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Detection of viral agents associated with Porcine Respiratory Disease Complex (PRDC) in pigs in North Kerala#

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Abstract

In pigs, stress factors such as pathogenic infections, environmental conditions, and various managemental practices can lead to a disease condition known as Porcine respiratory disease complex (PRDC). The main viral agents associated with this condition are Porcine circovirus 2 (PCV2), Porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), porcine respiratory coronavirus (PRCV), and pseudorabies virus. A study was undertaken to assess whether PCV2 and PRRSV, alone or in combination, are present in cases of respiratory ailments of pigs in North Kerala. The presence of PCV2 was detected by polymerase chain reaction (PCR) targeting the open reading frame 2 (ORF2) of the virus and PRRSV was detected by reverse transcriptase PCR (RT-PCR) targeting ORF6. A total of 54 tissue and lung samples were collected and screened for the presence of PCV2 and PRRSV. Of the samples tested, PCV2 could be detected in 22 (40.74 %) and PRRSV could be detected in 13 (24.07 %) samples. Two tissue samples showed mixed infections with PCV2 and PRRSV. The result shows that viral agents associated with PRDC are prevalent in pigs in North Kerala and that coinfections are also present.

Keywords: *Porcine respiratory disease complex, Porcine circovirus 2, Porcine reproductive and respiratory syndrome virus, polymerase chain reaction*

The condition known as Porcine respiratory disease complex (PRDC) affects the swine industry worldwide. Cough, dyspnea, fever, and anorexia are some of the clinical signs of PRDC. Along with is there can be reduced growth and feed conversion efficiency. It is a disease condition caused by a combination of various stress factors such as infectious pathogens, environmental stressors, and varying types of production systems and management practices. Porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus 2 (PCV2), swine influenza virus, porcine respiratory coronavirus, and pseudorabies virus are the viral pathogens associated with PRDC (Chae, 2016). The bacteria that cause PRDC include *Pasteurella multocida, Mycoplasma hyopneumoniae, Actinobacillus pleuropneumonia, Streptococcus* suis and *Haemophilus parasuis* (Zhu *et al*., 2021). Of the viral agents, PCV2 and PRRSV, usually in combination, play a significant role in the the pathogenesis of PRDC. The PCV2 is a DNA virus which is the causative agent of porcine circovirus-associated diseases (PCVADs), which includes post-weaning multisystemic wasting

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syndrome (PMWS) (Somashekara *et al*., 2023). PRRSV causes reproductive failure and respiratory disorders in pigs (Chaithra *et al.,* 2020). In North Kerala, there has been many cases of respiratory illness and subsequent mortality in pigs and in many of the cases, the symptoms and lesions observed were severe. This paper reports the results of a study conducted to detect the presence of viral agents *viz*. PRRSV and PCV2 associated with PRDC in pigs in North Kerala.

Materials and methods

Collection of samples

Tissue samples (lymph node and spleen, and lung samples separately) were collected from 34 animals brought for post-mortem to the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Pookode during the period from January 2022 to August 2023. The animals were from Wayanad, Kozhikode, Malappuram and Kannur districts of Kerala and had exhibited symptoms of respiratory illness prior to death. The tissue samples were placed in sterile screw-capped polypropylene vials and processed immediately or stored at –80 °C until processing to prevent DNA or RNA deterioration. Further, results of PCV2 and PRRSV testing of 20 tissue samples collected from pigs with respiratory ailments during the period from January 2020 to December 2021 were used to arrive at the percentage positivity for the period from January 2020 to August 2023.

Detection of PCV2 using conventional PCR using ORF2-specific primers

For detection of PCV2, total DNA was extracted from the 34 tissue samples, and lung samples using DNeasy Blood & Tissue Kit (Qiagen, Germany) as per the manufacturer's protocol. The extracted DNA was kept at –20 °C till further processing. The presence of PCV2 in the samples were detected by polymerase chain reaction (PCR), by employing primers specific to the open reading frame 2 (ORF2) (Ellis *et al.,* 1999). The 25 µL reaction mix consisted of 12.5 µL of 2X Emerald Amp GT PCR Mastermix containing Taq polymerase (DSS TaKaRa Bio, India), 1 µL each of 10 pmol PCV2 forward and reverse primers, 2 µL of DNA as template, and the rest NFW. The cycling conditions for the amplification of PCV2 were 95 °C for 2 min (initial denaturation), 34 cycles of 94 °C for 30 sec (denaturation), 55 °C for 45 sec (annealing), and 72 °C for 45 sec (polymerization) followed by single cycle at 72 °C for 5 min (final extension). The PCR products were electrophoresed in agarose gels containing ethidium bromide and visualized in a gel documentation system under UV illumination. Representative PCR products were outsourced for sequencing by Sanger dideoxy chain termination method. Nucelotide sequences obtained were analysed by Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm the identity of the virus.

Detection of PRRSV using conventional RT-PCR using ORF6-specific primers

For PRRSV detection, total RNA was extracted from the 34 tissue samples, and lung samples using a GeneJET RNA purification kit (Thermo Scientific, USA). Reverse transcription of total RNA into cDNA was carried out using RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Scientific, USA), as per the manufacturer's protocol. The extracted RNA was kept at –80 °C till further processing. The presence of PRRSV in the samples was detected by reverse transcriptase PCR (RT-PCR), by employing primers specific to the ORF6 region of the virus as previously described (Xu *et al.,* 2012). The 25 µL reaction mixture of the PCR comprised of 12.5 µL 2X EmeraldAmp GT PCR master mix (2X) (Takara), 1 µL each of forward and reverse primers (10 pmol), 2 µL of cDNA and rest NFW to make up the volume. The cycling conditions were 95°C for 2 min (initial denaturation), 35 cycles of 95°C for 45 sec (denaturation), 57°C for 45 sec (annealing) and 72°C for 45 sec (polymerisation) followed by a single cycle at 72°C for 2 min (final extension) and the PCR products were visualized as before. Representative PCR amplicons were sequenced and analysed by BLAST as indicated previously.

Results and discussion

Out of the 34 samples tested, 11 (32.35 per cent) samples were found to be positive for PCV2 targeting the ORF2 region of the genome as evidenced by the appearance of 481 bp amplicons (Fig. 1). In PRRSV testing, out of the 34 samples tested, 7 (20.58 per cent) samples were found to be positive as evidenced by the appearance of 451 bp amplicons (Fig. 2). When the lung samples were tested separately, only one sample was positive for PRRSV. Results of BLAST analysis indicated that the nucleotide sequences belonged to that of PCV2/PRRSV as the case may be. In the previously tested samples, the eleven and six samples were positive for PCV2 and PRRSV respectively. Thus the combined positivity of PCV2 is 40.74 per cent (22 out of 54 samples) and for PRRSV is 24.07 per cent (13 out of 54 samples).

In India, PCV2 was first detected in 2006 from cases of PMWS- associated PCV2 systemic disease (PCV2-SD) (Kumar *et al.,* 2006). The prevalence of PCV2 was 49.35 per cent in samples collected from the north eastern states of India (Rajesh *et al.,* 2019). The incidence of PCV2 infection in Kerala has been reported previously (Vijayaragavan *et al.,* 2021, Jessil *et al.,* 2022). In an earlier study carried out in North Kerala to detect PCV2, 58.06 per cent of the samples tested was found to be positive for the virus (Somashekara *et al.,* 2023) and hence the percentage positivity obtained in this study is lesser. In the case of PRRSV, the virus was detected in India in

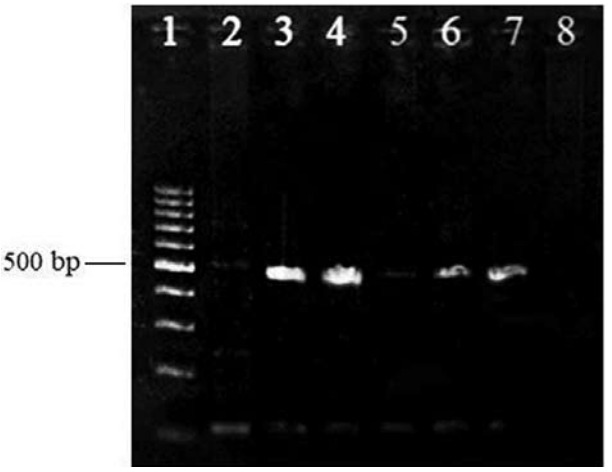

Fig. 1. Agarose gel picture showing 481 bp amplicons of PCV2 (Lane 1: 100 bp DNA marker; Lane 2: Negative sample; Lanes 3 to 7: Positive samples of which sample in lane 5 is weak positive; Lane 8: No template control)

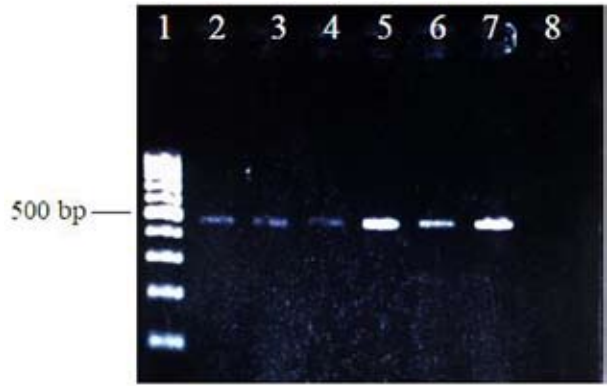

Fig. 2. Agarose gel picture showing 451 bp amplicons of PRRSV (Lane 1: 100 bp DNA marker; Lanes 2 to 7: Positive samples; Lane 8: No template control)

46 (90 %) of the 51 necropsy cases by using RT-PCR (Rajkhowa *et al.,* 2015). In 2016, the serological evidence of PRRSV was reported in Kerala (Kashyap *et al.,* 2016). In an earlier study to detect and characterise the virus in North Kerala, the percentage positivity reported was 32.2 per cent (Chaithra *et al.,* 2020) which is also higher than that obtained in this study.

In the present study, two tissue samples showed mixed infections with PCV2 and PRRSV. Co-infection with these two viruses has been reported previously. Drolet *et al.* (2003) reported coinfection rates of 42%, and 85.4% in lungs of post weaned pigs. Varying coinfection rates from 21.95 to 52.4 per cent have been reported (Liu *et al*., 2013; Zeng *et al*., 2014). Infection with these two viruses can impair host defenses and make the infected animal susceptible to other pathogens (Niederwerder *et al*., 2016). Also, PRRSV and PCV2 coinfection significantly increased viral pathogenicity and cytokine responses, resulting in severe clinical signs, lung pathology, and death (Zhang *et al*., 2022). It has been reported that the replications of PCV2 and PRRSV were increased and that very severe clinical symptoms and lesions were detected in piglets infected with the highly pathogenic strains of PRRSV followed by PCV2 infection (Fan *et al*., 2013). During co-infection, the PRRSV lengthens PCV2 viremia (Sinha *et al*., 2011) and PCV2 infection increases mutations of PRRSV. In pigs coinfected with PRRSV and certain PCV2 subtypes, mutation rates in PRRSV were significantly higher when compared to that in pigs infected with PRRSV only (Yin *et al*., 2013).

Conclusion

The PCV2 and PRRSV, viral agents associated with PRDC, are prevalent in pigs in North Kerala and the prevalence of the viral agents is lesser than those reported from the region in the recent past. Coinfection with these two agents also have been detected. Presence of these agents might play a role in the incidence of respiratory infection in pigs in the region.

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Conflict of interest

The authors declare that they have no conflict of interest.

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