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Structure analysis of capsid protein of *Porcine circovirus type 2* from pigs with systemic disease

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ABSTRACT

Economic losses with high mortality rate associated with *Porcine circovirus type 2* (PCV2) is reported worldwide. PCV2 commercial vaccine was introduced in 2006 in U.S. and in 2008 in Brazil. Although PCV2 vaccines have been widely used, cases of PCV2 systemic disease have been reported in the last years. Eleven nursery or fattening pigs suffering from PCV2 systemic disease were selected from eight PCV2-vaccinated farms with historical records of PCV2 systemic disease in Southern Brazil. PCV2 genomes were amplified and sequenced from lymph node samples of selected pigs. The comparison among the ORF2 amino acid sequences of PCV2 isolates revealed three amino acid substitutions in the positions F57I, N178S and A190T, respectively. Using molecular modeling, a structural model for the capsid protein of PCV2 was built. Afterwards, the mutated residues positions were identified in the model. The structural analysis of the mutated residues showed that the external residue 190 is close to an important predicted region for antibodies recognition. Therefore, changes in the viral protein conformation might lead to an inefficient antibody binding and this could be a relevant mechanism underlying the recent vaccine failures observed in swine farms in Brazil.

Introduction

Porcine circovirus type 2 (PCV2) is a small non-enveloped, icosahedral virus with single-stranded circular DNA that belongs to the *Circoviridae* family and genus *Circovirus*. PCV2 has been associated with different disease syndromes collectively named PCV diseases (PCVD) and is considered as one of the most economically important viral pathogens for swine worldwide.[1]

A genetic diversity within PCV2 strains has been described, although some isolates share high level of nucleotide identity among their genomes.[2,3] To date, three different PCV2 genotypes have been determinated[4]: PCV2a, PCV2b, and PCV2c. In

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2012, a variant PCV2 strain, named mutant PCV2b, showing an elongation of ORF2 by one amino acid (Lysine (K)), was detected in several PCVD cases in the United States.[5] This variant has been nowadays classified as PCV2d.

An epidemiological transition from sporadic to epidemic PCV2 systemic disease (PCV2-SD) under field conditions, has been suggested, based on a genotype shift of PCV2 isolates over time. PCV2a was predominant on pig farms with sporadic PCV2-SD in several countries prior 2000–2002, while PCV2b has been significantly more prevalent in epidemic PCV2-SD outbreaks from 2002 onwards.[4]

Besides several indirect control measures, prevention of PCV2-SD has been successfully achieved by means of PCV2 vaccination, since 2006. Up to this moment, several commercial vaccines are currently available; all of them are based on PCV2a strains.[6] Nevertheless, it has been shown that genotypes a and b share epitopes and the vaccines induce cross-protective immunity.[7,8] In Brazil, the PCV2 vaccination was introduced in commercial pig farms in 2008. However, despite of the continuous use of PCV2 vaccines, some authors have reported PCVD outbreaks in PCV2-vaccinated pigs.[9,10] The absence of a consistent model for PCV2-SD reproduction under experimental conditions[11] limits the studies to investigate the so called vaccine failures. In this regard, molecular modeling has been widely used to infer important structural information about viral proteins in the absence of experimental data. Over the last years, antigenic studies have identified six linear epitopes (A, B, C, D, E and F) from PCV2 as important sites to antibody recognition.[12,13] Also, several studies described a number of amino acids in different regions with possible role in antibody recognition.[12,14,15] In this sense, the PCV2 capsid and these epitopes have been used as a target for *in silico* structural analyses[16,17] and in experimental structural studies by Cryo electron microscopy[18] and X-ray crystallography.[13]

Aiming to study the possible alteration in the epitope conformation of the capsid protein of PCV2 owing to mutation, molecular modeling methodology was employed to build a model for the viral capsid from PCV2 isolates implicated in cases of PCV2-SD in vaccinated pig herds in Brazil.

Materials and methods

Case selection

Conventional nursery and fattening farms located in Southern Brazil with historical records of PCV2-SD were selected for this study. Pigs were vaccinated against PCV2 once or twice at weaning (21 days-old) with one out of four PCV2-vaccines available in Brazil, according to the farms' choice. Up to three pigs per farm, totalizing eleven pigs suffering from clinical signs of PCV2-SD, were euthanized and subsequent necropsied.

All selected pigs fulfilled the PCV2-SD diagnosis, according to individual disease case definition criteria that includes[1] clinical signs (growth retardation and wasting); moderate to severe histopathological lesions in lymphoid tissues (lymphocyte depletion with granulomatous inflammation); and moderate to high amount of PCV2 within microscopic lesion detected by immunohistochemistry (IHC).[19]

DNA sequencing and phylogenetic analysis

Sequencing was performed directly from PCV2 DNA amplified by PCR from lymph node samples from the eleven PCV2-SD affected pigs by the Sanger method using primers described by Mankertz et al.[2] and Dupont et al.[20] PCR products were gel purified using the MinElute Gel Extraction kit (Qiagen, Hilden, Germany), amplified using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and then purified with BigDye XTerminator Purification Kit (Applied Biosystems, Foster City, CA, USA). The nucleotide sequences were determined using an ABI3130xl Genetic Analyzer.

The obtained sequences were analyzed and assembled with the Phred/Phrap/Consed softwares.[21,22] For the phylogenetic analysis, 46 PCV2 complete genome sequences, including other representative sequences of PCV2a, PCV2b, PCV2c and PCV2d available in GenBank database (http://www.ncbi.nlm.nih.gov/GenBank) were aligned using ClustalW in MEGA 6.0 software.[23] Phylogenetic relationships among sequences were analyzed in MEGA 6.0[23] using the Maximum Composite Likelihood method. Confidence in the Neighbor-Joining (NJ) tree was estimated by 1000 bootstrap replicates.

Furthermore, amino acid sequence of the capsid protein of the eleven PCV2 isolates (Genbank accession number KT719404, KT819159, KT819160, KT819162, KT819163, KT819164, KT819165, KT819166, KT819167, KT819169 and KT819170) were aligned to the PCV2 crystallographic structure (Protein Data Bank ID: 3R0R:A) using the Clustal program[24] and plotted by EPSprint server.[25]

Molecular modeling of PCV2 capsid protein

A PCV2 sequence (KT719404) was selected to generate a representative model of the capsid protein monomer of the PCV2 by I-TASSER online server.[26] This model was built using as template the PCV2 monomer consensus sequence ($PCV2^{CS}$) structure (PDB ID: 3R0R:A) without the N-terminal portion.[13] Although the PDB structure does not contain the N-terminal portion in comparison with cryo-electron microscopy (Cryo-EM) reconstruction models,[18,27] it has the highest resolution (3R0R: 2.35 Å; 3JCI: 2.9 Å; non-deposited density map: 4.5 Å), which improves the atomic positions accuracy. The criteria of low energy folding were used to select the best model that was subsequently validated and used to build the capsid structure. Stereochemical quality and accuracy of the predicted model were evaluated using Ramachandran plot analysis.

The pentamer was generated by SYMMDOCK server[28] and the generated models were visualized and compared to $PCV2^{CS}$ capsid protein using the PyMOL Version 1.3 (Schrodinger, LLC). Additionally, the model was submitted to the structure analysis at PDBSum server,[29] in order to make structural inferences about the residues predicted to epitopes recognition.[13] The capsid structure was built using icosahedral symmetry by Chimera 1.10.2 software.[30]

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