



Porcine circovirus 2d associated with stillborn mummified foetus of pig

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Pig farming involves good economic returns due to intrinsic characteristics of pigs including shorter generation interval, good feed conversion efficiency and high prolific breeding rate. In last livestock census (2019) of India, a decline of about 12.03% was reported in swine population since 2012. One of the possible causes for this decline in pig population could be attributed to various disease conditions which are not addressed properly. Failure to identify the possible etiological agent/s will lead to improper control and management of the disease condition. Sow productivity is basically determined by the total number of live piglets born, piglets survived until weaning, weaning weight, piglets weaned per sow per year and piglets weaned per sow per litter (Angjelovski *et al.* 2014). The low reproductive performance can reduce sow productivity resulting in financial losses. Among various possible aetiologies causing reproductive problems in pigs, most important are those diseases caused by viruses. The viral infections caused by porcine circovirus 2, porcine parvo virus, porcine circovirus 3 and porcine respiratory and reproductive syndrome virus are most commonly associated with reproductive failure in pigs (Ritzmann *et al.* 2005, Sharma and Saikumar 2010; Bera *et al.* 2020). Porcine circovirus 2 (PCV2) is non-enveloped, small, circular, single stranded DNA virus associated with various clinical manifestations together termed as PCV-AD (PCV-associated diseases). PCV-RD (PCV- Reproductive disease) is one of the clinical manifestations caused by PCV2 (Saikumar and Das 2019). PCV2 is associated with reproductive failure including stillbirths, mummification, late term abortions and pre weaning mortality in pigs (Ritzmann *et al.* 2005). In view of the significance of reproductive health for the pig enterprise, the occurrence of stillborn mummified foetuses in the organized farm was investigated to find out the etiology involved.

In an organized pig farm of Uttar Pradesh of 100-120

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pig capacity, recurrent occurrence of stillborn piglets (2-3 piglets) with live births during each farrowing was noticed. One of the stillborn piglets was presented for necropsy at post mortem facility, Division of Pathology, IVRI to know the possible cause of the prevailing condition. Upon investigation, the animals were found to be not vaccinated against PCV2 infection. Detailed post mortem examination was carried out and all the findings were recorded. Tissue samples (heart, lungs, liver, kidney, spleen and intestine) from mummified foetus were collected in 10% neutral buffered formalin and also on ice and stored at -20°C after proper labelling.

Approximately 25 mg of pooled tissue sample was triturated in 500 µL of lysis buffer in a 1.5 mL sterile Eppendorf tube and DNA was further isolated using commercially available DNeasy Blood and Tissue Mini Kit (Qiagen, USA) as per manufacturer's protocol. RNA isolation was carried out from pooled tissue sample using TRIzol® method and cDNA was prepared using high-capacity cDNA synthesis kit (Invitrogen).

Polymerase Chain Reaction (PCR) was performed for Porcine Circovirus-2 (PCV-2) targeting *ORF2* region, porcine parvoviral infection (PPV) targeting *VP2* gene and PCV-3 targeting *ORF2* region. Reverse Transcriptase PCR (RT-PCR) performed for porcine respiratory and reproductive syndrome virus infection (PRRS) targeting *N* gene using previously published primers given in Table 1.

The obtained amplicon was analysed on 1.5% agarose gel after electrophoresis at 75 V for 40 min. The PCR amplicons obtained were further sequenced at sequencing facility of Eurofins, Bengaluru and the obtained sequence was aligned in MEGA 11 and phylogenetic tree was constructed and was compared with available sequences in the NCBI.

Externally, the foetus was found to be partially dehydrated and dark brown in colour with shrunken eyes. Hooves were curled up in both fore limbs and hind limbs. Internally, visceral organs including lungs, heart, kidneys, spleen, liver and intestine were completely developed, partially dehydrated and dark brown in colour (Fig. 1).

The PCR assay for PCV2 resulted in amplification of expected 481-bp fragment of *ORF2* gene (Fig. 2). Other

Table 1. Details of the primers used in the present study

Virus	Oligonucleotides sequence 5'-3'	Target region	Amplicon size	Reference
PCV2	CGGATATTGTAGTCCTGGTCCG ACTGTCAAGGCTACCACAGTCA	<i>ORF2</i>	481 bp	Ellis <i>et al.</i> 1999
PPV	CCAGCAGCTCACAAGAAAAGTTATCAC GTCCATGTTGGTAATCCATTGTAAATCT	<i>VP2</i>	226 bp	Arnauld <i>et al.</i> 1998
PRRSV	CCCGGGTTGAAAAGCCTCGTG GGCTTCTCCGGGTTTTTCTTCCTA	<i>N gene</i>	228 bp	Tian <i>et al.</i> 2010
PCV3	CCA CAG AAG GCG CTA TGT C CCG CAT AAG GGT CGT CTT G	<i>ORF2</i>	330 bp	Palinski <i>et al.</i> 2017

most possible infections PPV, PCV3 and PRRSV were found negative using PCR and RT-PCR techniques, respectively.

Phylogenetic analysis of obtained sequence (GenBank accession: OQ455174) was compared with available sequences in NCBI GenBank database, which confirms that PCV2 IVRI SDL sequence shared an evolutionary relationship with other PCV2d genotype (Fig. 3).

The association of PCV2 with reproductive failure has been well documented before from various countries (Bogdan *et al.* 2001, Oropeza-Moe *et al.* 2017) including India (Sharma and Saikumar 2008, Karuppanan *et al.* 2016). In unvaccinated sows/ gilts, the occurrence of PCV2 associated reproductive failure is evident (Oropeza-Moe *et al.* 2017). In the present case, upon investigation, the animals of the farm were found to be not vaccinated against PCV2 infection and the presence of PCV2 could be demonstrated by PCR. Other most possible causes including PPV, PCV3 and PRRSV infection were found negative using PCR and RT-PCR, respectively. This indicated the role of PCV2 as major pathogen in reproductive failure in pigs. Studies have associated porcine circovirus type 2 (PCV2) with reproductive failures with direct effects on embryo or foetus (Mateusen *et al.* 2007, Oropeza-Moe *et al.* 2017). On phylogenetic analysis, the obtained sequence was found to be similar to PCV2d genotype as reported earlier. PCV2d is most commonly associated with abortions, mummifications and foetal autolysis in sows (Unterweger *et al.* 2021).

Vaccination against PCV2 in pigs is found to be quite

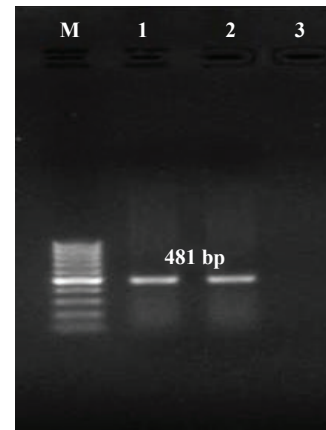


Fig. 2. Ethidium bromide stained 1.5 % agarose gel showing 481 bp PCR amplicon of *ORF2* region of PCV2. (Lane M: Ladder 100 bp, Lane 1: Positive control PCV2 (481 bp), Lane 2: Test sample (481 bp) and Lane 3: Negative control).

effective (da Silva *et al.* 2014). Presently, many PCV2 vaccines are available commercially and most of them are whole inactivated PCV2 based vaccines and also, subunit vaccines based on recombinant PCV2 capsid protein. Proper vaccination and good management practices can protect pigs from disease caused by PCV2 infection (Saikumar and Das 2019).

It is noticed that no further cases have been reported after taking proper precautions by initiating vaccination against PCV2 in pigs in the farm. This is suggestive of possible involvement of PCV2 as primary causative agent for the reproductive failure in sows/ gilts. Proper implementation



Fig 1. A. Partially dehydrated, dark brown foetus with shrunken eyes; B. Partially dehydrated, completely developed visceral organs; C-D. Curled up hooves in both fore limbs and hind limbs.

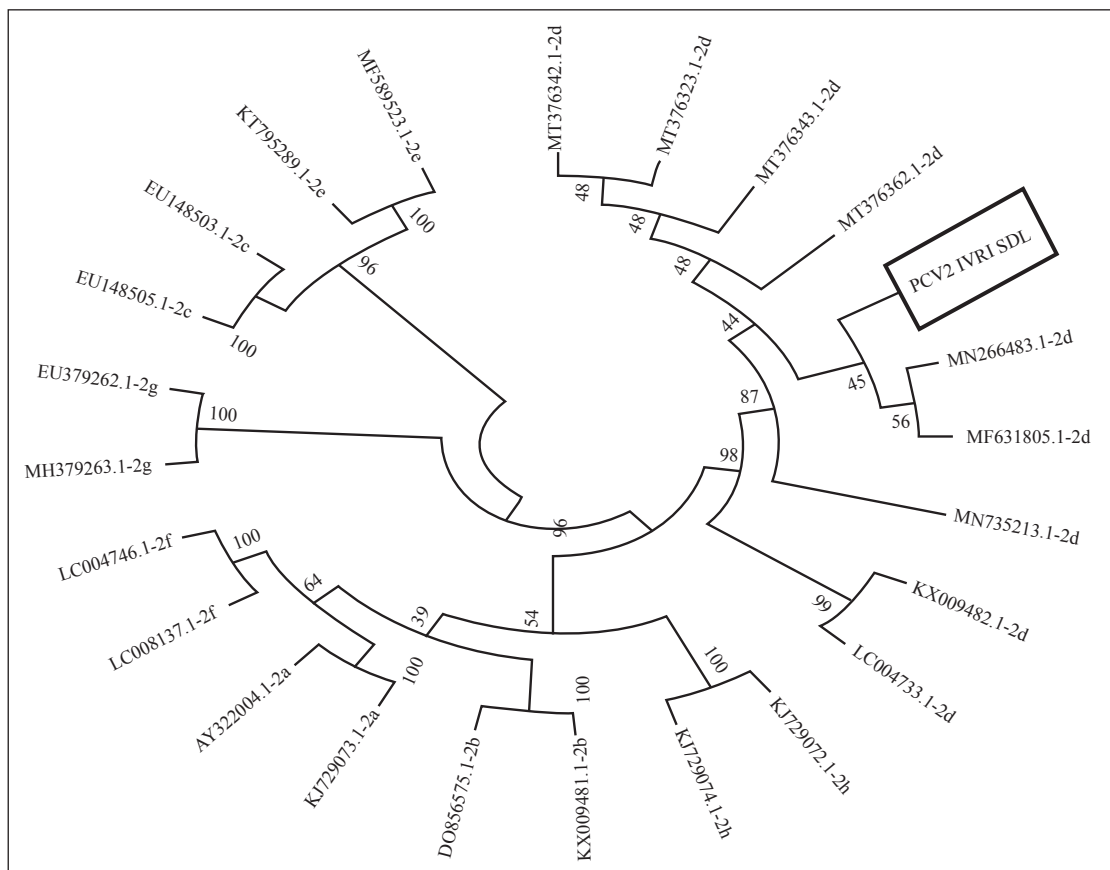


Fig. 3. Phylogenetic analysis of PCV2 sequence (The sequences alignment and phylogenetic tree construction was done using MEGA 11 software).

of protective vaccination against PCV2 along with good management practices including disinfection of the animal shed/ buildings, nutritious feed, and limiting contact between pigs helps in effective control of PCV2 infection in pigs. Vaccination of breeding pigs against PCV2 is also important to maintain the breeding population free of PCV2 as shedding PCV2 in semen of boar has been reported (Opriessnig *et al.* 2011).

Conclusively, positive polymerase chain reaction showed the presence of Porcine circovirus 2 in tissues of mummified swine foetus and further confirmation by genome sequencing is suggestive of PCV2d genotype as one of the possible etiological agents to cause stillbirth and mummification in pigs of porcine foetuses. PCV2 has to be considered and screened routinely in the reproductive failure cases. Proper precautions like regular vaccination against PCV2 need to be implemented for the prevention of the infection in pigs.

SUMMARY

Recurrent occurrence of stillborn piglets was noticed along with live births during farrowing in pigs in an organized pig farm of Uttar Pradesh. On detailed post mortem examination, externally, the foetus was found to be partially dehydrated and dark brown in colour with shrunken eyes. Internally, visceral organs including lungs, heart, kidneys, liver, spleen and intestine were partially

dehydrated and dark brown in colour. Upon molecular investigation, the pooled tissue samples were found positive for porcine circovirus 2 (PCV2) infection using PCR technique and further confirmed the PCV2d genotype by DNA sequencing. Other most possible infections including porcine parvoviral infection (PPV), PCV3 and porcine respiratory and reproductive syndrome virus infection (PRRS) were found negative using PCR and RT-PCR techniques, respectively. This result is suggestive of role of PCV2d as a possible etiological agent to cause stillbirth and mummification of porcine foetuses.

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