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Research Article

MOLECULAR PREVALENCE OF PORCINE CIRCOVIRUS 2 INFECTION: FOREMOST REPORT IN SOUTHERN STATES OF INDIA

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ABSTRACT: Porcine circovirus 2 (PCV2) is the emerging viral pathogen in the swine associated with multi-systemic clinical and subclinical outcomes. This study aimed to detect molecular and serological prevalence of PCV2 infection from southern states of India. A total of 434 random samples comprising of serum (n=273), pooled postmortem tissues (n=109) and rectal, vaginal and nasal swabs (n=52) and were collected from PCV2 suspected and healthy swine populations of Tamil Nadu, Kerala, Andhra Pradesh, Telangana and Puducherry states in India during 2019 to 2021 were screened for PCV2 by specific polymerase chain reaction (PCR) assay. Of 434 samples screened, 12.2% (n=53) showed positivity to PCV2 genome. Statistical analysis of molecular prevalence of PCV2 within breed, age, sex and vaccination status revealed no significant ($p>0.05$) difference but there was a significant ($p<0.05$) difference in the prevalence of PCV2 among healthy and suspected swine populations. Suspected pigs had significantly higher prevalence of PCV2 in comparison to healthy. ELISA based PCV2 antibody screening in 176 non-vaccinated serum samples revealed sero-positivity of 44.8% (n=79). The molecular and seroprevalence of PCV2 is alarming in southern states of India, which necessitates the need for genotypic characterization and phylogenetic analysis and development of candidate vaccine for implementation of suitable prevention and control measures.

Key words: PCV2, Molecular Prevalence, Sero-surveillance, Influencing factors, Southern India.

INTRODUCTION

Swine is one of the most valuable livestock reared in many parts of world for the meat production. Efficient feed conversion ratio (FCR) and high fecundity rates are the key players in the expansion of swine farming in many countries including India. Porcine circovirus 2 (PCV2) is first identified during 1998 in association with postweaning multisystemic wasting syndrome (PMWS) from Canada, USA and Europe (Allan *et al.* 1998, Ellis *et al*. 1998, Meehan *et al.* 1998). PCV2 belongs to the genus *Circovirus* of the family *Circoviridae,* is the smallest known vertebrate virus contains circular single standard DNA genome with 1767–1768 nucleotides. There are five major PCV2 genotypes reported worldwide, including PCV2a-e (Yang *et al.* 2018). PCV2a was the first reported genotype since its evolution, over a period of time PCV2b replaced the first one. In recent studies PCV2d is the major genotype reported around the world replacing PCV2b. PCV2c and PCV2e genotypes have been implicated only from limited geographical regions. The evolutionary dynamics of PCV2 is similar to that of single-stranded segmented RNA viruses (Zheng *et al.* 2020). PCV2 Infections are exhibited either as clinical or subclinical form in an unpredictable manner. The clinical outcomes of PCV2 infections are numerous which includes, PMWS, porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory disease complex (PRDC), enteric infections, necrotizing lymphadenitis, reproductive failure (abortions, still birth,

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mummification and neonatal deaths) and congenital tremor (Segalés 2012, Ren *et al.* 2016). PCV2 sub-clinical infection may results in poor weight gain, retarded growth rate, lowered production parameters and immune depletion promoting other infections. Sub-clinically infected swine may act as potential reservoir in spreading PCV2 infection to the healthy populations (Ladekjaer-Mikkelsen *et al*. 2002) PCV2 is the most widely distributed pathogen throughout the swine producing countries with surge in prevalence rate. PCV2 is the one among the emerging viral pathogen in swine populations throughout the world. In India, PCV2 infection associated with PMWS was first reported in few suspected cases of during 2006 from northern India (Kumar *et al.* 2006). In the year 2012, the first PCV2 infection associated with reproductive failure and stillborn piglets reported from Tamil Nadu (Karuppannan *et al*. 2016). Stringent surveillance of PCV2 through serology and molecular detection is highly essential to ascertain the impact of PCV2. Most of global PCV2 prevalence rates indicate the ubiquitous presence of PCV2 in swine herds. In India, northern and northeastern states reported prevalence of PCV2 infection at regular intervals but in southern states of India there are only few preliminary reports and pilot studies on PCV2 incidence. There is no detailed study on molecular and serological prevalence of PCV2 infection from southern states of India. This study addresses the above issue and documents PCV2 prevalence among swine populations in southern states of India.

MATERIALS AND METHODS

Collection and processing of samples

A total of 434 samples comprising of serum (n=273), pooled postmortem tissues (n=109) and rectal, vaginal and nasal swabs (n=52) were randomly collected irrespective of their breed, health status, age, sex, and vaccination status from PCV2 suspected and healthy swine populations of Tamil Nadu, Kerala, Andhra Pradesh, Telangana and Puducherry during the period 2019 to 2021 were used in this study (Fig. 1 and Table 1). Aseptically collected swab samples were emulsified

Table 1. Distribution of Clinical samples according to location, breed, health, age and sex.

Location (District)	State	Breed					Health Status		Age group			Sex	
		LWY	KPM Gold	Duroc	Cross	Desi	H	S	>1 y	3m-1y	<3m	M	F
Tirunelveli	Tamil Nadu	61	-	-	-	8	28	41	31	19	19	21	48
Chengalpattu		63	17	-	-	1	28	53	34	12	35	28	53
Chennai		56	-	-	-	-	14	42	23	30	3	25	31
Thanjavur		16	-	-	-	-	5	11	11	1	4	3	13
Tenkasi		3	-	12	11	-	18	8	8	8	10	7	19
Kanyakumari		10	-	-	-	-	2	8	5	4	1	3	7
Villupuram		-	-	-	-	20	9	11	9	11	-	9	11
Thiruvallur		10	-	-	-	-	-	10	5	5	-	4	6
Ranipet		-	-	-	-	2	2	-	-	2	-	1	1
Wayanad	Kerala	14	-	-	-	-	-	14	10	4	-	4	10
Kannur		9	-	-	-	-	-	9	5	4	-	5	4
Tirupati	Andhra Pradesh	20	-	-	-	-	15	5	-	20	-	8	12
Hyderabad	Telangana	49	-	-	-	-	-	49	33	16	-	6	43
Pondicherry	Puducherry	-	-	-	-	10	6	4	3	7	-	6	4
Bangalore	Karnataka	27	-	-	-	-	-	27	20	7	-	-	27
Chikkaballapur		-	-	-	15	-	-	15	13	2	-	-	15
Total		338	17	12	26	41	127	307	210	152	72	130	304

(LWY- Large White Yorkshir, KPM G-Kattupakkam Gold, Du-Duroc, De-Desi breed; H- Healthy, S-Suspected for PCV2; >1 y- More than One year, 3m-1y-3 months to 1 year old, <3 m- Under 3 months of age; M-male, F-Female).

in phosphate buffered saline (PBS) and post mortem tissues were triturated in pestle and mortar with sterile sand and PBS. The emulsified swab and triturated tissues were clarified at 6000 rpm for 15 min at room temperature to obtain cell free viral specimens. Blood samples were collected from ear, jugular and saphenous veins and transferred to vacutainer containing clot activator and kept for 4-6 hours at room temperature for serum separation. The tubes were further centrifuged at 3000 rpm at 10 minutes and clear serum samples were transferred into a sterile cryo-vial and labeled specifically and stored at -20°C till further use. All the 434 samples (serum/swabs/ tissue samples) were subjected to DNA extraction using DNeasy Blood and Tissue Kit (Qiagen, Germany) following the standard manufacturer's protocol and extracted nucleic acid was used as template for screening PCV2 genome.

Ethical statement

This research work was governed by the Institutional Biosafety Committee (IBSC) (approval number Lr.No. 0023/DFBS/3/IBSC/2020) and followed all the biosafety measures. All the methods were performed in accordance with the relevant guidelines and regulations of institutional biosafety committee. This study doesn't involve any animal trial studies hence institutional animal ethics committee approval not required for this study. Farm authorities and owners declared their oral consent before the collection of the blood samples as well to the related survey questions. The pigs were sampled by a qualified veterinarian following all applicable guidelines for the care and use of animal.

Molecular screening for PCV2 nucleic acid

All 434 samples (serum/swabs/tissue samples) were subjected to PCV2 detection using forward and reverse primer sequences 5'TAGGTTAGGGCTGTGGCCTT3' and 5'CCGCACCTTCGGATATACTG3' respectively and bind to the 1323-1342 and 1586-1567 nucleotide positions of *ORF2* gene in PCV2 and generates specific product of 264 bp (Larochelle *et al.* 1999). PCR reaction mixture includes 12.5 µl of 2X Taq DNA polymerase Master Mix RED (Ampliqon, Denmark), 1µl of each forward and reverse primer (with 10 pmol/µl concentration each), 4 µl of the template DNA with concentration of ~80-300 ng/µl and nuclease-free water was added to make-up a 25 µl reaction volume. PCR cycling condition includes initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 65°C for 1 min, 72°C for 1 min and final extension at 72°C for 10 min carried out in an automated thermal cycler (Eppendorf Master Cycler, Germany). The PCR products were analyzed by electrophoresis in 1.5 percent agarose gel in Tris acetate EDTA (TAE) buffer (1x) with ethidium bromide final concentration of 0.5 µg/ml. Ten microlitre of PCR product were loaded in the wells along with 100 bp molecular marker. Electrophoresis was carried out at 70V for one hour. The gel was visualized, and the images were documented in a gel documentation system (Bio-Rad Laboratories, USA).

Indirect ELISA based screening for PCV2 antibody

A total 176 serum samples from PCV2 non-vaccinated collected from commercial swine populations are de-complementation at 56 °C for 30 minutes and subjected to PCV2 antibody screening using commercial indirect ELISA kit from Elabscience Biotechnology Inc, USA. As per the manufactures protocol, recombinant PCV2-capsid protein pre-coated ELISA plates were used in screening PCV2 antibody from serum samples. PCV2

Table 2. Prevalence of PCV2 infection by PCR assay State wise sample details.

Sates in India	Number of samples collected	Number of samples positive for PCV2 by PCR	Number of samples negative for PCV2 by PCR	Chi-square value
Tamil Nadu	290	41 (14.1 %)	249 (85.9%)	17.313, p value 0.0039 **
Telangana	49	2 (4.09 %)	47 (95.91%)	
Karnataka	42	0 (0 %)	42(100%)	
Kerala	23	8 (34.8 %)	15(65.2%)	
Andhra Pradesh	20	2 (10 %)	18 (90%)	
Puducherry	10	0 (0 %)	10 (100%)	
Total	434	53 (12.2 %)	381 (87.8%)	

Figures in the parenthesis indicates percentage to total sample in the row.
**Statistically significant.

Table 3. Prevalence of PCV2 within breed, health status, age, and sex and vaccination status (Prevalence of PCV2 infection by PCR assay).

Particulars	Breed					Health Status		Age group			Sex		Vaccination Status	
	LWY	KPM Gold	Duroc	Cross	Desi	H	S	>1 year	3 M - 1year	< 3 M	M	F	V	NV
No.of samples collected	338	17	12	26	41	127	307	210	152	72	130	304	145	289
No. Positive to PCV2 genome	45	2	0	0	6	5	48	25	18	10	15	38	16	37
No. negative to PCV2 genome	293	15	12	26	35	122	259	185	134	62	115	266	129	252
Positivity Percentage	13.31	11.76	0	0	14.63	3.93	15.63	11.90	11.84	13.88	11.53	12.5	11.03	12.80
Chi-square value			3.73 p value 0.44^{NS}			11.47 p value 0.0007**		0.227 p value 0.89^{NS}			0.079 p value 0.78^{NS}		0.282 p value 0.59^{NS}	

**Statistically significant.
(LWY- Large White Yorkshir, KPM G-Kattupakkam Gold, Du-Duroc, De-Desi breed; H- Healthy, S-Suspected for PCV2; >1 y- More than One year, 3m-1y-3 months to 1 year old, <3 m- Under 3 months of age; M-male, F-Female).

specific antibody present in the serum sample binds to the pre-coated PCV2 antigen in the plate and anti-swine secondary antibody conjugated with horseradish peroxidase (HRP) were used to detect this specific PCV2 antigen and antibody complex. Upon addition of tetramethylbenzidine (TMB) /H2O2 chromogenic substrate to this reaction, HRP enzyme bound to antigen-antibody complex oxidizes the chromogen and results in formation of blue color. The color shade is of positive correlation with antibody levels in the samples. This reaction is stopped by adding stop solution to produce a yellow-colored product. The optical density (OD) of this reaction is measured using a microtitre plate Reader (Bio-Rad Laboratories, USA) at 450 nm wavelength. Absorbance value (A-value) of A450 ≥ 0.38, 0.38>A450 ≥ 0.2 and A450 < 0.2 are considered positive, suspicious and negative respectively for PCV2 antibody. Statistical analysis and interpretation.

Molecular and serological surveillance results were analyzed with suitable statistical method and descriptive statistics were used to estimate the molecular and seroprevalence of PCV2. Influence of epidemiological factors such as breed, health status, age, sex and vaccination status were considered and their difference with positivity was tested in chi square (χ^2) test. The relationship of epidemiological factors with PCV2 prevalence was analyzed by logistic regression with 95% confidence level. When the p value less than 0.05, it was considered as statistically significant.

RESULTS AND DISCUSSION

Molecular screening of PCV2 genome

Molecular based techniques are highly sensitive and have been widely employed in detection of PCV2 DNA in tissues and cell free samples. Molecular screening for PCV2 DNA using specific PCV2 primer (Larochelle *et al*. 1999) in 434 samples revealed, 12.2% (n=53) positivity by producing amplicon of 264bp in a PCR assay specific to PCV2 (Fig. 2). The details of results and state wise PCV2 occurrence are displayed in Table 2. State wise PCV2 prevalence by PCR assay revealed 34.8%, 14.1%, 10%, 4.09% in Kerala, Tamil Nadu, Andhra Pradesh and Telangana respectively.

PCR based PCV2 prevalence within breed, health, age, sex and vaccination status were shown in Table 3. Breed wise PCV2 prevalence revealed 14.63%, 13.31% and 11.76% in Desi, Large White Yorkshire breeds (LWY) and Kattupakkam (KPM) gold breeds respectively and nil prevalence was reported from Duroc and crossbred swine breeds. The PCV2 prevalence was 15.63% and

Table 4. Serological screening for PCV2 antibody by ELISA.

Districts	State	Type of farm	Serum sample from unvaccinated pigs (No.)	Sample Positive for PCV2 antibody (No.)
Tirunelveli	Tamil Nadu	Organized	37	25 (67.6%)
Chengalpattu		Organized	11	2 (18%)
Chennai		Organized	31	14 (45.16%)
Kanyakumari		Un-organized	9	2 (22.22 %)
Tenkasi		Un-organized	7	0 (0%)
Thiruvallur		Un-organized	10	2 (20%)
Kannur	Kerala	Organized	9	9 (100%)
Tirupati	Andhra Pradesh	Organized	20	17 (85%)
Bangalore and Chikkaballapur	Karnataka	Organized	42	8 (19.0%)
		Total	176	79 (44.8%)

3.93% in suspected animals and healthy animals respectively. The age wise PCV2 prevalence revealed 11.9%, 11.84% and 13.88% in above one year, between 3 months to one year and below 3 months of age respectively. The PCV2 prevalence in male and females were 11.53% and 12.5% respectively. PCV2 prevalence was 11.03% and 12.80% in vaccinated and non vaccinated animals respectively. The statistical analysis within breed, age, sex and vaccination status revealed no significant difference in the prevalence of PCV2 ($p>0.05$) but, there was a significant difference in the prevalence of PCV2 with regard to health status, suspected swine populations had significantly higher prevalence of PCV2 in comparison to healthy swine populations.

PCV2 infection was widely prevalent among swine population in India. There are regular PCV2 prevalence reports from north and north eastern states of India and all of them revealed molecular prevalence percentage ranging from 10-20% (Bhattacharjee *et al.* 2021). In Assam, screening 54 stillbirth and mummified fetuses collected during 2013–2014 revealed 16.6% (n=9) PCV2 positivity (Pegu *et al*. 2017). PCV2 prevalence percentages Mizoram and Nagaland are 16.4% (n=44) and 11.43% in 268 and 223 samples respectively (Varte *et al.* 2018, Kikon *et al.* 2017). Rajesh *et al.* (2019) tested 306 samples from north eastern hill (NEH) states of India during 2017-18 and found 13.7% (n=42) positivity to PCV2. Whereas most of the global reports from many countries revealed higher molecular prevalence rate of PCV2, ranging from 20-60% (Saporiti *et al.* 2020, Lv *et al.* 2020). A study in Thailand during 2009–2015 in 694 serum samples from different geographical regions revealed 44.09% (n=306) prevalence of PCV2 by PCR assay (Thangthamniyom *et al.* 2017). Lv *et al.* (2020) conducted a study in China in 279 samples collected during the period from 2016 to 2019 which revealed PCV2 molecular prevalence of 60.93% (n=170). In southern India, till date there are only four reports /pilot studies documented. In the year 2012 the incidence of PCV2 infection was first reported in Tamil Nadu in association with stillborn and weak piglets with involvement of PCV2b genotype (Karuppannan *et al.* 2016). Molecular and histopathological study evidenced PCV2 associated PMWS infection in Kerala is (Sairam *et al.* 2019). Another genome based screening study in Kerala revealed 15.38% (8/52) positivity of PCV2 infection (Keerthana *et al.* 2019). Recently Parthiban *et al.* (2021) screened 200 samples from Tamil Nadu for PCV2 nucleic acid and reported PCV2 positivity of 10.5% (n=21) with involvement of PCV2b, PCV2b-IM1 and PCV2d genotypes. The present study with reasonable sample size of 434 numbers from different states of southern India revealed overall molecular PCV2 prevalence rate of 12.2% (n=53) is concordance to that of earlier report from NEH region and Kerala (Rajesh *et al.* 2019, Keerthana *et al.* 2019).

All the swine breeds are equally susceptible to PCV2 infection. However, in field it has been observed that Landrace, Duroc, Large white Yorkshire and Pietrain swine populations show varying susceptibility. Opriessnig *et al.* (2006) conducted experimental study on PCV2 infection in different swine breeds and found that

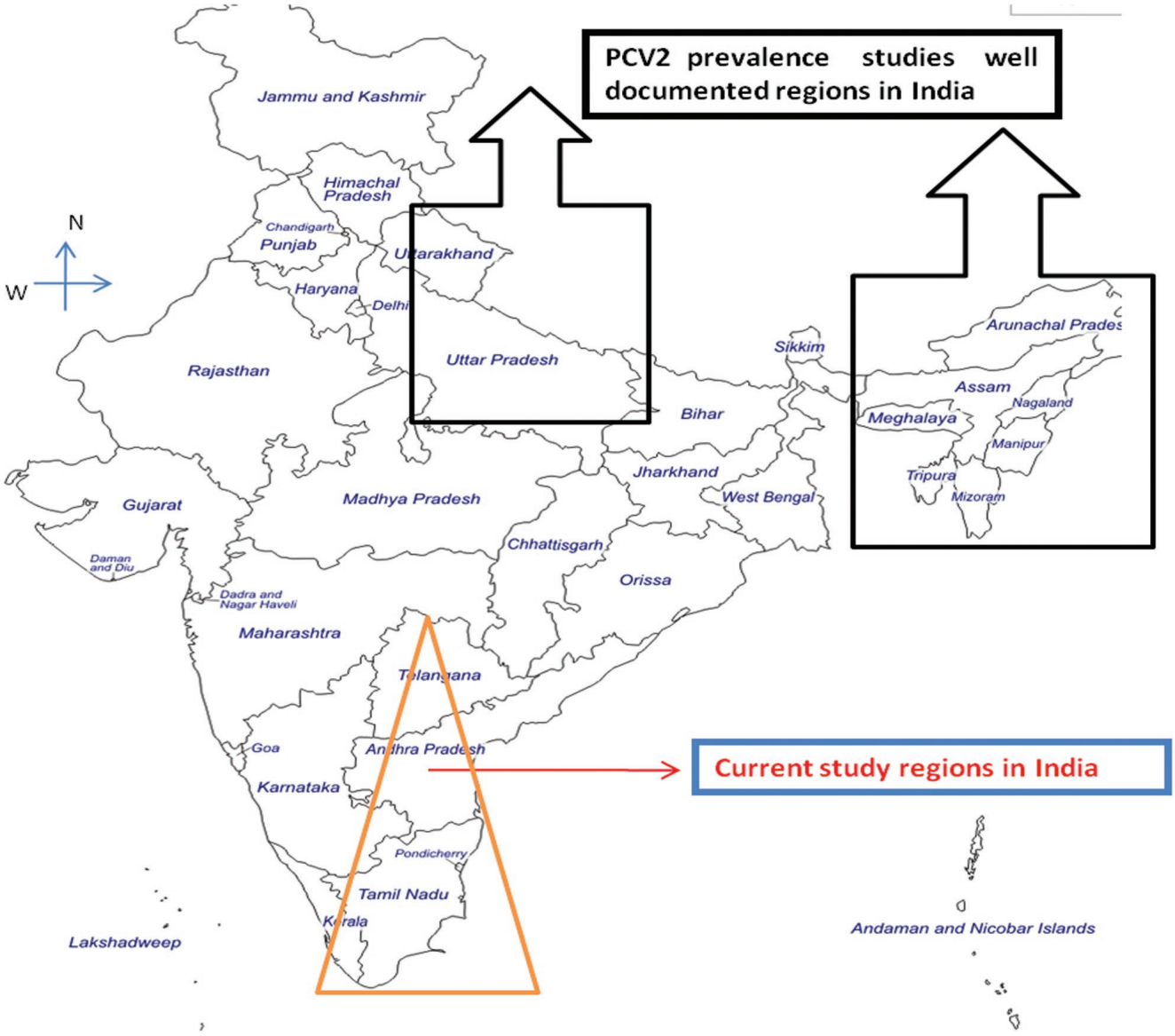

Fig. 1. Geographical regions included in this study.
(PCV2 prevalence well documented regions are marked in black color square box with arrowhead the geographical region of study is marked in orange color triangle).

Landrace breeds were more susceptible to PCV2 infections than Duroc and LWY breeds. But this study revealed 14.63% PCV2 prevalence in desi and 13.3% in LWY breeds and nil prevalence in Duroc and cross bred pigs contrary to the above report. Native swine populations are comparatively have better disease resistance than non-native breeds (Nidup *et al.* 2011). Due to movement of animals at international borders and coexistence of native breeds with exotic and cross breeds may with high load of novel pathogens may introduce newer pathogens to the native breeds and reduce its disease resistance potential. However, the higher prevalence of PCV2 in desi population and nil prevalence from Duroc and crossbred swine breeds needs to be further investigated involving a larger number of desi/ Duroc/cross bred swine populations belong to wider geographical area to substantiate this current observation. Shen *et al.* (2012) studied age related PCV2 susceptibility and found that maternal antibody level directly influences susceptibility to PCV2, 12–16 weeks aged swine populations are more susceptible than 2–7 weeks aged populations. A study by Kim *et al.* (2018) reported higher prevalence of PCV2 infection 24.0% and 21.1% in finisher and grower pigs respectively in comparison to 10.5% and 4.1% in weaning and suckling pigs respectively. The age wise PCV2 prevalence in this study

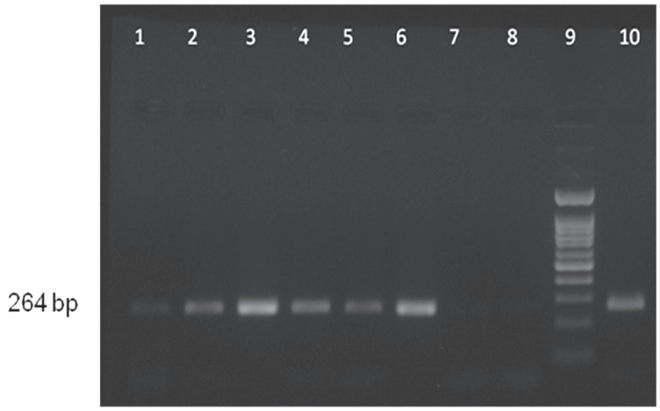

Fig. 2. Screening for PCV2 genome by PCR assay.
(Lane 1-7: Field samples; Lane 8- non-Template control; Lane 9: 100 bp ladder, Lane 10: PCV2 positive DNA).

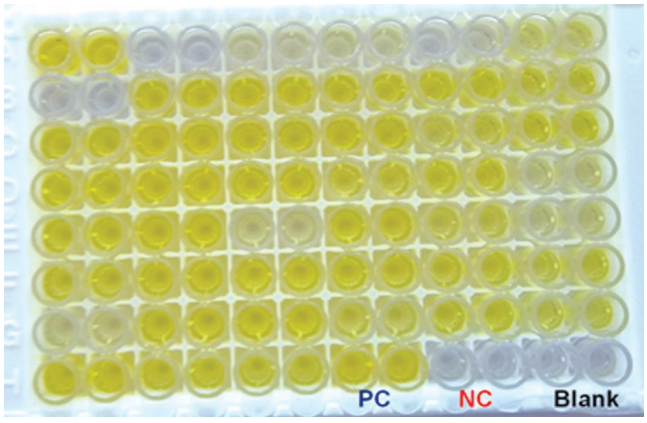

Fig. 3. Screening serum samples for PCV2 antibody by indirect ELISA.
(All the wells in rows A to G and H1 to H6 are filed samples, H7 & H8 wells are Positive control marked as PC in blue colour; Wells H9 & H10 are Negative controls marked as NC in red colour; Wells H11 &H12 are Blank controls marked in Black colour).

revealed almost equal occurrence in all the age groups and not aligning to the above-mentioned reports.

The PCV2 prevalence was comparatively higher in female than male animals the exact reason for this is not known but this may be attributed to maintenance of more female stock in organized farms than male stock. The PCV2 prevalence was significantly higher in PCV2 suspected animals in comparison to healthy animals. Of the 307 PCV2 suspected samples 113 were collected from PCV2 vaccinated herds. Out of 113 suspected samples collected from PCV2 vaccinated animals 14.1% (n=16) were found to be positive for PCV2 genome. Occurrence of PCV2 infection in vaccinated animals may be due to lower antibody titer or neutralizing antibody titer in vaccinated animals and vaccination mediated host defense responses may force the pathogen to alter its genetic nature and generates novel genotypes are the possible reasons for vaccine failure and reinfection (Xiao *et al.* 2016). The statistical analysis within breed, age, sex and vaccination status revealed no significant difference in the prevalence of PCV2 ($p>0.05$) but, there was a significant difference in the prevalence of PCV2 with regard to health status, suspected swine populations had significantly higher prevalence of PCV2 in comparison to healthy swine populations.

Detection of PCV2 specific antibody by indirect ELISA

PCV2 antibody screening in 176 PCV2 non-vaccinated swine serum samples using commercial ELISA kit (Elabscience Biotechnology Inc, USA) revealed PCV2 sero-positivity (as per manufactures OD cutoff values) of 44.8% (n=79) (Fig. 3 and Table 4). Out of 44.8% of seropositive samples from non-vaccinated animals, 97.46% (n=77) positivity was contributed by serum samples from organized farms and 2.53% (n=2) positivity was contributed by samples from unorganized swine populations.

The present research recorded 44.8% sero-prevalence of PCV2 when compared to its 12.2% molecular prevalence and besides this the higher seroprevalence were documented from serum samples collected from organized farms when compared to unorganized swine populations. Barman *et al.* (2018) who screened 5697 serum samples for PCV2 antibody from entire north eastern regions (NER) of India during the period from 2011 to 2017 and revealed mean seropositivity of 31.27%, with higher incidence in organized farms (65.7%) compared to unorganized farms (17.6%) supports the present study. Rajesh *et al.* (2019) explored seroprevalence of PCV2 from north eastern hill (NEH) states of India between the year 2017-2018 in 306 samples and found 49.35% (n=151) positivity. In Nagaland, analysis of 223 serum samples by commercial ELISA kit, revealed 51.57% seropositivity to PCV2 antibodies (Kikon *et al.* 2017). A study in Meghalaya with 1899 serum samples in different time periods reported 80.8%, 79.1% and 96.2% of PCV2 seroprevalence in 2014, 2015 and 2016 years respectively (Mukherjee *et al.* 2018). Another study in Meghalaya during the period 2016 to 2018 with 289 serum samples revealed mean seropositivity of 66.09% (Mukherjee *et al.* 2019). Most of the above studies from north and north-eastern parts India also evidenced higher seroprevalence favourably supporting this present study.

Most of the sero-prevalence studies around the globe

also documented higher prevalence of PCV2 antibody and making it as an alarming situation. Sero-surveillance in Australian National Pig Serum Bank in 2001 serum samples revealed 75.8% (n=1516) prevalence of PCV2 (Finlaison *et al.* 2007). In Canada, out of 386 serum samples screened 82.4% seropositivity recorded (Liu *et al.* 2002). The high prevalence rate of PCV2 might be attributed to stable nature of virus that can be shed through all the natural secretions and excretions including semen (Patterson *et al.* 2011). This may be also due to inability of commercial ELISA kits in differentiating mixtures of PCV1 and PCV2 antibodies in PCV1 and PCV2 co-infections and in PCV2-vaccinated pigs (Han *et al.* 2016). The overall 68 to 76% genetic homology between PCV1 and PCV2 viruses with 86 to 100% nucleotide sequence identities in ORF1 regions coding replicase protein in both PCV1 and PCV2 are the contributing factors for the antigenic cross reactivity between these two viruses (Ouardani *et al.* 1999, Rodriguez-Arrioja *et al.* 2000). Substantial vaccinated swine population (17.7%) were found to be seronegative for PCV2 antibody this may be due to lower longevity of PCV2 antibody titre. As per the manufactures guidelines the single dose recombinant commercial vaccine available India (Ingelvac CircoFLEX) provides at least 4 months of immunity and the antibody titre may start declining after that specified period of time and this may be the reason for sero-negativity in vaccinated swine populations.

CONCLUSION

This study is the first molecular and serological prevalence report on PCV2 infection from southern states of India. The present study documented 12.2% of molecular prevalence and 44.8% sero-prevalence of PCV2 in southern India. This study also revealed that within breed, age, sex and vaccination status the PCV2 prevalence is not statistically significant. PCV2 is widespread pathogen among swine populations of southern India which necessitates the need for implementation of suitable prevention and control measures.

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REFERENCES

Allan GM, Mcneilly F, Kennedy S, Daft B, Clarke EG *et al.* (1998) Isolation of Porcine circovirus-like viruses from pigs with a wasting disease in the USA and Europe. J Vet Diagn Invest 10: 03-10. DOI: 10.1177/104063879801000102.

Barman NN, Nath B, Kumar V, Sen A, Dutta TK *et al.* (2018) The emergence of porcine circovirus 2 infections in the Northeastern part of India: A retrospective study from 2011 to 2017. Transbound Emerg Dis 65(6): 1959-1967. DOI: 10.1111/tbed.12977.

Bhattacharjee U, Sen A, Sharma I (2021) A retrospective study reveals the porcine circovirus-2f genotype predominant in the indigenous pig population of North-eastern India. Infect Genet Evol 96: 105100.

Ellis J, Hassard L, Clark E, Harding J, Allan G *et al.* (1998) Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome. Can Vet J 39(1): 44-51.

Finlaison D, Kirkland P, Luong R, Ross A (2007) Survey of porcine circovirus 2 and postweaning multisystemic wasting syndrome in New South Wales piggeries. Aust Vet J 85(8): 304-310. DOI: 10.1111/j.1751-0813.2007.00181.x.

Han S, Xiao Y, Zheng D, Gu Y, Xuan Y *et al.* (2016) Establishment and application of a competitive enzyme-linked immunosorbent assay differentiating PCV2 antibodies from mixture of PCV1/PCV2 antibodies in pig sera. BMC Vet Res 26/12(1): 175. DOI: 10.1186/s12917-016-0802-9.

Karuppannan AK, Ramesh A, Reddy YK, Ramesh S, Mahaprabhu R *et al.* (2016) Emergence of porcine circovirus 2 associated reproductive failure in Southern India. Transbound Emerg Dis 63(3): 314-320. DOI: 10.1111/tbed.12276.

Keerthana J, Mammen J Abraham, Krithiga K, Priya PM (2019) Studies on naturally infected cases of post weaning multi systemic wasting syndrome (PMWS) in piglets. Pharm Innov 8(6): 485-488.

Kikon LJ, Rajkhowa TK, Arya RS, Singh YD, Ravindran R (2017) Seroprevalence of porcine circovirus type-2 (PCV2) and molecular diagnosis of PCV2 associated reproductive failure in pig population of Nagaland. Indian J Vet Pathol 41(2): 79-83.

Kim SC, Nazki S, Kwon S, Juhng JH, Mun KH *et al.* (2018) The prevalence and genetic characteristics of porcine circovirus

type 2 and 3 in Korea. BMC Vet Res 26/14(1): 294. DOI: 10.1186/s12917-018-1614-x.

Kumar GS, Sharma R, Paliwal OP (2006) 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, 83.

Ladekjaer-Mikkelsen AS, Nielsen J, Stadejek T, Storgaard T, Krakowka S *et al.* (2002) Reproduction of postweaning multisystemic wasting syndrome (PMWS) in immunostimulated and non-immunostimulated 3-week-old piglets experimentally infected with porcine circovirus type 2 (PCV2). Vet Microbiol 89(2-3): 97-114. DOI: 10.1016/s0378-1135(02)00174-8.

Larochelle R, Antaya M, Morin M, Magar R (1999) Typing of porcine circovirus in clinical specimens by multiplex PCR. J Virol Methods 80(1): 69-75. DOI: 10.1016/s0166-0934(99)00032-4.

Liu Q, Wang L, Willson P, O'Connor B, Keenliside J *et al.* (2002) Seroprevalence of porcine circovirus type 2 in swine populations in Canada and Costa Rica. Can J Vet Res 66(4): 225-231.

Lv N, Zhu L, Li W, Li Z, Qian Q *et al.* (2020) Molecular epidemiology and genetic variation analyses of porcine circovirus type 2 isolated from Yunnan province in China from 2016-2019. BMC Vet Res 23/16(1): 96. DOI: 10.1186/s12917-020-02304-8.

Meehan BM, Mcneilly F, Todd D, Kennedy S, Jewhurst VA *et al.* (1998) Characterization of novel circovirus DNAs associated with wasting syndromes in pigs. J Gen Virol 79: 2171-2179. DOI: 10.1099/0022-1317-79-9-2171.

Mukherjee P, Karam A, Chakraborty AK, Baruah S, Pegu R *et al.* (2019) Identification of a novel cluster of PCV2 isolates from Meghalaya, India indicates possible recombination along with changes in capsid protein. Infect Genet Evol 71: 7-15. DOI: 10.1016/j.meegid.2019.02.026.

Mukherjee P, Karam A, Singh U, Chakraborty AK, Huidrom S *et al.* (2018) Seroprevalence of selected viral pathogens in pigs reared in organized farms of Meghalaya from 2014 to 2016. Vet World 11(1): 42-47. DOI: 10.14202/vetworld.2018.42-47.

Nidup K, Tshering D, Wangdi S, Gyeltshen C, Phuntsho T, Moran C (2011) Farming and biodiversity of pigs in Bhutan. Animal Genetic Resources 48: 47-61. DOI:10.1017/S2078633610001256.

Opriessnig T, Fenaux M, Thomas P, Hoogland MJ, Rothschild MF *et al.* (2006) Evidence of breed-dependent differences in susceptibility to porcine circovirus type-2-associated disease and lesions. Vet Pathol 43(3): 281-293. DOI: 10.1354/vp.43-3-281.

Ouardani M, Wilson L, Jetté R, Montpetit C, Dea S (1999) Multiplex PCR for detection and typing of porcine circoviruses. J Clin Microbiol 37(12): 3917-3924. DOI: 10.1128/JCM.37.12.3917-3924.1999. Erratum in: J Clin Microbiol 38(4): 1707.

Parthiban S, Ramesh A, Karuppannan AK, Dhinakar Raj G, Johnson Rajeswar J *et al.* (2021) Emergence of novel porcine circovirus 2 genotypes in Southern India. Transboun Emerg Dis Online. DOI:10.1111/tbed.14158.

Patterson AR, Ramamoorthy S, Madson DM, Meng XJ, Halbur PG, Opriessnig T (2010) Shedding and infection dynamics of porcine circovirus type 2 (PCV2) after experimental infection. Vet Microbiol 21/149(1-2): 91-98. DOI: 10.1016/j.vetmic.2010.10.020.

Pegu SR, Sarma DK, Rajkhowa S, Choudhary MRS, Sarma D, Das JP (2017) Molecular detection of porcine circo virus type 2 and porcine parvo virus in pigs having reproductive problems and histopathological studies in the tissue of aborted pig foetuses. Indian J Anim Res 51 (4): 732-736.

Rajesh JB, Rajkhowa S, Dimri U, Prasad H, Pegu SR *et al.* (2019) Seroprevalence of PCV2 in north eastern hill states of India. Indian J Anim Sci 89: 119-122.

Ren L, Chen X, Ouyang H (2016) Interactions of porcine circovirus 2 with its hosts. Virus Genes 52(4): 437-444. DOI: 10.1007/s11262-016-1326-x.

Rodriguez-Arrioja GM, Segales J, Balasch M, Rosell C, Quintant J *et al.* (2000) Serum antibodies to porcine circovirus type 1 and type 2 in pigs with and without PMWS. Vet Rec 146(26): 762-764.

Sairam R, Dhanush Krishna B, Krithiga K, Sajitha IS, Priya PM *et al.* (2019) Molecular and pathological studies of post-weaning multi-systemic wasting syndrome among piglets in Kerala. India. Explor Anim Med Res 9(2): 137-144.

Saporiti V, Huerta E, Correa-Fiz F, Grosse Liesner B, Duran O *et al.* (2020) Detection and genotyping of porcine circovirus 2 (PCV-2) and detection of porcine circovirus 3 (PCV-3) in sera from fattening pigs of different European countries. Transbound Emerg Dis 67(6): 2521–2531. https://doi.org/10.1111/tbed.13596.

Segales J (2012) Porcine circovirus type 2 (PCV2) infections: clinical signs, pathology and laboratory diagnosis.

Virus Res 164(1-2): 10-19. DOI: 10.1016/ j.virusres.2011.10.007.

Shen HG, Loiacono CM, Halbur PG, Opriessnig T (2012) Age-dependent susceptibility to porcine circovirus type 2 infections is likely associated with declining levels of maternal antibodies. J Swine Health Prod 20(1): 17-24.

Thangthamniyom N, Sangthong P, Poolperm P, Thanantong N, Boonsoongnern A *et al.* (2017) Genetic diversity of porcine circovirus type 2 (PCV2) in Thailand during 2009-2015. Vet Microbiol 208: 239-246. DOI: 10.1016/j.vetmic.2017.08.006.

Varte L, Ravindran R, Chhangte L, Tonsing L (2018) Seroprevalence of porcine circovirus-2 associated reproductive failure in pigs of Mizoram, India. Int J Livest Res 8(12): 194-200.

Xiao CT, Harmon KM, Halbur PG, Opriessnig T (2016) PCV2d-2 is the predominant type of PCV2 DNA in pig samples collected in the U.S. during 2014-2016. Vet Microbiol. 197: 72-77. DOI: 10.1016/j.vetmic.2016.11.009.

Yang S, Yin S, Shang Y, Liu B, Yuan L *et al.* (2018) Phylogenetic and genetic variation analyses of porcine circovirus type 2 isolated from China. Transbound Emerg Dis 65(2): e383-e392. DOI: 10.1111/tbed.12768.

Zheng G, Lu Q, Wang F, Xing G, Feng H *et al.* (2020) Phylogenetic analysis of porcine circovirus type 2 (PCV2) between 2015 and 2018 in Henan Province, China. BMC Vet Res 16(1): 6. DOI: 10.1186/s12917-019-2193-1.

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