and the original draft. CZ finished data analyses and made contribute to writing the original draft. PL, XY, QS, FX made contribute to data analyses and data curation. PQ and QH managed the project and conceptualized this research with XK. All authors reviewed the manuscript. All authors read and approved the final manuscript.
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Availability of data and materials
The gene sequences sequenced during the current study are available in the GenBank database (accession numbers were in supplementary materials).

Declarations

Ethics approval and consent to participate
Declarations

Consent for publication
Not applicable.

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

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References
1. Phan TG, Giannitti F, Rossow S, Marthaler D, Knutson TP, Li L, Deng X, Resende T, Vannucci F, Delwart E. Detection of a novel circovirus PCV3 in pigs with cardiac and multi-systemic inflammation. Virology J. 2016;13:1–8.
2. Klaumann F, Correa-Fiz F, Franco G, Sibila M, Núñez JI, Segalés J. Current knowledge on porcine circovirus 3 (PCV-3): a novel virus with a yet unknown impact on the swine industry. Front Vet Sci. 2018;5:315.
3. Jiang H, Wang D, Wang J, Zhu S, She R, Ren X, Tian J, Quan R, Hou L, Li Z. Induction of porcine dermatitis and nephropathy syndrome in piglets by infection with porcine circovirus type 3. J Virol. 2019;93:e02045-e12018.
4. Jiang Z, Wu J, Jiang M, Xie Y, Bu W, Liu C, Zhang G, Luo M. A novel technique for constructing infectious cloning of type 3 porcine circovirus. Front Microbiol. 2020;11:1067.
5. Zheng S, Wu X, Zhang L, Xin C, Liu Y, Shi J, Peng Z, Xu S, Fu Y, Ju J. The occurrence of porcine circovirus 3 without clinical infection signs in Shandong Province. Transbound Emerg Dis. 2017;64:1337–41.
6. Dal Santo AC, Cezario KC, Bennemman PE, Machado SA, Martins M. Full-genome sequences of porcine circovirus 3 (PCV-3) and high prevalence in mummified fetuses from commercial farms in Brazil. Microb Pathog. 2020;141:104027.
7. Ha Z, Xie CZ, Li JF, Wen SB, Zhang KL, Nan FL, Zhang H, Guo YC, Wang W, Lu HJ. Molecular detection and genomic characterization of porcine circovirus 3 in pigs from Northeast China. BMC Vet Res. 2018;14:1–7.
8. Sun J, Wei L, Lu Z, Mi S, Bao F, Gao H, Tu C, Zhu Y, Gong WJ, Diseases E. Retrospective study of porcine circovirus 3 infection in China. Transbound Emerg Dis. 2020;67:915–35.
9. Franco G, Grassi L, Tucciarone CM, Drigo M, Martini M, Pasotto D, Mondin A, Menandro ML. A wild circulation: High presence of Porcine circovirus 3 in different mammalian wild hosts and ticks. Transbound Emerg Dis. 2019;66:1548–57.
10. Sun W, Wang W, Xin J, Cao L, Zhuang X, Zhang C, Zhu Y, Zhang H, Qin Y, Du Q. An epidemiological investigation of porcine circovirus 3 infection in dogs in the Guangxi Province from 2015 to 2017, China. Virus Res. 2019;270:197663.
11. Ha Z, Li JF, Xie CZ, Li CH, Zhou HN, Zhang Y, Hao PF, Nan FL, Zhang J-Y, Han J-C. First detection and genomic characterization of porcine circovirus 3 in mosquitoes from pig farms in China. Vet Microbiol. 2020;240:108522.
12. Ku X, Chen F, Li P, Wang Y, Yu X, Fan S, Qian P, Wu M, He Q. Identification and genetic characterization of porcine circovirus type 3 in China. Transbound Emerg Dis. 2017;64:703–8.
13. Minh BV, Schmidt HA, Chenomor Q, Schepman D, Woodham DM, Von Haeseler A, Lanfear R. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evolut. 2020;37:1530–40.
14. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evolut. 2018;35:1547.
15. Spilman SJ, pyphyl: Python package for facilitating the execution and parsing of HyPhy standard analyses. J Open Source Softw. 2018;3:514.
16. Jespersen MC, Peters B, Nielsen M, Marcotuli M, BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. Nucleic Acids Res. 2017;45:W24–9.