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Research Article

Molecular characterization of *Porcine circovirus* (PCV) field isolates from Punjab, India

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Abstract

Porcine circovirus (PCV-2) causes devastating losses to piggeries all over the world. It causes Porcine circovirus associated diseases (PCVADs) in pigs, a very common clinical sign of which is reproductive failure. The current study was based on the detection and molecular characterization of PCV-2 from aborted fetus samples. The samples were collected from different pig farms located in 7 districts of Punjab during July 2019 and March 2020. Diagnostic Rep protein ORF-1 gene PCR was used to detect the viral nucleic acid. Overall, 6 samples (6 out of 12) were found to be positive for PCVs. The ORF-2 (*Cap*) gene was characterized by cloning and sequencing, followed by phylogenetic analysis and selection pressure analysis of the sequenced gene. Phylogenetic analysis based on the ORF-2 gene showed that the PCV-2 virus isolates in this study belong to the PCV-2d strain. The selection pressure analysis on the ORF-*2* gene of the virus indicates evidence of both positive and negative selection. On the basis of this study, it was found that PCV-2 is circulating in the pig farms of Punjab which might be due to the combination of a lack of proper hygiene practices, limited routine testing and the absence of a vaccination program.

Keywords: PCV-2d strain, Porcine circovirus, Punjab, Reproductive failure, Swine

Highlights

- PCVs detected in 6 of aborted pig fetus samples (n=12) from Punjab farms.
- ORF-2 sequencing identified two species PCV-1 and PCV-2 in the isolates.
- Phylogenetic analysis confirmed PCV-2d as dominant strain in Punjab.
- Selection pressure analysis showed positive selection on PCV-2 *cap* gene.
- Study highlights need for vaccination to control PCVADs in India.

INTRODUCTION

Pig farming is a profitable business that provides livelihood to numerous farmers, not only in Punjab but pan-India. The pig population of Punjab including both, exotic and indigenous breeds was 52, 961 according to the 20th Livestock Census (2019) with a significant increase of 64.4% since the 19th Livestock Census (2012). Despite the adoption of proven scientific management skills in advanced countries, the piggery industry is susceptible to losses due to numerous pathogens that cause mortality or wasting in pigs, thereby producing huge economic losses. One such pathogen that is responsible for a multitude of complex multifactorial swine diseases is *Porcine circovirus* 2 (PCV-2). PCV-2, first described in Canada (Clark, 1997; Harding, 1997) is the causative agent of Porcine circovirus associated diseases (PCVADs), which are a major cause of concern for swine industries worldwide. PCVAD is an umbrella term used to describe the many disease syndromes associated with the virus, such as PCV2-subclinical infection (PCV2-SI), PCV2-systemic disease (PCV2-SD), porcine respiratory disease complex (PDRC), PCV2-enteric disease (PCV2-ED), etc. (Segalés, 2012). Factors such as import of the germplasm and exotic breeds, lack of proper diagnostic measures and low vaccination rates contribute towards the spread of infectious viral diseases such as PCVAD.

Four species of porcine circoviruses have been identified till now, including PCV1, PCV2, PCV3, and PCV4. PCV1 was discovered in 1974 as a picornavirus-like contaminant in PK-15 cells and has since been widely accepted as non-pathogenic. PCV2, first described in 1997, was isolated from a pig affected with postweaning multisystemic wasting syndrome (PMWS). It is the virus responsible for the majority of economic losses to the swine industry. Unlike conventional isolation methods, PCV3 and PCV4 were

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discovered in 2016 and 2019, respectively, by metagenomics sequencing on tissue samples from pigs with health conditions like porcine dermatitis and nephropathy syndrome (PDNS), myocarditis and reproductive failure (Opriessnig et al., 2020). The Genome of PCVs is circular ssDNA with about 1758-1760 (PCV1) and 1767-1768 (PCV2), 1999-2001(PCV3), 1770 (PCV4) nucleotides (Meehan et al., 1997; Guo et al., 2010; Maity et al., 2023). The genes that code for major products Replicase (*Rep*) and Capsid (*Cap*) proteins are ORF-1 and ORF-2, respectively (Lv et al., 2014). The nucleotide sequence identity between *Rep* and *Cap* gene of PCV1 and PCV2 is 83% and 67%, respectively (Palinski et al., 2017). The capsid protein is considered highly variable (Fenaux et al., 2000; Finsterbusch & Mankertz, 2009) and the most immunogenic (Nawagitgul et al., 2002) viral protein. Sequencing ORF-2 is less labor intensive than sequencing the whole genome, thus it is widely used for epidemiological and phylogenetic studies as a target fragment (Lv et al., 2014).

In India, the incidence of PCV-2 infections and PCV-2 associated reproductive failure have been reported by several studies (Sharma & Saikumar, 2008; Bhattacharjee et al., 2015; Karuppannan et al., 2016; Barman et al., 2018; Mukherjee et al., 2018), indicating a rise in the disease occurrence over the past decade. The typical signs that point towards the disease include wasting, dyspnea, enlarged lymph nodes, diarrhea, pallor, and jaundice (Harding, 2004), while it has also been associated with late term abortions, still births and mummifications (Brunborg et al., 2007; Madson et al., 2009). The present study aimed at the detection and molecular characterization of Porcine circovirus from cases of abortions and stillbirths of pigs at farms located in Punjab, India.

MATERIALS AND METHODS

Cases and samples: The aborted fetus samples (Fig. 1a & 1b), collected from organised piggeries (n=12) rearing large white Yorkshire, Landrace and crossbred pigs, located in 7 districts of Punjab (Barnala, Bhatinda, Ludhiana, Kapurthala, Muktsar, SAS Nagar and Sangrur), India, with complaints of neonatal mortality, stillbirths, abortions and mummifications were investigated. Organ samples (reproductive organs, lung, brain, liver, spleen, lymph nodes, stomach content, and placental fluid) from each aborted piglet were collected and aliquoted in PBS from necropsied animals during July 2019- March 2020. The samples were transported on ice from the farms to the molecular diagnostic laboratory, GADVASU, Ludhiana and stored at -80°C until further processing.

DNA extraction and PCV2 detection: Tissue samples such as reproductive organs, lungs, brain, liver, spleen, lymph nodes, stomach content and placental fluid were collected from the aborted fetus and triturated using a mortar and pestle. The triturated organ samples were subjected to DNA isolation procedure using the standard phenol-chloroform-isoamyl alcohol method (Sambrook & Russell, 2006). The isolated DNA was used for the initial screening of PCV-2 using previously described primers by Lee et al. (2007). The primers are based on the ORF-1 gene of PCV-2 and have an amplicon size of 565 base pairs.

Amplification and sequencing of the ORF-2(*Cap*) gene: The ORF-2 gene of the PCV-2 genome was amplified by using primers described by Monroy et al. (2014) with an amplicon size of 935 bp. PCR reactions were performed in 50 µL reaction volume containing 10X PCR Buffer (Mg^{2+}), 20 pM of each primer, 2.5 mM of each dNTP, 0.1 µg/µL BSA and 1.25U DNA Polymerase (Takara Prime STAR GXL DNA Polymerase). The PCR reaction conditions were initial denaturation at 98°C for 30s, followed by 35 cycles of denaturation at 98°C for 10s, annealing at 55°C for 45 seconds, extension at 72°C for 60s and final extension step at 72°C for 12 minutes. Finally, the PCR products were analysed by gel electrophoresis using 1.5% agarose gel. Following separation using electrophoresis, the amplified products were visualized using a UV transilluminator and documented using a UV gel documentation system (GBox, Syngene).

The PCR products of the relevant size (935bp) were purified from the gel using the QIAquick Gel Extraction Kit (Qiagen, Germany) by the procedure mentioned in the kit. The sequencing of the amplified products in both directions with forward and reverse primers used for PCR amplification of ORF-2 gene was outsourced (Agri Genome Labs Pvt. Ltd, Kakkanad, Kerala, India). The trimmed sequences of the forward and reverse complement of the reverse sequences obtained following sequencing were aligned using Clustal ω and the final assembled sequences were compared by BLAST analysis. The final sequences of ORF-2 gene of 4 samples were submitted to NCBI Genbank with accession numbers PCV-1 (OK349501, OK349502) and PCV-2 (OK349503, OK349504), respectively.

Phylogenetic analysis: A phylogenetic analysis based on the *Cap* gene sequences was performed to establish the genotypes of the PCV2 and PCV1 strains in this study.

Multiple sequence alignment was carried out using the Clustal ω programe of the Molecular Evolutionary

Genetics Analysis (MEGA X) software (Kumar et al., 2018). The best evolutionary model was selected using the same software based on the lowest Bayesian information criterion (BIC) scores and the Akaike information criterion (AICc)-corrected scores. For PCV-2, a phylogenetic tree was constructed using the Hasegawa-Kishino-Yano nucleotide substitution and five discrete Gamma categories for rates of substitution among sites with Maximum Likelihood (ML) as the statistical method. Similarly for the group of PCV-1, PCV-2, PCV-3, PCV-4 and Chicken cyclovirus (CCV) sequences, a phylogenetic tree was also constructed using the HKY+G evolutionary model with the ML statistical model. The reliability of the trees was determined by using 1000 bootstrap replicates. MEGA X software was also used for the estimation of evolutionary divergence and for determining the selection pressure on the PCV variants. CCV sequences were included as outgroup as this genus belongs to the same family as circoviruses (Rosario et al., 2017).

Selection pressure analysis: Selection pressure on the ORF-2 gene of PCV-2 virus was detected using the online Datamonkey Server using three statistical methods, namely Mixed Effects Model of Evolution (MEME), Fast Unconstrained Bayesian App Roximation (FUBAR) and Internal-branch Fixed Effects Likelihood (IFEL). Significance level was set separately for each of the analyses, i.e. $p<0.1$ for MEME and FUBAR, and posterior probability ≥ 0.9 for FUBAR.

RESULTS

Screening for PCV prevalence in clinical samples: Tissue samples such as lungs, liver, spleen, brain, lymph nodes, reproductive organs as well as stomach content and placental fluid were collected from the aborted fetuses. Among the 12 collected samples, 6 were positive (one each from SAS Nagar, Kapurthala, Barnala, Muktsar, and two from Ludhiana district) for PCV-2 based on PCR amplification of the ORF-1. The diagnostic primers used were designed in the conserved region, which could amplify both PCV1 and PCV2. The ORF-2(*Cap*) gene of 4 of these viral isolates was cloned and sequenced. Both PCV-1 and PCV-2 were detected in the samples.

Sequence analysis: The sequences of the 4 viral isolates obtained post-sequencing were analyzed using NCBI Nucleotide BLAST. Out of the four, 2 sequences showed close nucleotide identity with PCV-1 genotype and the other 2, with the PCV-2 genotype.

Phylogenetic analysis: To analyze the genetic relationship of PCV-1 and PCV-2 strains circulating in the Punjab region of India, the ORF-2 (Cap) gene of 2 isolates each of PCV-1 and PCV-2 were cloned and sequenced. The sequences thus obtained were subjected to phylogenetic analysis using the MEGA X software along with reference PCV-1,-2,-3,-4 and CCV sequences taken from GenBank as mentioned in Table 1. The resulting phylogenetic tree (Fig. 2) showed that the PCV-2 isolates in this study have a close relationship with the PCV-2d genotype. This was confirmed by comparing the amino-acid sequence of the isolates in this study to PCV-2d strains mentioned in the study by Wei et al. (2019) as certain amino acids are unique to this strain. The phylogenetic analysis of PCV-1,-2,-3,-4 and CCV sequences taken from NCBI-GenBank, along with PCV-1 and PCV-2 isolates from this study, is presented in Fig. 3.

Table 1. Details of PCV sequences along with their accession numbers, used in this study for bioinformatics analysis

SL. No.	Accession No.	Length (bp)	Year of collection	Country	Genotype
1	MK770354	699	2019	India	PCV1
2	JN133303	671	2011	Belgium	PCV1
3	KJ808815	674	2013	China	PCV1
4	KU697141	505	2015	USA	PCV2
5	KJ729075	654	2013	India	PCV2
6	MT814846	699	2020	South Korea	PCV2
7	LC004742	654	2013	India	PCV2
8	GU799576	651	2009	USA	PCV2b
9	JN133304	699	2002	Belgium	PCV2a-2C
10	LC004732	699	2014	India	PCV2
11	MF139077	654	1999	China	PCV2f

Cont. Table 1.

Table 1., Cont. ...

SL. No.	Accession No.	Length (bp)	Year of collection	Country	Genotype
12	MF314313	654	2015	Thailand	PCV2
13	MG715490	702	2016	South Korea	PCV2d
14	MK545052	702	2019	China	PCV2d
15	MT376317	702	2015	South Korea	PCV2
16	KT870146	666	2015	USA	PCV2e
17	KF035066	702	2012	China	PCV2
18	KX831483	654	2015	China	PCV2d
19	MF314318	702	2015	Thailand	PCV2
20	MW790263	702	2021	India	PCV2d
21	MT501730	702	2017	South Korea	PCV2
22	LC383445	702	2014	Japan	PCV2
23	MF142267	702	2012	China	PCV2d
24	MT376321	702	2017	South Korea	PCV2
25	LC004742	654	2013	India	PCV2
26	LC010926	651	2014	India	PCV2
27	LC008136	654	2013	India	PCV2
28	KX831481	651	2015	China	PCV2b-1B
29	JN662682	651	1999	France	PCV2
30	HM565923	651	2007	Argentina	PCV2
31	GU799576	651	2009	USA	PCV2b
32	KT795287	666	2014	USA	PCV2e
33	KT795290	666	2015	USA	PCV2e
34	KY806023	651	2009	England	PCV2b
35	KT867799	666	2006	USA	PCV2e
36	LC383448	651	2015	Japan	PCV2
37	JF927976	651	2002	Taiwan	PCV2b
38	KY806061	651	2014	England	PCV2b
39	JN006462	651	2010	Romania	PCV2
40	JQ866914	651	2008	Thailand	PCV2b
41	EU146505	656	2008	Denmark	PCV2c
42	KC620516	651	2011	South Korea	PCV2
43	MF39077	654	1999	China	PCV2f
44	MF278779	654	1999	China	PCV2f
45	EU148503	656	2008	Denmark	PCV2c
46	MK347405	699	2018	China	PCV2a
47	JF927979	699	2002	Taiwan	PCV2a
48	KC699529	651	2012	USA	PCV2
49	MN593289	618	2017	Poland	PCV2a
50	JN133305	699	2009	Slovakia	PCV2a-2D
51	FJ998185	645	2005	China	PCV2g
52	JX099786	654	2013	Vietnam	PCV2g
53	KP420197	654	2010	Poland	PCV2g
54	KM042398	702	2009	Vietnam	PCV2h
55	JQ81592	702	2011	Vietnam	PCV2h
56	MT350519	564	2017	Spain	PCV3
57	MK645716	564	2018	Brazil	PCV3
58	MN725080	564	2018	Malayasia	PCV3
59	MT311854	684	2018	China	PCV4

Cont. Table 1.

Table 1., Cont. ...

SL. No.	Accession No.	Length (bp)	Year of collection	Country	Genotype
60	MW600958	684	2012	China	PCV4
61	MN162710	624	2019	China	PCV4
62	MN379600	684	2017	USA	CCV
63	MN379599	684	2017	USA	CCV
64	MN379598	678	2017	USA	CCV

Table 2. The codon sites detected under statistically significant (in bold) diversifying selection by different methods for *Orf*-2 gene of PCV-2 isolates

Codon	MEME (p<0.1)		FUBAR (Post pr.≥0.9)		IFEL (p<0.1)		Statistical method consensus
	ω^+	p-value	dN-dS	P(α<β)	Normalised dN-dS	p-value	
14	0.124	**0.092**	0.183	0.754	0.087	**0.045**	2
33	0.10	0.19	0.118	0.705	0.088	**0.082**	1
36	0.184	**0.083**	0.041	0.736	0.113	**0.041**	2
37	0.323	**0.097**	0.317	0.784	0.174	**0.044**	2
43	430.603	**0.047**	-2.631	0.436	-	-	1
52	22.383	**0.091**	0.736	0.621	-	-	1
53	-	-	-0.063	0.572	0.378	**0.098**	1
59	119.499	**0.016**	0.562	0.657	-	-	1
68	68.338	**0.017**	2.159	0.744	1.514	**0.058**	2
113	-	-	-0.008	0.720	0.123	**0.067**	1
118	6.671	**0.0006**	-0.618	0.519	0.904	**0.047**	2
137	268.314	**0.076**	-4.544	0.392	-	-	1
148	0.223	**0.089**	-0.092	0.701	0.127	**0.060**	1
153	22.487	**0.077**	2.142	0.756	-	-	1
169	-	-	14.624	**0.927**	5.330	**0.099**	2

Selection pressure analysis: The selection pressure analysis of the cap gene of PCV2 sequences by the online Datamonkey server revealed that evidence of positive selection was detected by all three statistical methods, i.e., MEME (Fig.4), FUBAR (Fig. 5a and 5b) and IFEL (Fig. S1). The results of all the three methods have been summarized in Table 2.

DISCUSSION

PCV-2 was first detected in India in the year 2008 by Sharma and Saikumar (2008) in clinical samples of a pig farm located in North India. Subsequently, PCV2 was detected in Uttar Pradesh (Anoopraj et al., 2015), Tamil Nadu (Karuppannan et al., 2016) and Meghalaya (Mukherjee et al., 2018). In the current study, the presence of PCVs was demonstrated by ORF 1 gene PCR in 6 of the total 12 tissue samples examined. All the samples included in this study were aborted fetus/ mummified/stillbirth samples from various pig farms of Punjab, as also reported by Sharma and Saikumar (2008), and Karuppannan et al. (2016). Vaccination of swine herds is still not a common practice in India, despite the presence of a wide variety of vaccines such as CIRCOVAC® (Merial), Porcilis® PCV (Merck), Fostera ™ PCV (Pfizer Animal Health), etc. Vaccination plays an important role in the prevention and control of PCVADs, and the occurrence of several outbreaks (Anoopraj et al., 2015; Karuppannan et al., 2016; Mukherjee et al., 2018) across the country indicates the need for incorporation of vaccination in pig rearing.

This study also characterized and phylogenetically analyzed the ORF-2 of two PCV-1 (OK349501, OK349502), and two PCV-2 (OK349503 and OK349504) strains obtained from tissues of clinical samples obtained in this study. As the cap gene is highly variable and immunogenic, it is considered suitable for the construction of a phylogenetic tree. Thus, molecular characterization of the isolates was done based on the analysis of the cap gene. The PCV-2a genotype was most prevalent until 2003, when a genotype shift occurred in favor of PCV-2b, with an increase in outbreak severity. A similar genotype shift

Porcine circovirus-2d circulating in Punjab



Fig. 1. Aborted fetus samples suspected of PCV-2 infection

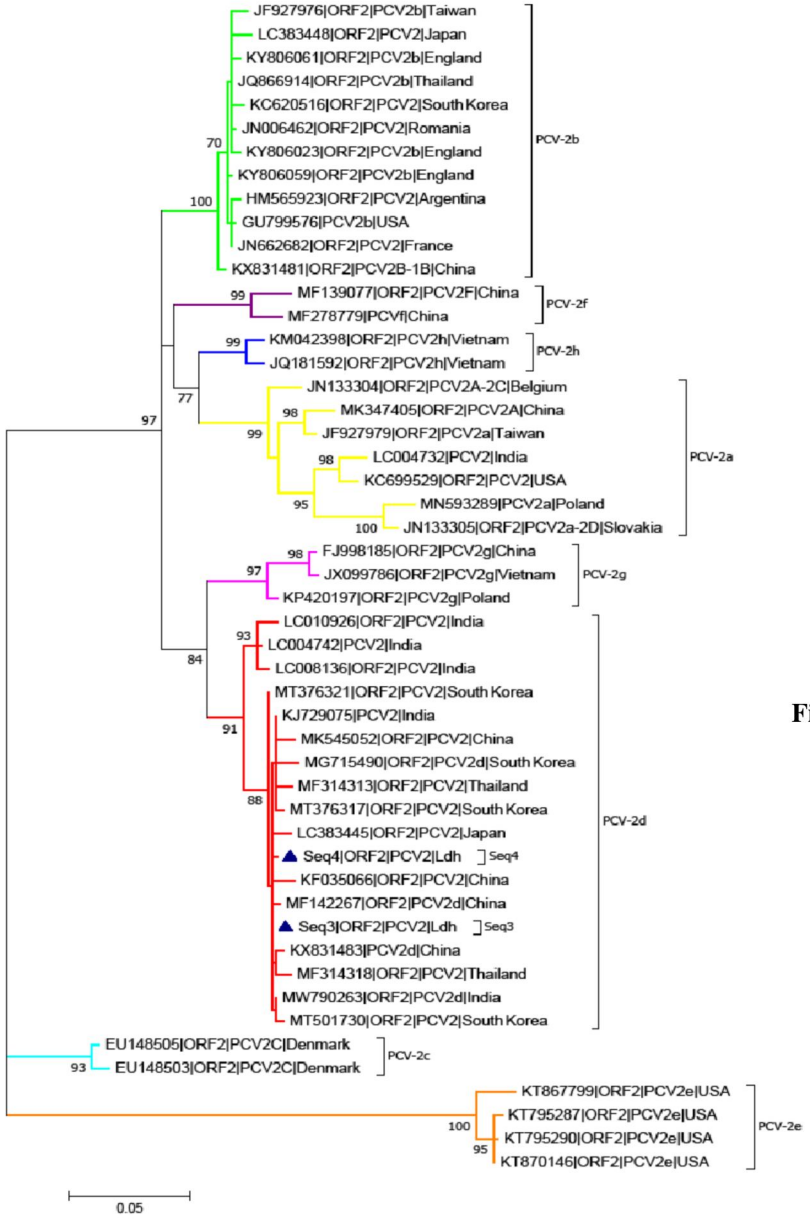


Fig. 2. The phylogenetic analysis based on the nucleotide sequences of the cap gene of the viral isolates of this study (denoted by▲) with PCV-2 reference strains downloaded from NCBI-GenBank, constructed using the MEGA-X software. ML with HKY+G evolutionary model was used to construct the tree with bootstrap values (based on 1000 replicates) showing if >70%

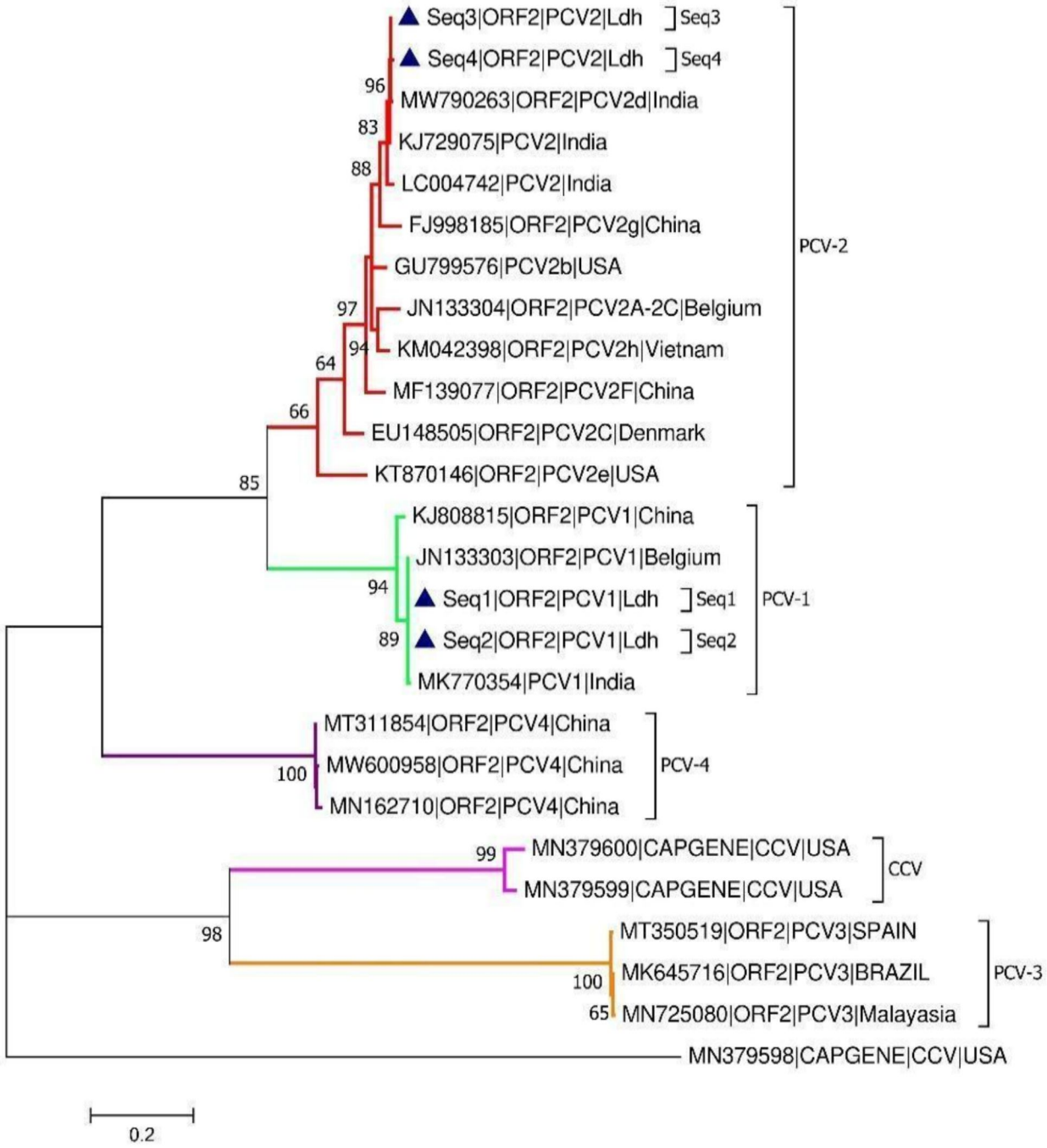


Fig. 3. The phylogenetic analysis based on the nucleotide sequences of the cap gene of the viral isolates of this study (denoted by ▲) with PCV-1,-2,-3,-4 and CCV reference strains downloaded from NCBI-GenBank, constructed using the MEGA-X software. ML with HKY+G evolutionary model was used to construct the tree with bootstrap values (based on 1000 replicates) showing if >60%

occurred in 2010, this time in favor of PCV-2d strain (Franzo et al., 2016). The PCV-2 viral isolates of the current study have also been found to be of the PCV-2d strain. Apart from this study, the PCV-2d strain has been detected in the states Uttar Pradesh (Anoopraj et al., 2015), Meghalaya (Mukherjee et al., 2018) and Assam (Barman et al., 2018). The rapid shifts in genotype of the PCV-2 viruses are a result of their high evolutionary rate (10^{-3}-10^{-4} substitutions/site/year), and a huge population size, and vaccination-induced immunity escape, optimally allowing natural selection to act (Firth et al., 2009; Shen et al., 2012).

The selection pressure analysis of the cap gene of PCV-2 isolates of this study revealed evidence of positive selection by three statistical methods i.e. MEME, FUBAR and IFEL. This study corroborated the results of a Belgian study by Wei et al. (2019), which reported the codons 59 (also reported to be under positive selection pressure in our study), 139 and 191 to be evolving under positive selection pressure, identified by MEME ($P<0.1$) in the PCV-2 strains of Belgium.

In conclusion, this study detected the presence of PCV-1 and PCV-2 in aborted fetuses of piglets in Punjab, India. Phylogenetic analysis based on the ORF-2 (Cap) gene showed that the PCV-2 virus isolates from this study belong to the PCV-2d strain. The selection pressure analysis on the ORF-2 gene of the virus indicated evidence of both positive and negative selection.

Conflict of interest: The Authors have no conflict of interest in this study.

Author's contribution: SD: Involved in investigation, data generation, preparing original draft; DD: Engaged in conceptualization, data curation, supervision and final editing; CSM: Involved in bioinformatics analyses, and methodology; RV: Involved in conceptualization and editing.

Data availability statement: The dataset generated during research will be available from the corresponding author on reasonable request.

Ethical approval: Since aborted fetal samples of pigs were used in this study, no animal care and use approval was required.

Use of artificial intelligence tools: The authors have not used any artificial intelligence tools in this study.

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REFERENCES

19th Livestock Census. (2012). 19th Livestock Census – 2012, Animal Husbandry Department, Govt. of Punjab. http://www.husbandrypunjab.org/pages/livestock.htm

20th Livestock Census. (2019). 20th Livestock Census – 2019, Animal Husbandry Department, Govt. of Punjab. http://husbandrypunjab.org/census/Pig%20Census%202019.pdf

Anoopraj, R., Rajkhowa, T. K., Cherian, S., Arya, R. S., Tomar, N., Gupta, A., Ray, P. K., Somvanshi, R., & Saikumar, G. (2015). Genetic characterisation and phylogenetic analysis of PCV2 isolates from India: Indications for emergence of natural inter-genotypic recombinants. *Infection Genetics and Evolution*, *31*, 25-32. https://doi.org/10.1016/j.meegid.2015.01.006

Barman, N. N., Nath, B., Kumar, V., Sen, A., Dutta, T. K., Dutta, B., Rahman, T., & Kumar, S. (2018). The emergence of porcine circovirus 2 infections in the Northeastern part of India: A retrospective study from 2011 to 2017. *Transboundary and Emerging Diseases*, *65*(6), 1959-1967. https://doi.org/10.1111/tbed.12977

Bhattacharjee, U., Ahuja, A., Sharma, I., Karam, A., Chakraborty, A. K., Ghatak, S., Puro, K., Das, S., Shakuntala, I., Giri, S., Pegu, R. K., Laha, R., Pattanayak, A., Ngachan, S. V., & Sen, A. (2015). Complete genome sequence of emerging porcine circovirus types 2a and 2b from India. *Genome Announcements*, *3*(2). https://doi.org/10.1128/genomea.00087-15

Brunborg, I. M., Jonassen, C. M., Moldal, T., Bratberg, B., Lium, B., Koenen, F., & Schönheit, J. (2007). Association of myocarditis with high viral load of porcine circovirus type 2 in several tissues in cases of fetal death and high mortality in piglets. A case study. *Journal of Veterinary Diagnostic Investigation*, *19*(4), 368-375. https://doi.org/10.1177/104063870701900405

Clark, E. G. (1997). Post-weaning multisystemic wasting syndrome. In *Proceedings of the 28th Annual Meeting of the American Association of Swine Practitioners* (pp. 499-501). American Association of Swine Practitioners.

Fenaux, M., Halbur, P. G., Gill, M., Toth, T. E., & Meng, X. (2000). Genetic characterization of type 2 porcine circovirus (PCV-2) from pigs with postweaning multisystemic wasting syndrome in different geographic regions of North America and development of a differential PCR-restriction fragment length polymorphism assay to detect and differentiate between infections with PCV-1 and PCV-2. *Journal of Clinical Microbiology*, *38*(7), 2494-2503. https://doi.org/10.1128/jcm.38.7.2494-2503.2000

Finsterbusch, T., & Mankertz, A. (2009). Porcine circoviruses – Small but powerful. *Virus Research*, *143*(2), 177–183. https://doi.org/10.1016/j.virusres.2009.02.009

Firth, C., Charleston, M. A., Duffy, S., Shapiro, B., & Holmes, E. C. (2009). Insights into the evolutionary

history of an emerging livestock pathogen: Porcine circovirus 2. *Journal of Virology*, *83*(24), 12813–12821. https://doi.org/10.1128/jvi.01719-09

Franzo, G., Tucciarone, C. M., Cecchinato, M., & Drigo, M. (2016). Porcine circovirus type 2 (PCV2) evolution before and after the vaccination introduction: A large scale epidemiological study. *Scientific Reports*, *6*(1), 39458. https://doi.org/10.1038/srep39458

Guo, L. J., Lu, Y. H., Wei, Y. W., Huang, L. P., & Liu, C. M. (2010). Porcine circovirus type 2 (PCV2): genetic variation and newly emerging genotypes in China. *Virology Journal*, *7*(1), 273. https://doi.org/10.1186/1743-422x-7-273

Harding, J. (2004). The clinical expression and emergence of porcine circovirus 2. *Veterinary Microbiology*, *98*(2), 131-135. https://doi.org/10.1016/j.vetmic.2003.10.013

Harding, J. C. (1997). Post-weaning multisystemic wasting syndrome (PMWS): Preliminary epidemiology and clinical presentation. In *Proceedings of the 28th Annual Meeting of the American Association of Swine Practitioners* (p. 503). American Association of Swine Practitioners.

Karuppannan, A. K., Ramesh, A., Reddy, Y. K., Ramesh, S., Mahaprabhu, R., Jaisree, S., Roy, P., Sridhar, R., Pazhanivel, N., Sakthivelan, S. M., Sreekumar, C., Murugan, M., Jaishankar, S., Gopi, H., Purushothaman, V., Kumanan, K., & Babu, M. (2016). Emergence of porcine circovirus 2 associated reproductive failure in southern India. *Transboundary and Emerging Diseases*, *63*(3), 314–320. https://doi.org/10.1111/tbed.12276

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution*, *35*(6), 1547-1549. https://doi.org/10.1093/molbev/msy096

Lee, C. S., Moon, H. J., Yang, J. S., Park, S. J., Song, D. S., Kang, B. K., & Park, B. K. (2007). Multiplex PCR for the simultaneous detection of pseudorabies virus, porcine cytomegalovirus, and porcine circovirus in pigs. *Journal of Virological Methods*, *139*(1), 39–43. https://doi.org/10.1016/j.jviromet.2006.09.003

Lv, Q. Z., Guo, K. K., & Zhang, Y. M. (2014). Current understanding of genomic DNA of porcine circovirus type 2. *Virus genes*, *49*(1), 1-10. https://doi.org/10.1007/s11262-014-1099-z

Madson, D. M., Patterson, A. R., Ramamoorthy, S., Pal, N., Meng, X. J., & Opriessnig, T. (2009). Reproductive failure experimentally induced in sows via artificial insemination with semen spiked with porcine circovirus type 2. *Veterinary Pathology*, *46*(4), 707–716. https://doi.org/10.1354/vp.08-vp-0234-o-fl

Maity, H. K., Samanta, K., Deb, R., & Gupta, V. K. (2023). Revisiting porcine circovirus infection: recent insights and its significance in the piggery sector. *Vaccines*, *11*(8), 1308. https://doi.org/10.3390/vaccines11081308

Meehan, B. M., Creelan, J. L., Stewart McNulty, M., & Todd, D. (1997). Sequence of porcine circovirus DNA: affinities with plant circoviruses. *Journal of General Virology*, *78*(1), 221-227. https://doi.org/10.1099/0022-1317-78-1-221

Monroy Rincón, M. A., Ramirez-Nieto, G. C., Vera, V. J., Correa, J. J., & Mogollón-Galvis, J. D. (2014). Detection and molecular characterization of porcine circovirus type 2 from piglets with Porcine Circovirus Associated Diseases in Colombia. *Virology Journal*, *11*(1), 143. https://doi.org/10.1186/1743-422x-11-143

Mukherjee, P., Karam, A., Barkalita, L., Borah, P., Chakraborty, A. K., Das, S., Puro, K., Sanjukta, R., Ghatak, S., Shakuntala, I., Laha, R. G., Sen, A., & Sharma, I. (2018). 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 Tropica*, *182*, 166-172. https://doi.org/10.1016/j.actatropica.2018.02.025

Nawagitgul, P., Harms, P. A., Morozov, I., Thacker, B. J., Sorden, S. D., Lekcharoensuk, C., & Paul, P. S. (2002). Modified indirect porcine circovirus (PCV) type 2-based and recombinant capsid protein (ORF2)-based enzyme-linked immunosorbent assays for detection of antibodies to PCV. *Clinical and Vaccine Immunology*, *9*(1), 33–40. https://doi.org/10.1128/cdli.9.1.33-40.2002

Opriessnig, T., Karuppannan, A. K., Castro, A. M., & Xiao, C. T. (2020). Porcine circoviruses: current status, knowledge gaps and challenges. *Virus Research*, *286*, 198044. https://doi.org/10.1016/j.virusres.2020.198044

Palinski, R., Piñeyro, P., Shang, P., Yuan, F., Guo, R., Fang, Y., Byers, E., & Hause, B. M. (2016). A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure. *Journal of Virology*, *91*(1). https://doi.org/10.1128/jvi.01879-16

Rosario, K., Breitbart, M., Harrach, B., Segalés, J., Delwart, E., Biagini, P., & Varsani, A. (2017). Revisiting the taxonomy of the family *Circoviridae*: establishment of the genus *Cyclovirus* and removal of the genus *Gyrovirus*. *Archives of Virology*, *162*(5), 1447-1463. https://doi.org/10.1007/s00705-017-3247-y

Sambrook, J., & Russell, D. W. (2006). Purification of nucleic acids by extraction with phenol: Chloroform. *Cold Spring Harbor Protocols*, *2006*(1), Article pdb.prot4455. https://doi.org/10.1101/pdb.prot4455

Segalés, J. (2012). Porcine circovirus type 2 (PCV2) infections: Clinical signs, pathology and laboratory diagnosis. *Virus Research*, *164*(1–2), 10–19. https://doi.org/10.1016/j.virusres.2011.10.007

Sharma, R., & Saikumar, G. (2008). Porcine circovirus 2 associated reproductive failure in Indian pigs. *Indian Journal of Animal Sciences*, *78*(11), 1238–1240. https://epubs.icar.org.in/index.php/IJAnS/article/view/4960

Shen, H., Halbur, P. G., & Opriessnig, T. (2012). Prevalence and phylogenetic analysis of the current porcine circovirus 2 genotypes after implementation of widespread vaccination programmes in the USA. *Journal of General Virology*, *93*(6), 1345-1355. https://doi.org/10.1099/vir.0.039552-0

Wei, R., Xie, J., Theuns, S., & Nauwynck, H. J. (2019). Changes on the viral capsid surface during the evolution of porcine circovirus type 2 (PCV2) from 2009 till 2018 may lead to a better receptor binding. *Virus Evolution*, *5*(2), https://doi.org/10.1093/ve/vez026

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