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Isolation and molecular phylogenetic analysis of porcine circovirus 2 from Indian pigs with respiratory lesions

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Abstract

Porcine circovirus type 2 (PCV2) is a major viral pathogen of pigs, causing significant economic losses to the swine industry worldwide. In India, PCV2 is widely prevalent and is a key etiological agent of the porcine respiratory disease complex. Porcine circoviruses are non-enveloped viruses with a single-stranded circular DNA genome and are among the smallest known animal viruses. The present study aimed to detect and molecularly characterize PCV2 from pigs exhibiting respiratory lesions in the Hassan region. A total of 108 pig carcasses were examined during post-mortem inspection, of which 87 samples showing prominent respiratory lesions were subjected to molecular analysis. Genomic DNA extracted from tissue samples was screened for PCV2 using nucleocapsid gene-specific polymerase chain reaction (PCR). A specific 481 bp amplicon was detected in 61 samples, indicating a prevalence of 70.1%. Sequence and BLAST analysis revealed high nucleotide similarity between the field isolates and previously reported Indian and global PCV2 strains. Phylogenetic analysis confirmed that the circulating PCV2 strains clustered with known PCV2 lineages, indicating conservation of viral genetic characteristics. The study highlights the high prevalence of PCV2 in pigs with respiratory lesions. It underscores the utility of molecular diagnostics and phylogenetic analysis in understanding PCV2 epidemiology, which is essential for effective disease surveillance and control strategies.

Keywords: Porcine circovirus type 2, respiratory disease complex, polymerase chain reaction

Introduction

Porcine circovirus type 2 (PCV2) is a small, non-enveloped virus belonging to the family Circoviridae and the genus Circovirus, characterised by a circular single-stranded DNA genome of approximately 1.7-2.0 kb (Franzo *et al.*, 2016) [1]. The family Circoviridae comprises viruses infecting a wide range of avian and mammalian hosts, including important pathogens such as chicken anaemia virus (CAV), porcine circovirus (PCV), and psittacine beak and feather disease virus (PBFD). Among these, PCV2 is recognised as one of the most economically significant viral pathogens affecting the global swine industry (Murphy *et al.*, 1999) [2].

PCV2 is the primary etiological agent of porcine circovirus-associated diseases (PCVD), a multifactorial disease complex characterised by systemic illness, enteritis, interstitial pneumonia, and reproductive failure (Segales, 2012) [3]. In addition to overt clinical disease, PCV2 infection commonly occurs in a subclinical form, which may result in reduced growth performance and increased susceptibility to secondary infections (Segales, 2012) [3]. Owing to the substantial economic and health impact of PCVD, reliable and sensitive diagnostic methods are essential. Polymerase chain reaction (PCR)-based assays provide a practical and sensitive approach for the routine detection and epidemiological surveillance of PCV2 infection in veterinary diagnostic laboratories (Hamel *et al.*, 2000) [4].

In this context, the present study was undertaken to investigate respiratory infections in pigs in and around the Hassan region and to confirm PCV-2 using molecular diagnostic techniques.

Materials and Methods

Sample collection

Pigs exhibiting respiratory infections were included in the study for the confirmation of porcine circovirus type 2 (PCV-2) by polymerase chain reaction (PCR) during the period from January to December 2022 in Hassan district, Karnataka, India. A total of 108 samples (Table 1) were collected, along with relevant epidemiological data, including age, sex, breed, and gross pathological lesions observed in the lungs and other organs. Tissue samples

comprising lungs, tonsils of the soft palate, lymph nodes, heart, and spleen were collected aseptically and stored at -20°C until further molecular analysis.

Polymerase chain reaction: Using the HiPurA® Mammalian Genomic DNA Purification Kit (HiMedia Laboratories Pvt. Ltd), total DNA was recovered from pooled organ tissue samples. The sequence of primers used are as given below (Ellis *et al.*, 1999) [5].

Gene	Primer name	Sequence 5' to 3'	Length (bp)	Base pair
PCV 2	Forward	CGGATATTGTAGTCCTGGTCG	21	481bp
	Reverse	ACTGTCAAGGCTACCACAGTCA	22	

The PCR master mix was prepared as follows:

Sl. No.	Component	Volume per reaction
1	2X PCR Master mix	10.00 μL
2	Primers (0.5 μL each)	1.00 μL
3	Template	2.00 μL
4	Sterile water	7.00 μL
	Total	20.00 μL

PCR program for the amplification of PCV 2

PCR step		Temperature	Duration	Cycle
1	Initial denaturation	94 $^{\circ}\text{C}$	1 minute	1
2	Cycling	Denaturation	94 $^{\circ}\text{C}$	35
		Annealing	60 $^{\circ}\text{C}$	
		Elongation	72 $^{\circ}\text{C}$	
3	Final extension	72 $^{\circ}\text{C}$	10 minutes	1

After 45 minutes of electrophoresis at 80 V, the amplicons were examined on a 2% agarose gel. Image Lab Software (Version 5.1, Bio-Rad Laboratories Inc.) was used to document the PCR product in the gel.

Custom sequencing: The PCR-amplified product of the PCV-2 capsid protein gene obtained from tissue samples was subjected to custom nucleotide sequencing using a single-pass sequencing approach, employing the same primers used for PCR amplification. Sequencing was performed using an Applied Biosystems genetic analyzer (Inst Model/Name: 3730xl/XE3-IOT-2019-1406-029). Sequence data were generated using both forward and reverse primers.

Sequence data analysis and phylogenetic analysis: The forward and reverse read sequence chromatograms were initially examined to resolve ambiguous bases and trim the sequence ends of each chromatogram. The obtained PCR sequences from different locations were then aligned using the Clustal W method (DNASTAR Lasergene programme). The programme also calculated sequence similarity and divergence. All of the putative genes included in the current investigation of Indian strains of PCV-2 capsid protein gene sequences were downloaded from the Genbank data base for comparative sequencing analysis. Sequences from both India and other countries were included. The MegAlign programme of DNASTAR Lasergene software was used to generate the maximum likelihood phylogenetic tree and the phylogenetic tree using the Porcine reproductive and respiratory syndrome virus strain with the highest homology.

Results

Out of the 108 pig samples collected from the slaughterhouses of the Hassan region, 87 animals showed respiratory-related lesions.

Molecular confirmation of PCV-2 by PCR

In the present study, tissue samples comprising lung, tonsils, bronchial lymph nodes, spleen, heart, and brain were collected and screened for PCV-2 using a nucleocapsid gene-specific PCR assay. Out of the 87 samples analyzed, 61 yielded the expected amplicon of 481 bp (Fig.1) indicating an overall PCV-2 prevalence of 70.11%.

The sequences of the five positive samples revealed similarities to other PCV-2 isolates, confirming the presence of PCV-2 in the samples. In terms of similarity, the field isolates matched the PCV-2 completely. Using NCBI blast, the nucleotide sequences obtained from the 481-base pair PCV-2 capsid protein PCR products coincided with the PCV-2 nucleotide sequences available in GenBank. In order of highest similarity, the field isolates revealed 100% resemblance to the PCV-2 previously reported (Table 2).

BLAST analysis of sequences from different livestock species revealed genetic homology of Indian strains of PCV-2 with reported sequences of capsid protein gene like India_KHpur4_2022 and India_KHpur5_2022 showed 100% similarity with strains from China, Vietnam, Denmark, and USA. The strain India_KHpur1_2022 shared 99.77% similarity with strains from South Korea, China, Thailand, and India; India_KHpur5_2022 shared 99.76% similarity with strains from China, Taiwan, and Austria. India_KHpur3_2022 shared 99.52% of its DNA with strains from Taiwan, China, Thailand, and India; India_KHpur2_2022 shared 99.28% of its DNA with strains from China, Nigeria, Chile, and Denmark, indicating the capsid protein gene of PCV-2 as one of the most conserved genes (Table 3) and multiple nucleotide sequence alignment of Indian strains of PCV-2 with the reported sequences of capsid protein gene was aligned using Clustal W method (Lasegene).

The results of phylogenetic analysis based on capsid protein gene sequences of PCV-2 isolates from India and foreign isolates are shown in Fig 2. The presence of PCV-2 was verified by phylogenetic analysis in the representative samples. Another phylogenetic analysis based on Porcine Reproductive and Respiratory Syndrome Virus strain 5617-3w-3 capsid protein gene (GenBank accession OM779073.1) was compared with capsid protein gene with PCV-2 isolates due to PCV-2 co-infection with PRRSV (Fig 3). Homology of Indian strains of PCV-2 with reported

sequences of capsid protein gene (MegAlign, Lasergene) is depicted in the Fig 4.

The majority of affected animals (59.01%) ranged in age from 3 to 6 months. Local breeds had the highest rate of PCV-2 infection (70.49%), followed by Large White

Yorkshire (14.75%), Duroc (9.83%), and Landrace (4.91%). The research areas were located in four distinct agro-climatic zones; most of the affected animals were from Hassan Taluk, which was located in the district's Southern dry zone (74.41%).

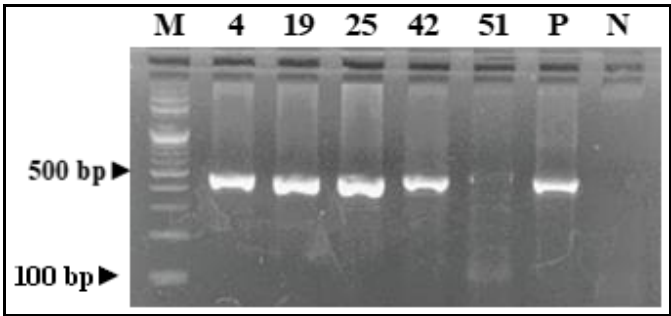


Fig 1: Agarose gel electrophoresis of capsid protein gene of PCV 2 isolates

Lanes M) 100 bp DNA ladder; 4, 19, 25 and 42 positive samples; 51 negative sample; P) positive control and N) no template control

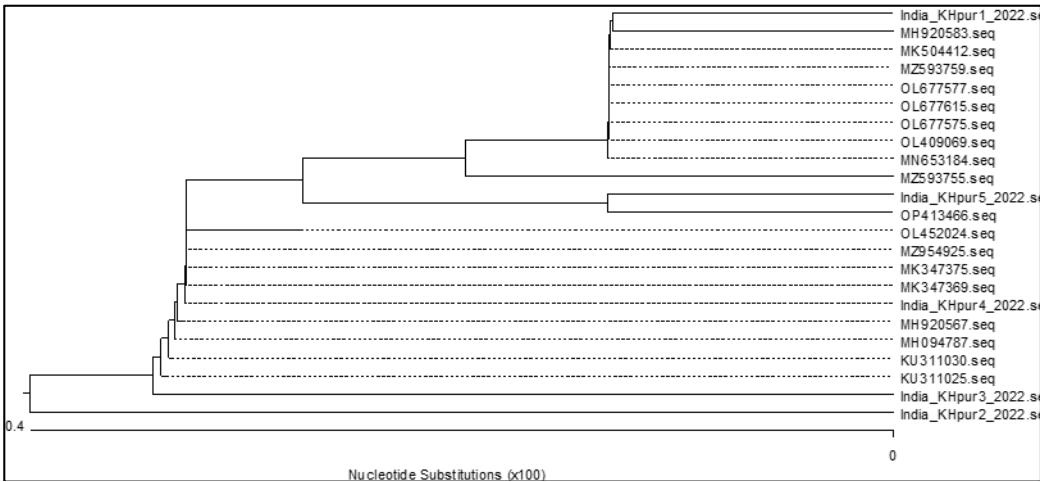


Fig 2. Phylogenetic tree of Indian strains of PCV 2 aligned with highest homology reported sequences of capsid protein gene using Clustal W method (MegAlign, Lasergene)

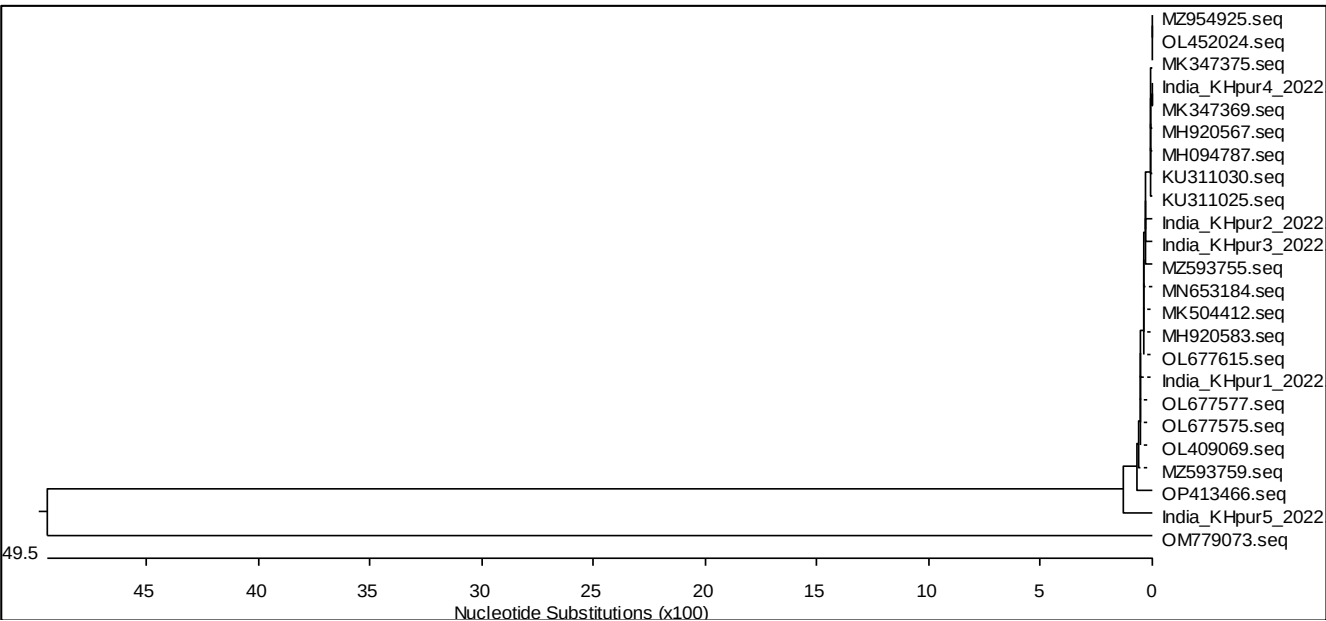


Fig 3. Phylogenetic tree with Porcine Reproductive and Respiratory Syndrome Virus strain 5617-3w-3 capsid protein gene (GenBank accession OM779073.1) as an outlier

		Percent Identity																								
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
Divergence	1	1	95.3	97.7	82.9	95.3	96.0	96.0	96.0	99.8	82.9	82.9	99.8	99.8	96.0	98.1	82.9	98.1	82.9	98.1	98.1	98.1	95.6	1	India_KHpur1_2022.seq	
	2	1.2	1	98.3	81.3	96.9	99.3	99.3	99.3	99.3	97.8	81.3	81.3	97.8	97.8	99.3	97.8	81.3	97.8	81.3	97.8	97.8	97.8	97.1	2	India_KHpur2_2022.seq
	3	0.7	0.5	1	82.6	96.7	97.9	97.9	97.9	97.9	99.5	82.6	82.6	99.5	99.5	97.4	99.5	82.6	99.5	82.6	99.5	99.5	99.5	96.9	3	India_KHpur3_2022.seq
	4	0.6	0.6	0.6	1	95.5	95.8	95.8	95.8	95.8	99.7	100.0	100.0	99.7	99.7	95.5	97.7	100.0	97.8	100.0	97.8	97.8	97.8	95.5	4	India_KHpur4_2022.seq
	5	0.7	1.5	1.0	0.3	1	99.3	99.3	99.3	99.3	99.5	82.9	82.9	99.5	99.5	99.3	99.5	82.9	99.5	82.9	99.5	99.5	99.5	99.8	5	India_KHpur5_2022.seq
	6	0.5	0.7	0.2	0.0	0.7	1	100.0	100.0	100.0	98.6	81.8	81.8	98.6	98.6	99.5	98.6	81.8	98.6	81.8	98.6	98.6	98.6	97.8	6	KU311025.seq
	7	0.5	0.7	0.2	0.0	0.7	0.0	1	100.0	100.0	98.6	81.8	81.8	98.6	98.6	99.5	98.6	81.8	98.6	81.8	98.6	98.6	98.6	97.8	7	KU311030.seq
	8	0.5	0.7	0.2	0.0	0.7	0.0	0.0	1	100.0	98.6	81.8	81.8	98.6	98.6	99.5	98.6	81.8	98.6	81.8	98.6	98.6	98.6	97.8	8	MH094787.seq
	9	0.5	0.7	0.2	0.0	0.7	0.0	0.0	0.0	1	98.6	81.8	81.8	98.6	98.6	99.5	98.6	81.8	98.6	81.8	98.6	98.6	98.6	97.8	9	MH920567.seq
	10	0.2	1.0	0.5	0.3	0.5	0.2	0.2	0.2	0.2	1	83.1	83.1	100.0	100.0	96.3	98.4	83.1	98.4	83.1	98.4	98.4	98.4	95.8	10	MH920583.seq
	11	0.6	0.6	0.6	0.0	0.3	0.0	0.0	0.0	0.0	0.3	1	100.0	99.7	99.7	95.5	97.8	100.0	97.8	100.0	97.8	97.8	97.8	95.5	11	MK347369.seq
	12	0.6	0.6	0.6	0.0	0.3	0.0	0.0	0.0	0.0	0.3	0.0	1	99.7	99.7	95.5	97.8	100.0	97.8	100.0	97.8	97.8	97.8	95.5	12	MK347375.seq
	13	0.2	1.0	0.5	0.3	0.5	0.2	0.2	0.2	0.2	0.3	0.3	1	100.0	96.3	98.4	83.1	98.4	83.1	98.4	98.4	98.4	98.4	95.8	13	MK504412.seq
	14	0.2	1.0	0.5	0.3	0.5	0.2	0.2	0.2	0.2	0.3	0.3	0.0	1	96.3	98.4	83.1	98.4	83.1	98.4	98.4	98.4	98.4	95.8	14	MN653184.seq
	15	0.5	0.7	0.7	0.3	0.7	0.5	0.5	0.5	0.5	0.2	0.3	0.2	0.2	1	98.6	81.5	98.6	81.5	98.6	98.6	98.6	98.6	97.8	15	MZ593755.seq
	16	0.2	1.0	0.5	0.3	0.5	0.2	0.2	0.2	0.2	0.3	0.3	0.0	0.0	0.2	1	82.9	100.0	82.9	100.0	100.0	100.0	97.4	16	MZ593759.seq	
	17	0.6	0.6	0.6	0.0	0.3	0.0	0.0	0.0	0.0	0.3	0.0	0.3	0.3	0.3	0.3	1	97.8	100.0	97.8	97.8	97.8	97.8	95.5	17	MZ954925.seq
	18	0.2	1.0	0.5	0.3	0.5	0.2	0.2	0.2	0.2	0.3	0.3	0.0	0.0	0.2	0.0	0.3	1	82.9	100.0	100.0	100.0	97.4	18	OL409069.seq	
	19	0.6	0.6	0.6	0.0	0.3	0.0	0.0	0.0	0.0	0.3	0.0	0.3	0.3	0.3	0.3	0.3	0.3	1	97.8	97.8	97.8	97.8	95.5	19	OL452024.seq
	20	0.2	1.0	0.5	0.3	0.5	0.2	0.2	0.2	0.2	0.3	0.3	0.0	0.0	0.2	0.0	0.3	0.0	0.3	1	100.0	100.0	97.4	20	OL677575.seq	
	21	0.2	1.0	0.5	0.3	0.5	0.2	0.2	0.2	0.2	0.3	0.3	0.0	0.0	0.2	0.0	0.3	0.0	0.3	0.0	1	100.0	97.4	21	OL677577.seq	
	22	0.2	1.0	0.5	0.3	0.5	0.2	0.2	0.2	0.2	0.3	0.3	0.0	0.0	0.2	0.0	0.3	0.0	0.3	0.0	0.0	1	97.4	22	OL677615.seq	
	23	0.5	1.2	0.7	0.3	0.2	0.5	0.5	0.5	0.5	0.2	0.3	0.3	0.2	0.2	0.5	0.2	0.3	0.2	0.3	0.2	0.2	0.2	1	23	OP413466.seq
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		

Fig 4. Homology of Indian strains of PCV 2 with reported sequences of capsid protein gene (MegAlign, Lasergene)

Table 1: Details of samples collected from the slaughter houses

Sl. No.	Taluka	Samples collected
1.	Aluru	06
2.	Arasikere	24
3.	Arakalagudu	04
4.	Beluru	07
5.	Channarayapatna	14
6.	Hassan	43
7.	Holenarsipura	08
8.	Sakleshpura	02
	Total	108

Table 2: Nucleotide sequences of Indian strains of PCV-2 of capsid protein gene

S. No.	Strain	PCV-2 capsid protein gene sequence
1)	India_KHpur1_2022	ACGCAGTGCCGAGGCCTACATGGTCTACATTTCCAGTAGTTTGTAGTCTCAGCCAGAGTTGATTTCTTTTGTATTGGGTTGGAAGTAATCGATTGTCCCATCAAGGACAGGTTTCGGGGTAAAGTACCGG GAGTGGTAGGAGAAGGGCTGGGTTATGGTATGGCGGGAGGAGTAGTTTACATAGGGGTCATAGG TTAGGGCATTGGCCTTTGTTACAAAGTTATCATCTAGAATAACAGCAGTGGAGCCCACTCCCCTGT CTCCCTGGGTGATTGGGGAGCAGGGCCAGAATTCAACCTTAACCTTCCTTATTCTGTAGTATTCAA AGGGCACAGTGAGGGGGTTTGAGCCCCCTCTGGGGGAAGAAAATCATTAAATATTAAATCTCATC ATGTCCACATTCCAGGAGGGCGTTCTGACTGTGGT
2)	India_KHpur2_2022	ITCGAACGCAGTGCCGAGGCCTACATGGTCTACATTTCCAGTAGTTTGGAGTTTCAGCCAGAGTTG ATTTCTTTTGTATTGGGTTGGAAGTAATCGATTGTCCCATCAAGGACAGGTTTCGGGGTAAAGTA ACGGGAGTGGTAGGAGAAGGGCTGGGTTATGGTATGGCGGGAGGAGTAGTTTACATAGGGGTCAT TAGGTTAGGGCATTGGCCTTTGTTACAAAGTTATCATCTAGAATAACAGCAGTGGAGCCCACTCC CCTGTCAACCTGGGTGATTGGGGAGCAGGGCCAGAATTCAACCTTAACCTTCCTTATTCTGTAGTA TTCAAAGGGCACAGTGAGGGGGTTTGAGCCCCCTCTGGGGGAAGAAAATCATTAAATATTAAATC TCATCATGTCCACATTCCAGGAGGG
3)	India_KHpur3_2022	ACGCAGTGCCGAGGCCTACATGGTCTACATTTCCAGTAGTTTGGAGTCTCAGCCAGAGTTGATTTCTTTTGTATTGGGTTGGAAGTAATCGATTGTCCCATCAAGGACAGGTTTCGGGGTAAAGTAACGG GAGTGGTAGGAGAAGGGCTGGGTTATGGTATGGCGGGAGGAGTAGTTTACATAGGGGTCATAGG TTAGGGCATTGGCCTTTGTTACAAAGTTATCATCTAGAATAACAGCAGTGGAGCCCACTCCCCTGT CACCTGGGTGATTGGGGAGCAGGGCCAGAATTCAACCTTAACCTTCCTTATTCTGTAGTATTCAA AGGGCACAGTGAGGGGGTTTGAGCCCCCTCTGGGGGAAGAAAATCATTAAATATTAAATCTCATC ATGTCCACATTCCAGGAGGGCGTTCTGACTGTGGT
4)	India_KHpur4_2022	TTATTGGGTTGGAAGTAATCGATTGTCCCATCAAGGACAGGTTTCGGGGTAAAGTACCGGGAGTG GTAGGAGAAGGGCTGGGTTATGGTATGGCGGGAGGAGTAGTTTACATAGGGGTCATAGGTTAGG GCATTGGCCTTTGTTACAAAGTTATCATCTAGAATAACAGCAGTGGAGCCCACTCCCCTGTCAACCC TGGGTGATTGGGGAGCAGGGCCAGAATTCAACCTTAACCTTCCTTATTCTGTAGTATTCAAAGGG CACAGTGAGGGGGTTTGAGCCCCCTCTGGGGGAAGAAAATCATTAAATATTAAATCTCATCATGT CCACATTCCAGGAGGGGGTTCTGACTGTGGT
5)	India_KHpur5_2022	GCAGTGCCGAGGCCTACATGGTCTACATTTCCAGTAGTTTGTAGTCTCAGCCAGAGTTGATTTTTTT TTGTTATTGGGTTGGAAGTAATCGATTGTCCCATCAAGGACAGGTTTCGGGGTAAAGTACCGGGA GTGGTAGGAGAAGGGCTGGGTTATGGTATGGCGGGAGGAGTAGTTTACATAGGGGTCATAGGTT AGGGCATTGGCCTTTGTTACAAAGTTATCATCTAGAATAACAGCAGTGGAGCCCACTCCCCTGTC ACCCTGGGTGATTGGGGAGCAGGGCCAGAATTCAACCTTAACCTTCCTTATTCTGTAGTATTCAA GGGCACAGTGAGGGGGTTTGAGCCCCCTCTGGGGGAAGAAAATCATTAAATATTAAATCTCATCA TGTCCACATTCCAGGAGGG

Table 3: Genetic homology of Indian strains of PCV-2 capsid protein gene sequences

Sl.	sequence	PCV-2 strain	Similarity	Accession	Place
1)	India_KHpur1_2022	CN9910	99.77%	MT376362.1	South Korea
		GG10691 C	99.77%	MT376343.1	South Korea
		GG10694	99.77%	MT376342.1	South Korea
		GW11028	99.77%	MT376323.1	South Korea
		NY	99.77%	MZ593759.1	China
		Thailand/2020/20RBR395	99.77%	OL677615.1	Thailand (Ratchaburi)
		Thailand/2019/19NPT269	99.77%	OL677577.1	Thailand (Nakornpathom)
		Thailand/2019/19CMI162	99.77%	OL677575.1	Thailand (Chiang Mai)
		PCV-2D	99.77%	OK349503.1	India
2)	India_KHpur2_2022	PCV-2/CN/Anhui/Sinder202110-2	99.77%	OL409069.1	China
		LN-2	99.28%	MH920567.1	China
		SCADC17-DY3	99.28%	MH094787.1	China
		YiY-2-8-2	99.28%	KU311030.1	China
		YiY-2-2	99.28%	KU311025.1	China
		JZ4	99.28%	MZ593755.1	China
		PCV-2/Nigeria/KAF189/2020	99.28%	ON098566.1	Nigeria
		Chile_D21-23744-3d	99.28%	OL377675.1	Chile
		Den_80_2_22d capsid protein (ORF2)	99.28%	OL377466.1	Denmark
3)	India_KHpur3_2022	PCV-2/CN/Jiangxi-4/201801	99.28%	MW089684.1	China
		PCV-2/serum/Farm_U/Chile/2021	99.28%	OK322708.1	Chile
		YiY-2-2	99.76%	KU311025.1	China
		109-1810	99.52%	OM743460.1	Taiwan
		108-627	99.52%	OM677738.1	Taiwan
		108-165LD	99.52%	OM677732.1	Taiwan
		NY	99.52%	MZ593759.1	China
		Thailand/2020/20RBR395	99.52%	OL677615.1	Thailand (Ratchaburi)
		Thailand/2019/19NPT269	99.52%	OL677577.1	Thailand (Nakornpathom)
4)	India_KHpur4_2022	Thailand/2019/19CMI162	99.52%	OL677575.1	Thailand (Chiang Mai)
		PCV-2D	99.52%	OK349503.1	India
		PCV-2/CN/Anhui/Sinder202110-2	99.52%	OL409069.1	China
		LN-2	100.00%	MH920567.1	China
		XT2807	100.00%	MK347375.1	China
		YK1805	100.00%	MK347369.1	China
		SCADC17-DY3	100.00%	MH094787.1	China
		YiY-2-8-2	100.00%	KU311030.1	China
		SDRS	100.00%	OL452024.1	China
5)	India_KHpur5_2022	Chile_D21-23744-3d	100.00%	OL377675.1	Chile
		Den_80_2_22d	100.00%	OL377466.1	Denmark
		DongNai7/2016	100.00%	MZ954925.1	Viet Nam (Dong Nai)
		PCV-2/CN/Jiangxi-4/201801	100.00%	MW089684.1	China
		IA-234	100.00%	KU697015.1	USA
		Viet_MT432436_12d	100.00%	OL377530.1	Viet Nam
		DTD-CH4/BT_2019	100.00%	MT432436.1	Viet Nam (Binh Thuan)
		PCV-2-CH-SDHaiYang-44-2022	99.76%	OP413466.1	China
		108-1605	99.76%	OM681463.1	Taiwan
		108-764	99.76%	OM677742.1	Taiwan
		107-2631 S	99.76%	OM677729.1	Taiwan
		107-1758 MIX	99.76%	OM677719.1	Taiwan
		107-1643 KD	99.76%	OM677716.1	Taiwan
		AUT174/2019	99.76%	OM460170.1	Austria

Discussion

In the present study, 61 of 87 animals exhibiting respiratory lesions tested positive for PCV-2 by PCR, resulting in a prevalence of 70.11%, which is higher than the prevalence rates of 26-60% reported in earlier studies (Deka *et al.*, 2021) [6]. These findings indicate that PCV-2 is highly prevalent among pigs affected with porcine respiratory disease complex (PRDC) and should be considered a major respiratory pathogen, thereby supporting the observations reported previously (Deka *et al.*, 2021; Opriessnig *et al.*, 2006) [6, 7]. The results of the present investigation are also consistent with earlier studies documenting significant respiratory disease in pigs from the Hassan district, with comparable findings reported by researchers from different

regions of the country (Yaeger *et al.*, 2019) [8].

Age-wise distribution of PCV-2-positive animals showed that 16 animals (26.22%) were between two and three months of age, 36 animals (59.01%) were between three and six months, and 9 animals (14.75%) were between six and twelve months of age. No statistically significant difference in prevalence was observed among the different age groups. Although PCV-2 infection has been reported in piglets aged one to six months, the disease most frequently affects pigs between two and four months of age (Satheesha *et al.*, 2021; Segalés *et al.*, 2005) [9, 10]. Increased susceptibility in younger animals has been attributed to immature or insufficient immunity. Nevertheless, PCV-2 can affect pigs of all age groups, with disease expression influenced by

factors such as health status, nutrition, immune competence, and management practices (Barman *et al.*, 2018) ^[11].

The polymerase chain reaction (PCR) assay is a rapid and essential tool for the detection of porcine circovirus type 2 (PCV-2), demonstrating greater sensitivity and faster turnaround than virus isolation, immunohistochemistry, and in situ hybridization. Confirmation of PCV-2 infection has been achieved through the detection of 481 bp and 225 bp amplicons corresponding to the ORF2 region in pig samples collected between 1985 and 1998 (Jacobsen *et al.*, 2009) ^[12]. The detection of PCV-2 nucleic acids using PCR with specific primers designed to amplify defined genomic fragments has also been previously reported (Ellis *et al.*, 1999) ^[5]. In addition, PCR assays targeting the nucleocapsid gene and yielding a 481 bp amplicon in pooled organ samples have been described, which is consistent with the findings of the present study (Keerthana *et al.*, 2019; Sairam *et al.*, 2019) ^[13, 14]. Respiratory disease in pigs represents a major health concern for swine producers, with reports from the National Animal Health Monitoring System identifying it as the leading cause of mortality in nursery and grower-finisher operations (Brockmeier *et al.*, 2002) ^[15].

Conclusion

A high occurrence of porcine circovirus type 2 (PCV-2) among pigs with respiratory lesions highlights its important role in the porcine respiratory disease complex. The effective detection of PCV-2 using polymerase chain reaction underscores the value of molecular diagnostics for accurate surveillance. Future efforts should focus on large-scale epidemiological studies, continuous molecular monitoring, and the evaluation of targeted prevention and management strategies to reduce the impact of PCV-2-associated respiratory disease in swine production systems.

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References

1. Franzo G, Tucciarone CM, Cecchinato M, Drigo M. Porcine circovirus type 2 (PCV2) evolution before and after the vaccination introduction: A large scale epidemiological study. *Sci Rep.* 2016;6:39458.
2. Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ. Circoviridae. *Virol J.* 1999;357-361.
3. Segalés J. Porcine circovirus type 2 (PCV2) infections: Clinical signs, pathology and laboratory diagnosis. *Virus Res.* 2012;164:10-19.
4. Hamel AL, Lin LL, Sachvie C, Grudeski E, Nayar GP. PCR detection and characterization of type-2 porcine circovirus. *Can J Vet Res.* 2000;64(1):44-52.
5. Ellis J, Krakowka S, Lairmore M, Haines D, Bratanich A, Clark E, *et al.* Reproduction of lesions of postweaning multisystemic wasting syndrome in gnotobiotic piglets. *J Vet Diagn Invest.* 1999;11(1):3-14.
6. Deka D, Barman NN, Deka N, *et al.* Sero-epidemiology of porcine parvovirus, circovirus and Classical Swine Fever Virus infections in India. *Trop Anim Health Prod.* 2021;53:1-12.
7. Opriessnig T, Janke BH, Halbur PG. Cardiovascular lesions in pigs naturally or experimentally infected with porcine circovirus type 2. *J Comp Pathol.* 2006;134:105-110.
8. Yaeger MJ, Alstine WG. Respiratory system. In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, Zhang J, editors. *Diseases of swine.* 11th ed. Hoboken: John Wiley and Sons; 2019. p. 393-407.
9. Satheesha SP, Bhat MN, Ramesh PT, *et al.* A retrospective study on various clinical complications related to the body systems in pigs with special reference to respiratory complications in Hassan district, Karnataka state. *J Entomol Zool Stud.* 2021;9:509-514.
10. Segalés J, Allan GM, Domingo M. Porcine circovirus diseases. *Anim Health Res Rev.* 2005;6:119-142.
11. Barman NN, Nath B, Kumar V, *et al.* The emergence of porcine circovirus 2 infections in the North-Eastern part of India: A retrospective study from 2011 to 2017. *Transbound Emerg Dis.* 2018;65:1959-1967.
12. Jacobsen B, Krueger L, Seeliger F, Bruegmann M, Segalés J, Baumgaertner W. Retrospective study on the occurrence of porcine circovirus 2 infection and associated entities in Northern Germany. *Vet Microbiol.* 2009;138:27-33.
13. Keerthana J, Abraham MJ, Krithiga K, Priya PM. Studies on naturally infected cases of post-weaning multi systemic wasting syndrome (PMWS) in piglets. *Pharma Innov J.* 2019;8:485-488.
14. Sairam R, Krishna BD, Krithiga K, *et al.* Molecular and pathological studies of post-weaning multi-systemic wasting syndrome among piglets in Kerala, India. *Explor Anim Med Res.* 2019;9:137-144.
15. Brockmeier SL, Halbur PG, Thacker EL. Porcine respiratory disease complex. In: *Polymicrobial diseases.* Washington (DC): ASM Press; 2002. p. 231-258.