ORIGINAL RESEARCH PAPER

Veterinary Science

UNVEILING THE GENOMIC DIVERSITY OF PORCINE CIRCOVIRUS 4 BY BIOINFORMATICS ANALYSIS

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ABSTRACT

Porcine Circovirus 4 (PCV4) is the latest type of Porcine Circoviruses (PCVs) and found to be associated with several clinical manifestations in pigs, although exact pathogenesis is still unclear. This study aims to reveal the genetic diversity of different strains of PCV4 that were detected in different locations. Accordingly, seventy numbers of complete genome of PCV4 and 13 genome sequences of other PCVs were retrieved from public database and phylogenetic analysis, pairwise identity and intergenomic diversity analysis were done with different online and offline bioinformatics tools. Phylogenetic analysis showed that the PCV4 sequences formed a separate clade and the genetic variation among different PCV4 strains were between 0.0-2.3%. The genetic diversity analysis presented in this study will be helpful to conduct future research in designing molecular diagnostics for detection of PCV4, however, more detailed study in future is required for better genomic characterization of this virus.

INTRODUCTION:
Earlier there were three types of Porcine circoviruses (PCV1, PCV2 and PCV3), but in 2019 a novel PCV has been identified which was designated as Porcine circovirus 4 (PCV4) [1]. PCV4 primarily infects pigs. PCV4 is a DNA virus which is non-enveloped and have icosahedral capsid. The genome of PCV4 is single stranded circular DNA. The length of PCV4 genome is 1770 bases and contains two major ORFs, *viz.*, ORF1 and ORF2. The ORF1 (*rep* gene) encodes replicas protein and ORF2 or *cap* gene encodes capsid protein which is the only structural protein as well as the most immunogenic protein [2].

In 2019, PCV4 was first detected in pigs from China [1]. Subsequently, it has been detected in several other countries like Korea [3], Thailand [4], Malaysia [5] and Europe [6]. Although, majority of PCV4 cases were reported from China but gradually it is being detected in many other countries either in fresh samples or in archived samples. So, it is important to reveal the genetic diversity among the PCV4 strains that were detected in several geographical locations. Therefore, this study aims to analyze the genomic diversity among different strains of PCV4 by using bioinformatics tool.

MATERIALS AND METHODS:
Seventy (70) numbers of PCV4 complete genome sequences were retrieved from NCBI virus database (https://www. ncbi.nlm.nih.gov/labs/virus/vssi/) and similarly 5, 4, and 4 numbers of complete genome sequences of PCV1, PCV2 and PCV3 were retrieved respectively, for comparison.

Phylogenetic analysis was carried out with 83 numbers of PCVs complete genome sequences. Phylogenetic analysis was done in MEGA 11 software [7]. At first, the 83 sequences were subjected to multiple sequence alignment by using ClustalW program present in MEGA 11. The phylogenetic tree was then constructed by Neighbor-Joining method [8]. Substitution model used was Tamura-Nei [9] and test of phylogeny was done with bootstrap method using 1000 replicates.

The pairwise identity and molecular grouping of PCVs was determined by using Sequence Demarcation Tool Version 1.2 (SDTv1.2) program [10]. The intergenomic distances among 70 strains of PCV4 were calculated by using VIRIDIC web portal [11].

RESULTS AND DISCUSSION:
The genome length of all the 70 sequences of PCV4 strains retrieved from NCBI virus database were 1770 nucleotides and earlier studies also revealed similar length of PCV4 genome [1, 2, 12]. The major ORFs of PCV4 were ORF1 and ORF2 and the observed length of ORF1 and ORF2 were 891 and 687 nucleotides, respectively [1].

The phylogenetic analysis done in MEGA 11 software revealed that the all the types of PCVs (PCV1, 2, 3 and 4) formed separate clades (Figure 1). Similar results were observed in previous studies and it was demonstrated that PCV4 and mink circoviruses shares common ancestor rather than with other PCVs [1,2].
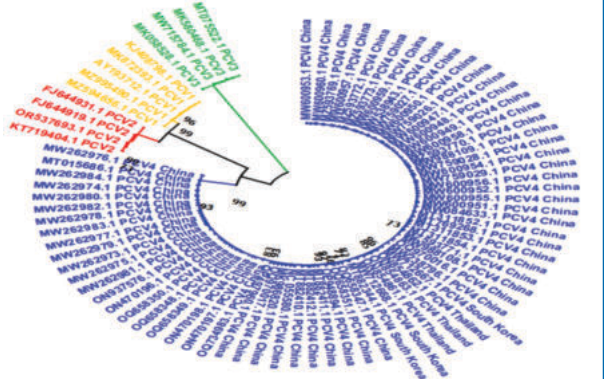

Figure 1: Phylogenetic tree based on complete genome of PCV4 was constructed with MEGA 11 software. Each clade with different colour represents different types of PCVs. Seventy sequences of PCV4 with GenBank accession nos. and country are shown in navy blue colour.

The pairwise identity of PCV1, 2, 3 and 4 was determined by SDT software and it was observed that all the four PCVs from four different molecular group (Figure 2) and the genomic

identities among different types of PCVs is low. Earlier investigations also showed that PCV4 has very low identities with other PCVs which ranges from 43.2 to 51.5% [1]. Moreover, identity analysis of *cap* gene sequences of four PCVs revealed that PCV4 has lowest identity with PCV3 followed by PCV1 and PCV2 [1]. Again, the intergenomic distance among PCV4 strains as determined by VIRIDIC ranges from 0.0- 2.3 % (Figure 3).
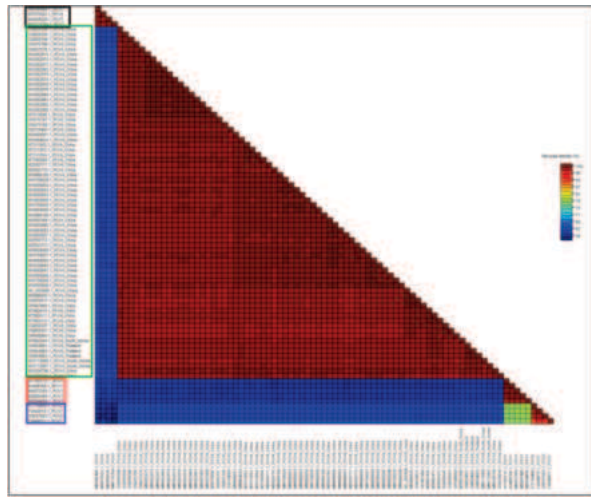

Figure 2:
Pairwise identity analysis of different types of PCVs in SDT software. Sequences represented with GenBank accession nos. belonging to different molecular groups (types of PCVs) are demarcated with different coloured solid boxes. Sequences in red, blue, black and green boxes represents PCV1, PCV2, PCV3 and PCV4, respectively.
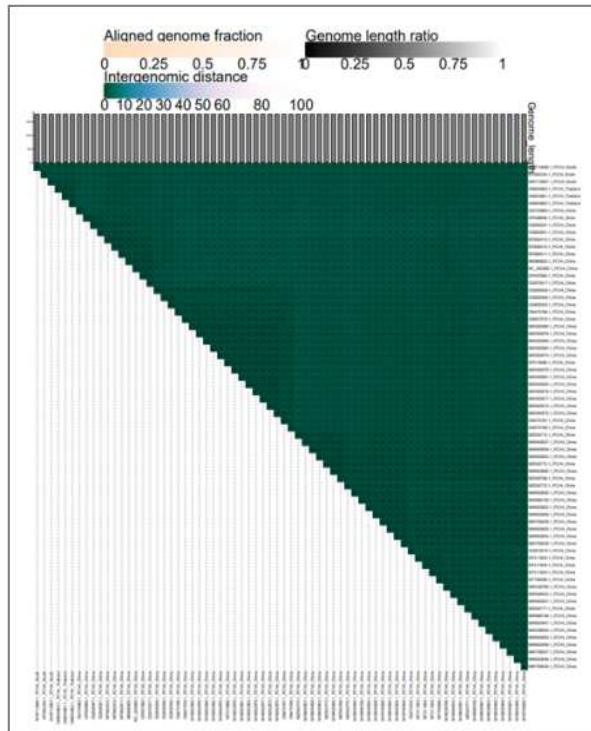

Figure 3:
Nucleotide diversity analysis of various strains of PCV4 by VIRIDIC web portal.

Hence, from this study it can be concluded that strains of PCV4 circulating in different countries were of different origin and they were genetically diverse. And, the findings of this study will aid in designing molecular diagnostics for detection of PCV4 in future.

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