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A comprehensive analysis of circulating porcine circovirus 2 (PCV2) genotypes from 2008 to 2023 in India

K Anbu Kumar, PL Sujatha and P Devendran

Abstract

Porcine circovirus-2 (PCV2) infections in pigs are endemic in India and cause a variety of adverse health effects ranging from ill-thrift, growth retardation to reproductive failure. The virus is present as sub-clinical infection even in healthy herds. Worldwide, eight genotypes of PCV2 have been identified from PCV2a to 2h and additional genotypes and recombinant types have been proposed. This study reveals that six genotypes of PCV2, *viz*, PCV2a, PCV2b, PCV2d, PCV2f, PCV2g, PCV2h and a few recombinant strains, are reported from India. However, genotypes PCV2c and PCV2e are not detected in India. Based on data from Genbank, strains of PCV2d genotype are the predominant circulating genotype in India (55.64%), with two distinct clades; one of which is found predominantly in the north east and another in south of India. Among the recently delineated genotypes, PCV2f and PCV2g are detected in the north east of India, with PCV2g strains being phylogenitically related to PCV2d strains and PCV2f being phylogenetically related to PCV2a strains. The PCV2h strains, another recently categorized genotype, are so far not detected in the north east of India. The PCV2h are phylogenitically aligned with PCV2b strains. The PCV2b and PCV2a genotypes are lesser in proportion among the circulating Indian PCV2 strains.

Keywords: Porcine circovirus, circulating porcine, sero-prevalence, immunological pressure

Introduction

Porcine circovirus 2 (PCV2) is an important viral pathogen of farmed pigs and wild pigs worldwide first identified in 1998, almost simultaneously in North American and then in Europe [1]. PCV2 was first recorded in India in 2007 [2]. PCV2 is spread by the oro-nasal route of infection. The virus is shed in feces and other secretions from the infected pigs. Notably, PCV2 is shed in the infected boar's semen and sows milk. The infection is vertically transmitted. PCV2 causes a chronic infection in pigs of all ages, which leads to loss of productivity manifested as poor growth rate, immune suppression leading to other secondary infection and reproductive failure in adult pigs [1]. PCV2 infection can lead to early embryonic mortality, mummified fetuses, and weak piglets which do not survive. PCV2 infected gilts are more prone to reproductive failure than sows which develop resistance in due course. PCV2 infection in post weaning piglets, especially from 3 weeks of age to 16 weeks of age, causes severe growth retardation and ill thrift. The sero-prevalence of PCV2 is relatively high worldwide, including India, and can reach upto 80% of swine population [3]. The PCR positivity rate varies on the sensitivity and specificity of the assay employed and can also reach over 70%. The capsid protein of PCV2 is the antigen conferring neutralizing immune response. Cell mediated immune response against the capsid and replicase proteins of PCV2 in infected pigs has been documented [1]. Inactivated and recombinant protein vaccines against PCV2 have been demonstrated to be beneficial under experimental and farm conditions [1]. Live attenuated vaccines are not available for immunization against PCV2. The capsid protein of PCV2 has considerable variation, with the number of amino acid residues ranging from 233, 234 or a few more. The genetic diversity of PCV2 capsid gene has led to the classification of the virus in genotypes from PCV2a through to PCV2h [4, 5]. A recent publication has even proposed PCV2i clade [6]. However, the current consensus is that there are no serological distinctions among PCV2 strains circulating worldwide. The absence of serotypes among PCV2 is also evidenced by vaccination trials which show vaccination with one genotype based vaccine confers protective immunity against other genotypes [1, 5]. In addition current serological assays cannot distinguish the genotypes of PCV2.

The molecular epidemiology of PCV2 virus has been a subject of keen observation for the past decade in India [1], where vaccination against PCV2 is minimal and vaccines are not available. This poses an interesting opportunity to look into the population structure of PCV2 in the absence of vaccine induced immunological pressure. This study reports a comprehensive analysis of the circulating genotypes in India as of May 2023.

2. Materials and Methods

2.1. Dataset

The full length sequences of PCV2 ORF2 gene, submitted from India, upto end of May 2023 were retrieved from the NIH genetic sequence database, Genbank using the search terms “India + PCV2 + capsid + complete”. The search retrieved 133 complete gene sequences of PCV2 ORF2 originating from India. The sequences were manually examined and found to meet the criteria of being submitted from India. The list of sequences analyzed in this study are provided in Table 1. Partial sequences of PCV2 ORF2 submitted from India were not included in this study.

2.2. Sequence Alignment and Phylogenetic Analysis

Nucleotide acid sequences of the PCV2 ORF2 submitted from India were analyzed using Molecular Evolutionary Genetics Analysis software suite version 11 (MEGA XI) [7]. The sequences were aligned with ClustalW algorithm in the MEGA XI package. Maximum Likelihood tree was reconstructed with the indicated best-fitting model and 1000 bootstrap replicates. Genotyping was then performed using a phylogenetic approach according to Franzo *et al.* 2015. The phylogenetic tree visualization was generated and annotated using interactive tree of life version 6 [8] (iToL v6).

2.3. Minimum spanning tree visualization

The minimum spanning tree visualization was constructed using GrapeTree SA version [9]. Precalculated maximum likelihood phylogenetic tree in Newick format (with 133 PCV2 ORF2 sequences) was uploaded into the GrapeTree SA and the metadata containing information on genotype, region and year of characterization were provided.

3. Results

3.1. PCV2 genotype diversity

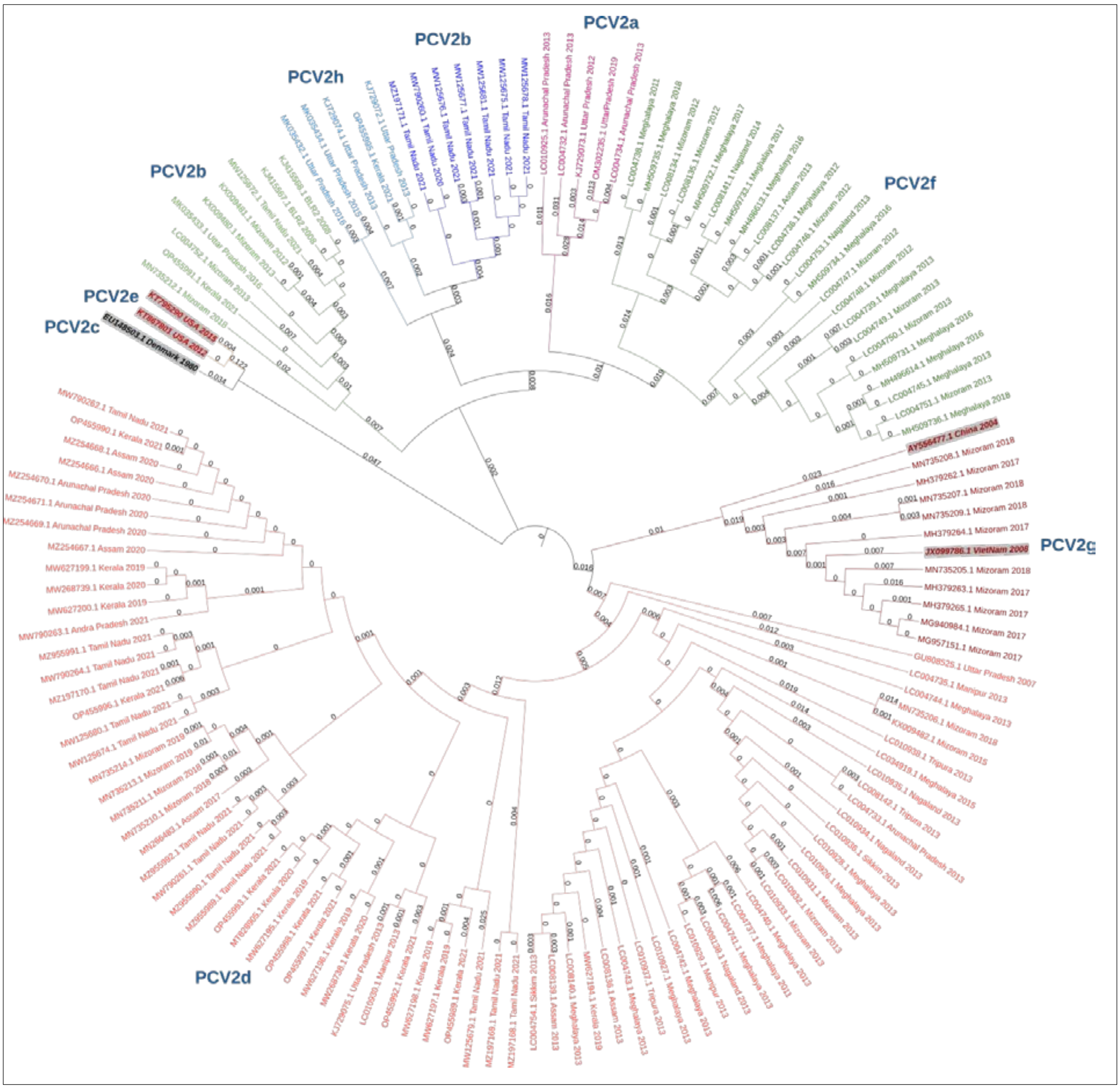


Fig 1: A maximum likelihood tree constructed from 133 complete PCV2 ORF2 (capsid) genes. The Genbank accession number, place and year of sampling of each strain are indicated.

The classification of the PCV2 strains circulating in India, based on their complete ORF2 (capsid) gene sequence (N=133), into genotypes (Figure 1), indicate that the PCV2d is the predominant genotype in circulation (55.63%), with at least two major clades (subtypes). The PCV2g strains, which account for 7.5% of circulating strains, are phylogenetically related to PCV2d strains. PCV2a and PCV2f strains are phylogenetically related and account for 3.75% and 17.29%, respectively, of all circulating strains. PCV2b strains, which account for 12.03% of all circulating strains, fall into two separate clades. One of the PCV2b clades, only consisting of strains from south of India, are closely related to PCV2h strains, which account for 3.75% for all circulating strains. The PCV2c and PCV2e genotypes were not recorded in India.

3.2. Regional and temporal prevalence

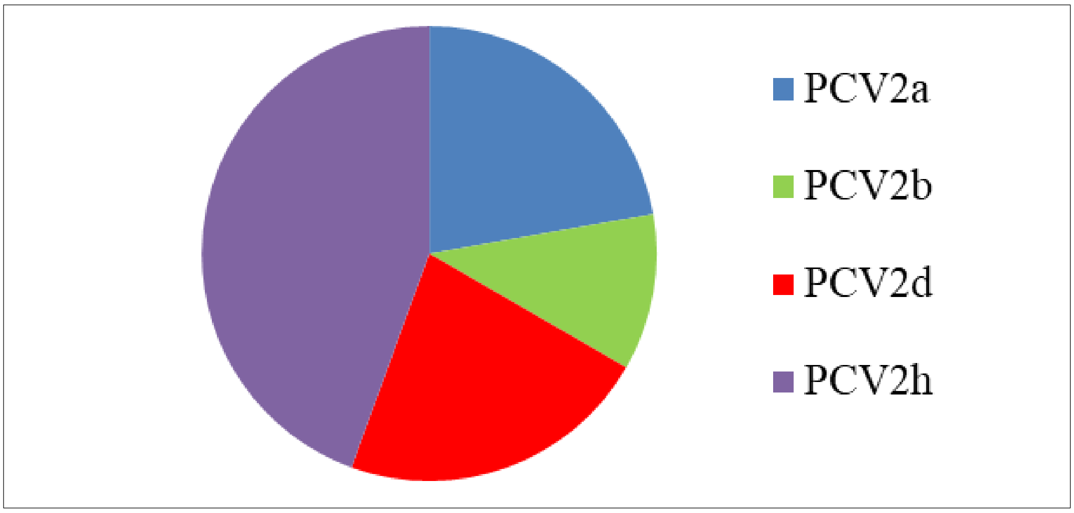


Fig 2: Pie chart of PCV2 strains (N=9) from the Northern States of India

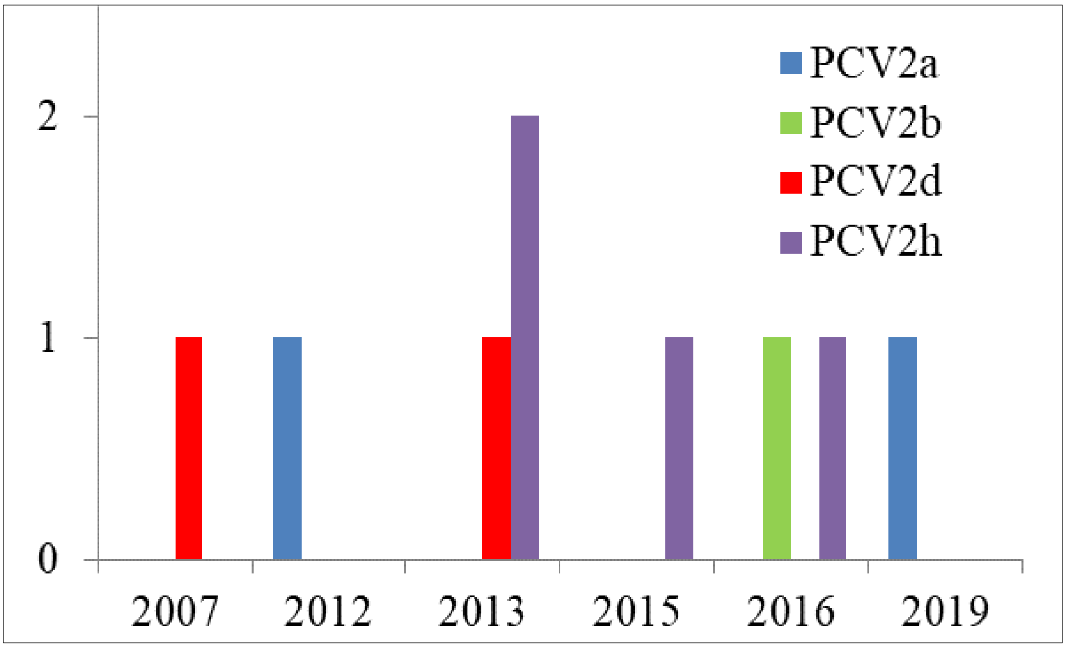


Fig 3: Temporal distribution of PCV2 strains (N=9) of different genotypes in the Northern States of India

The small sample size (N=9) of the complete ORF2 sequences of PCV2 from north, all from Uttar Pradesh, consisted of PCV2a, PCV2b, PCV2d and PCV2h. The newly delineated [4, 5] genotype PCV2h was reported from 2013 onwards, whereas PCV2g and PCV2f were not recorded. Notably, the earliest PCV2 detection in India is from sample collected from Uttar Pradesh.

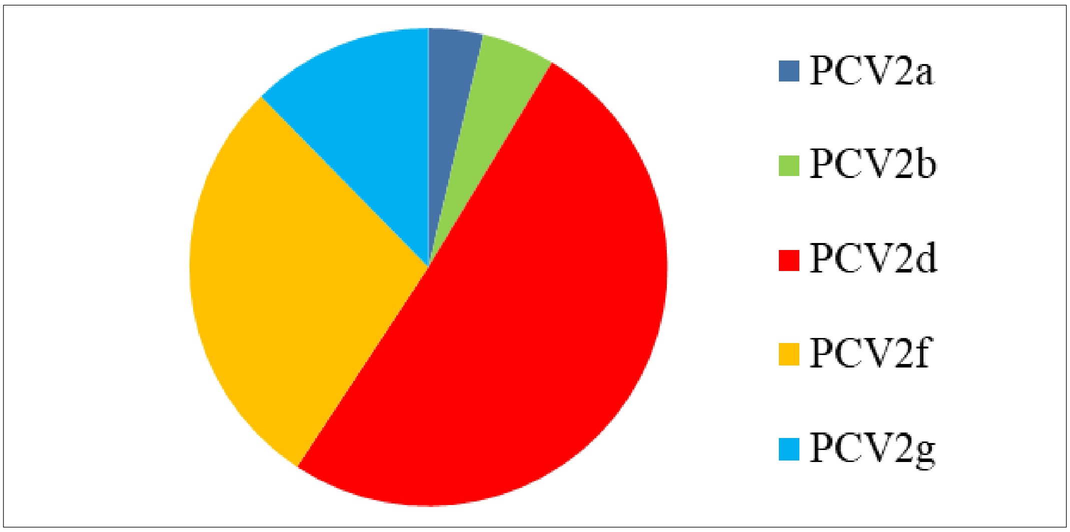


Fig 4: Pie chart of PCV2 strains (N=81) from the North Eastern States of India

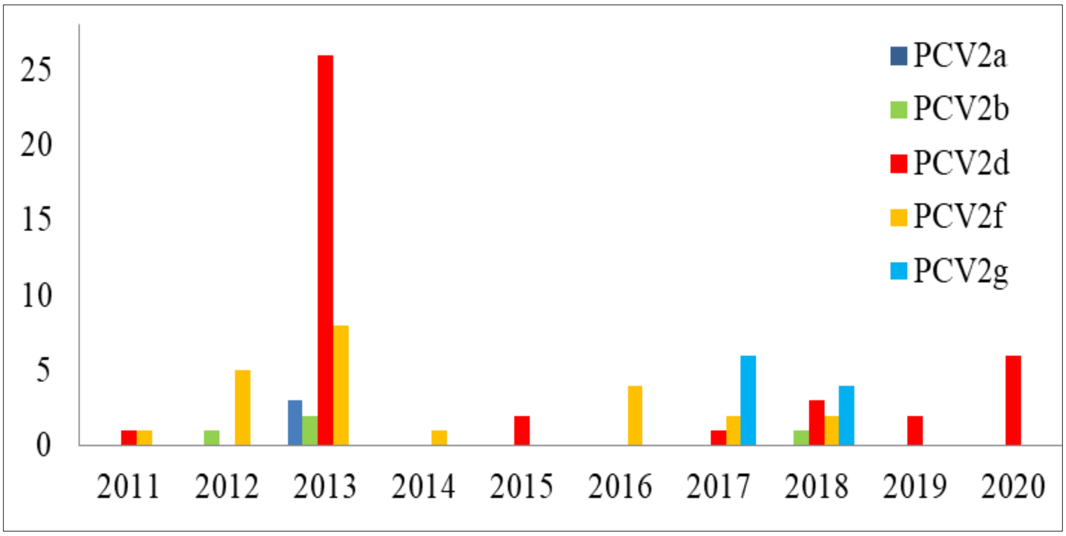


Fig 5: Temporal distribution of PCV2 strains (N=81) of different genotypes in the North Eastern States of India

PCV2f are detected from the north eastern states of Assam, Meghalaya, Mizoram and Nagaland in samples collected from 2012 onwards. Whereas, the PCV2g are reported only from Mizoram during the years 2017 and 2018. Interestingly, there is a high reporting of strains of PCV2d genotype in the year 2013 from north east.

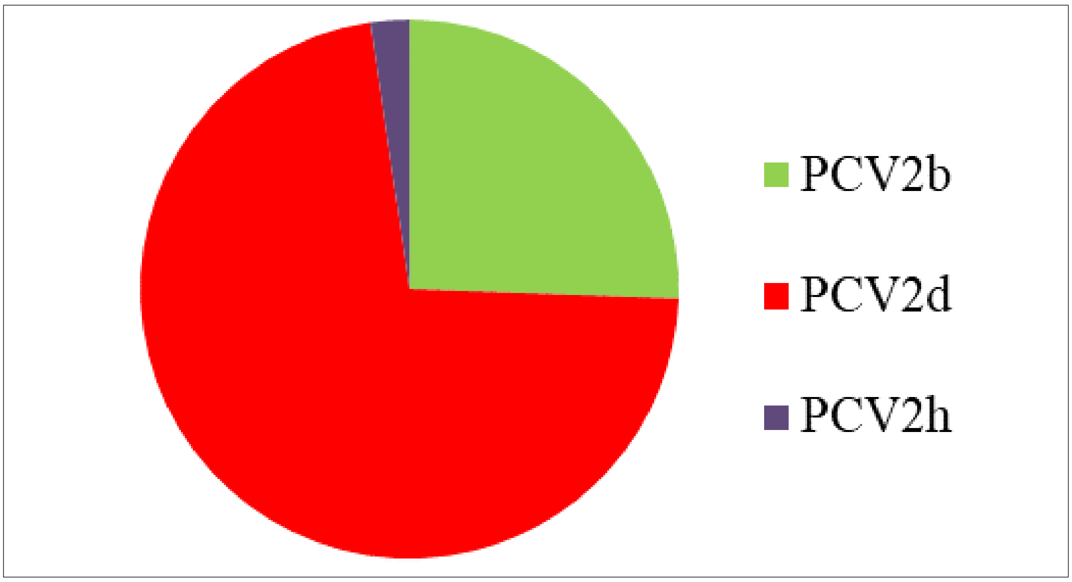


Fig 6: Pie chart of PCV2 strains (N=43) from the Southern States of India

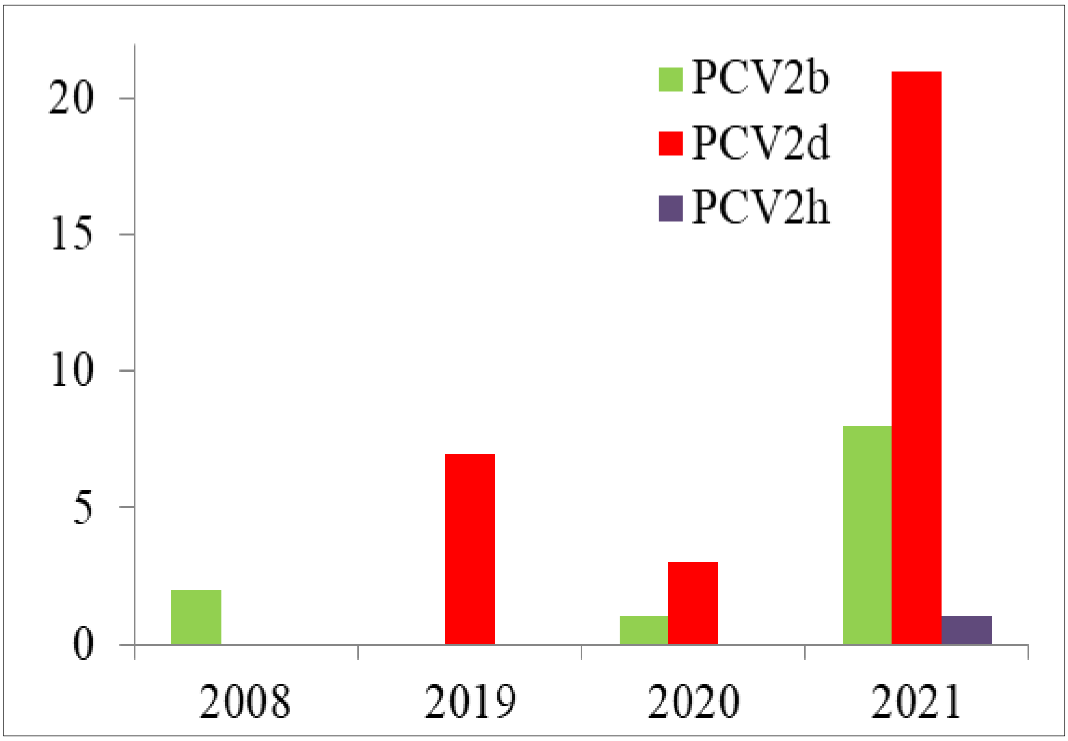
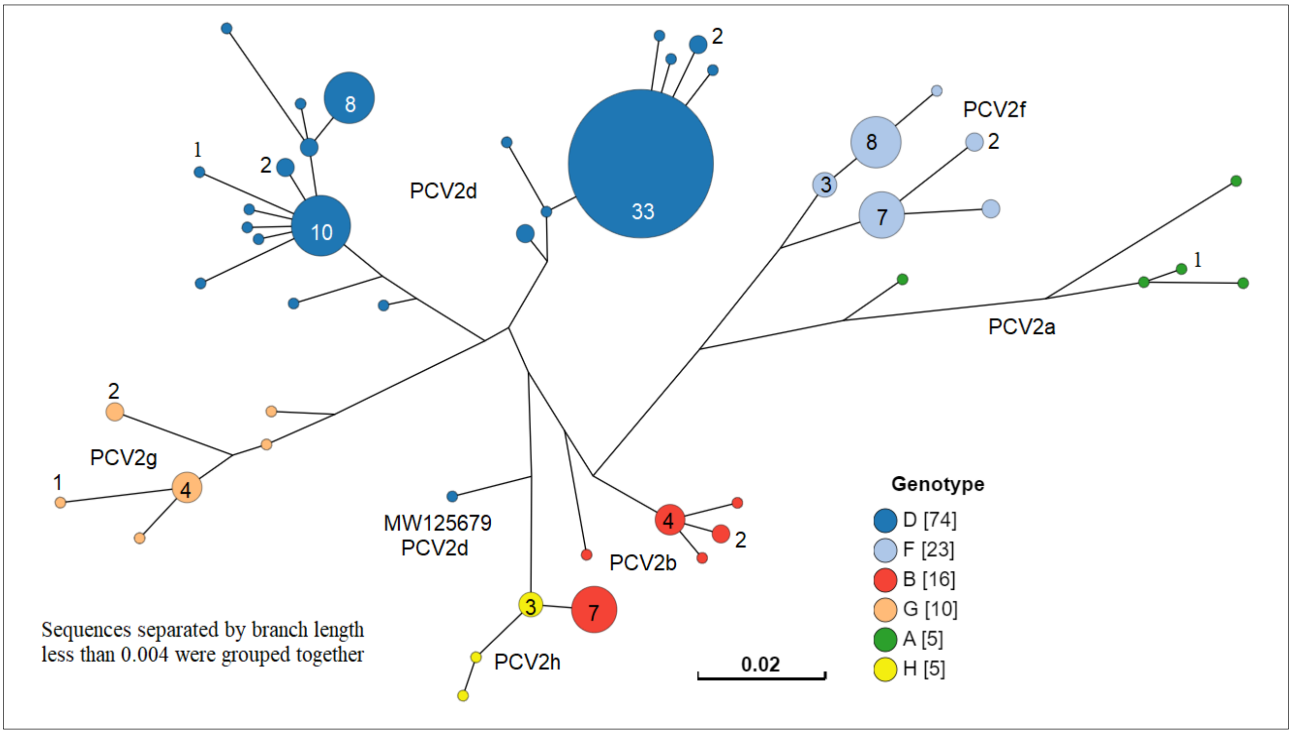


Fig 7: Temporal distribution of PCV2 strains (N=43) of different genotypes in the Southern States of India

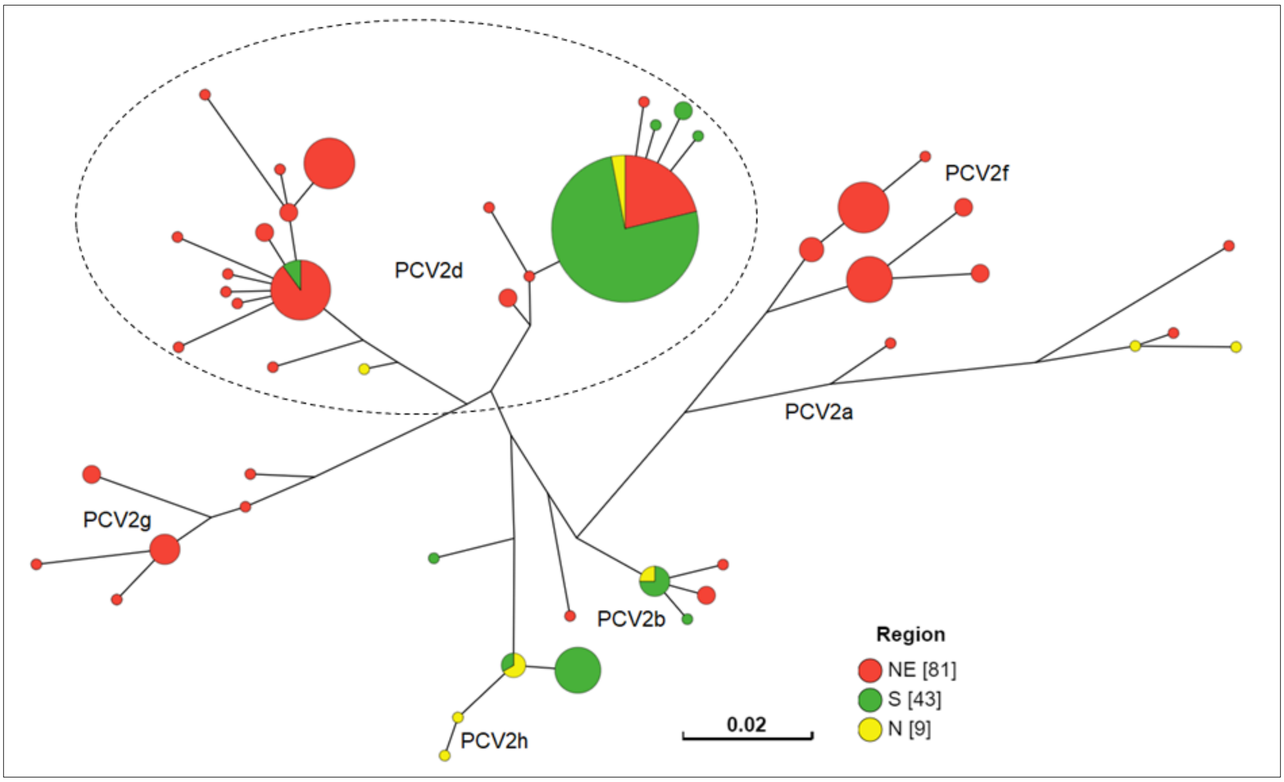
The circulating PCV2 strains reported in the southern states are of PCV2d and PCV2b genotypes, with one PCV2h reported. 72% of the PCV2 strains reported from Southern States of Tamil Nadu, Kerala and Andhra Pradesh are of PCV2d genotype.

3.3. Population structure of PCV2 circulating strains



Sequences separated by branch length less than 0.004 were grouped together

Fig 8: Population structure of PCV2 strains with genotype metadata generated using Grape Tree SA. The genetic distance is indicated in the scale



Fige 9: Population structure of PCV2 strains with region metadata (NE –North East, S – South, N – North) generated using GrapeTree SA. The genetic distance is indicated in the scale.

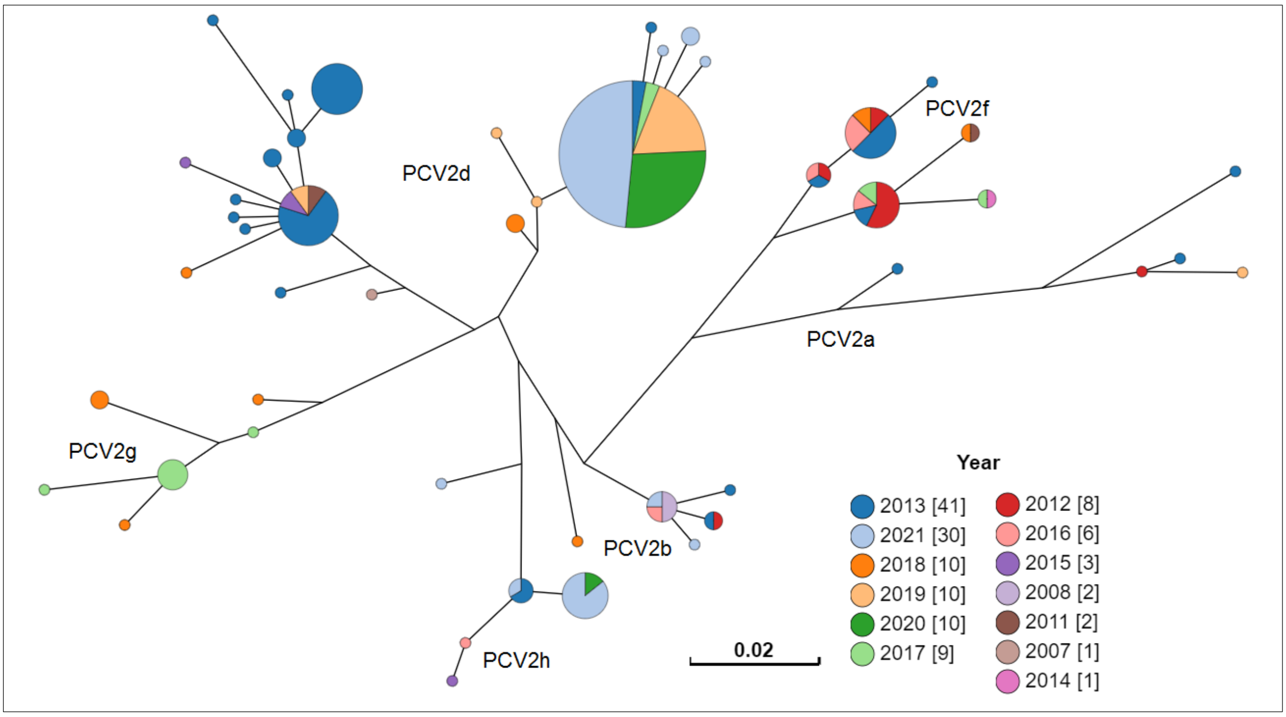


Fig 10: Population structure of PCV2 strains with temporal metadata generated using Grape Tree SA. The genetic distance is indicated in the scale.

The PCV2d strains reported from India upto May 2023 branch into two clades (Figure 8) and interestingly one of the clades is predominant north eastern states and another predominant in the southern states (Figure 9). The majority of the strains from PCV2d clade which is predominant in the southern states are reported from 2019 onwards (Figure 10), whereas the strains of other PCV2d clade which is predominant in the north east are reported in 2013. The strains of the genotypes PCV2f and PCV2g are only reported from north eastern states as of May 2023 (Figure 8 & 9). Similarly, the PCV2b strains also branch into two clades, one of which is phylogenetically close to PCV2h strains (Figure 8).

4. Discussion

The first report of PCV2 infection in India was made in 2006 [2]. Subsequently, the role of PCV2 in reproductive failure, post weaning wasting and ill thrift was documented around the country [1, 2, 10-16] PCV2 ORF2 gene sequences have been submitted to the Genbank from India in increasing numbers since the first report of PCV2 in 2006/2007. This analysis of complete ORF2 sequences in Genbank submitted from India, upto May 2023, reveals that PCV2 strains belonging to six genotypes, *viz*, PCV2a, PCV2b, PCV2d, PCV2f, PCV2g, PCV2h and some recombinant strains, are found. However, strains of genotypes PCV2c and PCV2e are not detected in India. Two distinct clades are found in the analysis of strains of PCV2d genotype, which is the predominant circulating genotype in India. Among the two PCV2d clades, one is more prevalent in the southern states (Figure 9) and has been reported mostly in the last five years (Figure 10). The other clade of PCV2d is predominantly reported from north eastern states (Figure 9) in the year 2013. This indicates the potential differences in the different origins of transmission of the two PCV2d populations. The north eastern states owing to their proximity to South East Asia, where pig rearing is common with high pig population, could have acquired the PCV2d infection from across the border through cross border trade or wild boars. The source of PCV2d infection in the southern states could be ascribed to trade, especially, in breeding animals, as the shore line of the south does not favours cross border transmission. Among the recently delineated genotypes [4, 5], PCV2f strains, which are phylogenetically related to PCV2a strains are reported only in the north east of India. Interestingly, the PCV2f strains are not reported after 2018. Similarly, PCV2g strains, another newly delineated strain which are phylogenitically related to PCV2d genotype, are reported only from north eastern states. The geographical limitation of PCV2 strains belonging to genotypes PCV2f and PCV2g to only north eastern states again indicates the potential transmission by trans boundary routes from South East Asia. On the same note, the PCV2h strains, another recently categorized genotype [4, 5], are so far not detected in the north east of India (Figure 9). The PCV2h are phylogenitically aligned with PCV2b strains.

The population structure of PCV2 strains in India reflects the global scenario [1, 4, 5] with increase in PCV2d genotype population, especially after 2013 in the Indian sub-continent. The PCV2d genotype accounts for 55.63% of all reported full length ORF2 sequences in the Genbank from India. Overall, the PCV2b and PCV2a genotypes are lesser in proportion among the reported circulating Indian PCV2 strains. Though the current inactivated or recombinant PCV2 vaccines based on strains of PCV2a or PCV2b genotype are effective against the other genotypes, it would be preferable to use vaccines based on strains of PCV2d genotype [1] to prevent vaccine induced virus evolution.

5. References

1. Karuppannan AK, Opriessnig T. Porcine Circovirus Type 2 (PCV2) Vaccines in the Context of Current Molecular Epidemiology. Viruses. 2017;9(5):99.
2. Kumar GS, Sharma R, Paliwal OP. Occurrence and pathology of postweaning multisystemic wasting syndrome. In: Compendium of Invited Papers and Abstracts of XXIII Annual Conference of Indian Association of Veterinary Pathologists; c2006. p. 83.
3. Karuppannan AK, Ramesh A, Reddy YK, Ramesh S, Mahaprabhu R, Jaisree S, *et al.* Emergence of Porcine Circovirus 2 Associated Reproductive Failure in Southern India. Transbound Emerg Dis. 2016;63(3):314-20.
4. Franzo G, Cortey M, Olvera A, Novosel D, Castro AM, Biagini P, *et al.* Revisiting the taxonomical classification of Porcine Circovirus type 2 (PCV2): Still a real challenge. Virol J. 2015;12:131.
5. Franzo G, Segalés J. Porcine Circovirus 2 Genotypes, Immunity and Vaccines: Multiple Genotypes but One Single Serotype. Pathogens. 2020;9(12):1049
6. Wang Y, Noll L, Lu N, Porter E, Stoy C, Zheng W, *et al.* Genetic diversity and prevalence of porcine circovirus type 3 (PCV3) and type 2 (PCV2) in the Midwest of the USA during 2016-2018. Trans bound Emerg Dis. 2020;67(3):1284-1294.
7. Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol. 2021;38(7):3022-3027.
8. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res. 2021;49(W1):W293-W296.
9. Zhou Z, Alikhan NF, Sergeant MJ, Luhmann N, Vaz C, Francisco AP, *et al.* Grape Tree: Visualization of core genomic relationships among 100,000 bacterial pathogens. Genome Res. 2018;28(9):1395-1404.
10. Anoopraj R, Rajkhowa TK, Cherian S, Arya RS, Tomar N, Gupta A, *et al.* Genetic characterisation and phylogenetic analysis of PCV2 isolates from India: indications for emergence of natural inter-genotypic recombinants. Infect Genet Evol. 2015;31:25-32.
11. Mukherjee P, Karam A, Barkalita L, Borah P, Chakraborty AK, Das S, *et al.* Porcine circovirus 2 in the North Eastern region of India: Disease prevalence and genetic variation among the isolates from areas of intensive pig rearing. Acta Trop. 2018;182:166-172.
12. Barman NN, Nath B, Kumar V, Sen A, Dutta TK, Dutta B, *et al.* The emergence of porcine circovirus 2 infections in the Northeastern part of India: A retrospective study from 2011 to 2017. Trans bound Emerg Dis. 2018;65(6):1959-1967.
13. Mukherjee P, Karam A, Chakraborty AK, Baruah S, Pegu R, Das S, *et al.* Identification of a novel cluster of PCV2 isolates from Meghalaya, India indicates possible recombination along with changes in capsid protein. Infect Genet Evol. 2019;71:7-15.
14. John JK, Kattoor JJ, Sethi M, Tomar N, Das T, Saikumar G. Genetic diversity of Indian porcine circovirus type 2 (PCV2) isolates (2006-2018). The Indian Journal of Animal Sciences. 2020;90(6):842-846.
15. Rajkhowa TK, Lalnunthanga P, Rao PL, Subbiah M, Lalrohlua B. Emergence of porcine circovirus 2g (PCV2g) and evidence for recombination between genotypes 2g, 2b and 2d among field isolates from non-vaccinated pigs in Mizoram, India. Infect Genet Evol. 2021;90:104775.
16. Parthiban S, Ramesh A, Karuppannan AK, Dhinakar Raj G, Johnson Rajeshwar J, Hemalatha S, *et al.* Emergence of novel Porcine circovirus 2 genotypes in Southern India. Transbound Emerg Dis. 2022;69(4):1804-1812.