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Porcine dermatitis and nephropathy syndrome on natural infection of Porcine Circovirus Type-2 in pigs in Kerala, southern India

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Abstract

Porcine circovirus type-2 (PCV-2), is a widely studied pathogen in the swine population during the last decade. Even though, PCV-2 has many disease manifestations, the objective of the current study was to investigate the cutaneous and renal lesions in PCV-2 infected cases. Swine carcasses presented for post-mortem examination with a history of skin lesions, inappetance, dyspnoea and diarrhoea, during September 2021 to April 2022 from different parts of Kerala were utilised in this study. The samples were collected and screened with Polymerase chain reaction (PCR) after which, histological and immunohistochemical examination were performed. Three samples were found positive for PCV-2 out of 25 samples using PCR. Microscopically, skin revealed congestion, haemorrhages and infiltration of mononuclear infiltration cells and neutrophils in dermis and spongiosis in epidermis. Kidney revealed haemorrhages, congestion, tubular necrosis and degenerative alterations, interstitial mononuclear cell infiltration with occasional glomerulitis and vasculitis in the renal pelvis. Localisation of PCV-2 antigen was observed in renal tissue and lymph node with immunohistochemistry. Altogether, these findings were suggestive of porcine dermatitis and nephropathy syndrome (PDNS) in pigs. These results indicate the importance of including PCV-2 in the differential diagnosis of cutaneous lesions caused by bacterial and viral etiologies in pigs.

Keywords: *PCV-2, skin, porcine dermatitis, nephropathy syndrome*

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Swine industry is one of the fastest growing sectors in the Indian agriculture sector. Swine production is affected by various viral diseases that greatly reduce productivity (Barman *et al.,* 2018). Porcine circovirus type -2 is identified as a dominant emerging viral pathogen causing appalling economic impact in the swine industry worldwide (Allan *et al*., 1999; Wang *et al.,* 2016). The virus is an invariably evolving virus, giving rise to multiple new genetic variants (Kang *et al*., 2022).

PCV-2 is now endemic in India with multiple disease manifestations such as porcine respiratory disease, post-weaning multi-systemic wasting syndrome, reproductive disease, porcine dermatitis and nephropathy syndrome (PDNS), exudative dermatitis, enteritis, myocarditis, vasculitis and necrotizing lymphadenitis (Rosell *et al*., 2000; Krakowka *et al*., 2008).The pathogenesis has been related to a multitude of factors such as virus, host immunity, co-infections and other environmental factors. Laboured respiration with coughing progressive weight loss, paleness or icterus, ill thrift, diarrhoea or decreased rate of weight gain are the clinical signs of PCV2-associated diseases (PCVADs) (Keerthana *et al.,* 2017). Because of the immunosuppressive nature of the virus, the incidence of PCVAD is relevant in swine population (Wang *et al.,* 2016). Direct contact with an infected pig, contaminated vectors and fomites are considered as major routes of virus transmission according to Imai *et al.*(2006).

Among these distinct manifestations, PDNS was first reported in the United Kingdom in 1993 (Smith *et al.,* 1993). Later, other countries also reported this condition indicating its global presence (Rosell *et al.,* 2000). However, PDNS is a rare syndrome reported among swine herds in India. Hence, the present study attempted to undertake gross, histopathological lesions and IHC localisation of the virus on the one of the emerging manifestations of PCV-2 infection such as PDNS in the swine population of Kerala.

Materials and methods

Twenty-five swine carcasses, ranging in age from one week to three months, presented for necropsy at Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy between September, 2021 to April, 2022 formed the material of the present study. Carcasses were selected based on clinical history of anorexia, respiratory distress, anuria, diarrhoea and pale mucus membrane and gross lesions. Gross lesions were noted in the various organs. For histopathology and immunohistochemistry, tissue samples with gross lesions were collected in 10 % neutral buffered formalin. The tissues exhibiting gross lesions were collected in phosphate buffer solution and kept at -18°C for the molecular detection of PCV-2. Total DNA was recovered from the pooled samples from 25 carcasses suspected of harbouring PCV-2 using a Qiagen D Neasy blood and tissue kit. The primers (forward primer 5'CGGATATTGTAGTCCTGGTCG3' and reverse primer 5'ACTGTCAAGGCTACCACAGTCA3') used in the study were designed from nucleocapsid gene segment -*Open reading frame* (ORF-2) of PCV-2 (Ellis *et al.,* 1999). The primers allowed the amplication of 481 bp fragment specific to PCV-2 and generated amplicons were examined on a 2 per cent agarose gel. Positive controls were obtained from the positive PCV-2 DNA samples from previous studies of Vijayaragavan *et al.* (2021) and sterile nuclease free water was used as negative control.

The paraffin tissue sections were taken and subjected to haematoxylin and eosin staining. PCV-2 capsid antibody with a dilution of 1:200 from Invitrogen (ThermoFisher Scientific, USA) was used for the detection of PCV-2 antigen in immunohistochemistry. Avidin- biotin complex method (Mouse and rabbit specific HRP/DAB detection IHC kit-Abcam-ab64264, USA) was used to demonstrate antigen antibody reaction (Bancroft and Gamble, 2008; Sairam *et al*., 2019).

Microbiological cultural examination was performed on organs such as liver, heart and lungs to rule out bacterial infection associated with PDNS.

Results and discussion

A total of 25 pig carcasses pooled

tissue samples were screened for PCV-2 by PCR targeting 481 bp of ORF-2 region, along with known positive samples. Three among the 25 carcasses that underwent PCR screening for PCV-2 yielded amplicons of 481 bp size and were positive (Fig 1). The affected pigs were aged between 1-2 months.

PCV-2 may be accurately diagnosed using clinical symptoms, necropsy findings, PCR and immunohistochemistry (Sairam *et al.,*2019).The carcasses were found to have anaemia, dry hard hair coats, elevated purple skin lesions, multifocal erythematous lesions or red to purple coloured scabs with black cores, which were most noticeable on the facial regions, ear, fore limbs, vulva, perineum and to a lesser extent in thighs and lower parts of hind legs (Fig 2). The lesions were similar to the findings of Duran *et al*. (1997) and Kim and Chae (2004). Congestion and petechial haemorrhages were seen in the kidneys and a pale yellowish discoloration of the surface and renal papillae (Fig 3 & 4) was present in three cases similar to the findings of Sharma and Saikumar (2010) and Palinski *et al.* (2016).

The spleen showed considerable enlargement with multi-focal congestion. The heart was typically found to have haemorrhages, congestion in epicardium and mild hydropericardium. All cases had non-collapsed, firm lungs with different degrees of congestion and petechial to ecchymotic haemorrhages (Drolet *et al.,* 1999; Mahe *et al*., 2000; Sarli *et al.,* 2008). In all the cases, the small intestine displayed haemorrhage and mucosal congestion. Mesenteric lymph nodes were found to be congested and oedematous. The liver displayed moderate to severe congestion, jaundice and enlargement. Other internal organs, such the soft palate tonsil, frequently exhibited significant oedema, congestion and ulceration (Segales *et al.,* 2004).

The histopathology of the skin revealed congestion, haemorrhages, vasculitis, luminal thrombosis and infiltration of mononuclear infiltration cells in the dermis. In the epidermis, spongiosis was observed in two cases (Fig 5, 6 and 7). Kidney revealed congestion, multifocal petechial haemorrhages, tubular necrosis and

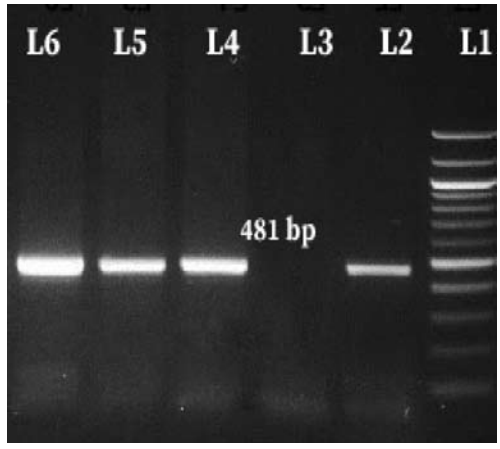

Fig. 1. Agarose gel electrophoresis picture showing 481 bp PCR amplified product of PCV-2 (lane 1-DNA ladder lane L2-positive control lane L3-negative control lane and L4, L5 and L6 positive samples)

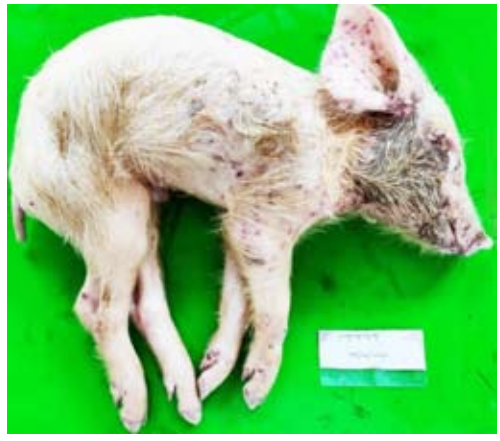

Fig. 2. Emaciated and blanched carcasses with rough to dry hair coat, poor body condition and visible bony prominences

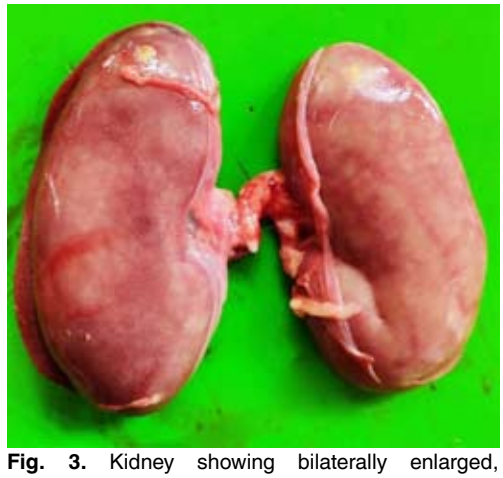

Fig. 3. Kidney showing bilaterally enlarged, oedematous with pale areas with mild petechial haemorrhages

degenerative alterations, mononuclear cell infiltration with mild glomerulitis and occasional vasculitis in the renal parenchyma. There was occasionally fused visceral epithelium to Bowman's capsule (synechia). Disruption of glomerular tufts was also evident (Fig 8, 9 and 10) (Jong *et al.,* 2004; Cadar *et al*., 2007; Sahoo *et al*., 2022).

Microscopic examinations of lungs revealed congestion, haemorrhages, alveolar oedema, septal necrosis, vasculitis and infiltration of mononuclear cells in all cases.The liver displayed diffuse haemorrhages, severe sinusoidal congestion, damaged hepatic cords, lympho-histiocytic infiltration around periportal vein and extensive hepatocellular necrosis which were similar to the observations of Sairam *et al*. (2019). In all cases, the spleen displayed severe peri-arteriolar lymphoid depletion, congestion and botryoid inclusion bodies as previously reported by Sairam *et al.* (2019). With necrotizing lymph-adenitis, histiocytic infiltration and congestion, mesenteric lymph nodes had substantial lymphoid cellular depletion in the follicular area. With lympho-histiocytic infiltration and congestion, the soft palate tonsils also showed significant lymphoid depletion (Vijayaragavan *et al.,* 2021).

Immunohistochemistry was performed to demonstrate PCV-2 viral antigen in the kidney and other organs using PCV-2 polyclonal antibodies (Sairam *et al*., 2019). This technique was successful in identifying PCV-2 antigen in the kidney (Fig 11) as well as in lymph nodes (Fig 12) of pigs with positive PCR results. Both renal tubular cells and interstitial inflammatory infiltrates in the kidney revealed PCV-2 antigen which were similar to the observations of Sahoo *et al*. (2022). Porcine dermatitis and nephropathy syndrome, characterised by blotchy purple skin lesions and nephropathy in pigs, is thought to be an immune-mediated (type III hypersensitivity) injury against PCV-2 infection. The pathogenesis of PCV-2 and the associated formation of immune complexes are not explored so far (Phaneuf *et al*., 2007).

In this study, we noticed enlarged and pale kidneys with multifocal petechiae. Even though, the remarkable lesions of PDNS are necrotizing skin lesions and severe

Fig. 4. Kidney showing enlargement, cortical haemorrhage, necrosis and congestion

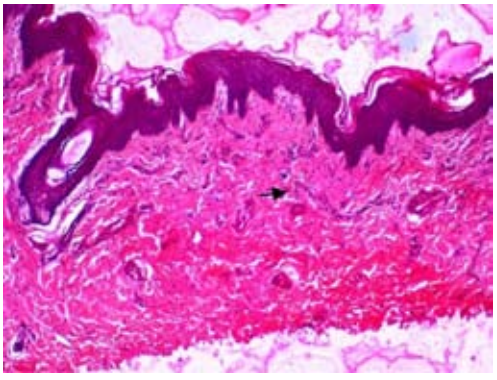

Fig. 5. Congestion and infiltration of mononuclear cells (arrow) in skin (H&E X 100)

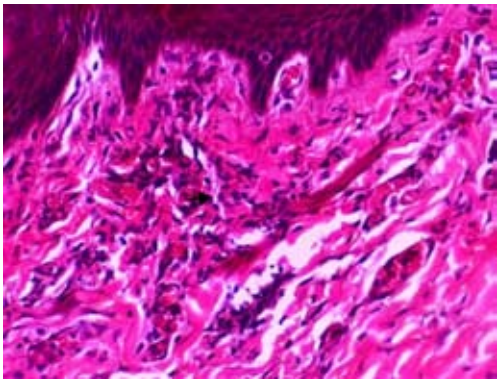

Fig. 6. Severe congestion, haemorrhages, luminal thrombosis, spongiosis and infiltration of mononuclear cells and neutrophils (arrow) in skin (H&E X 400)

non-suppurative interstitial and necrotizing glomerular nephritis to chronic glomerular sclerosis due to necrotizing vasculitis (Thibault *et al.,* 1998; Wellenberg *et al.,* 2004). The present study did not reveal similar severity of the lesion but was mild. The severity of lesions

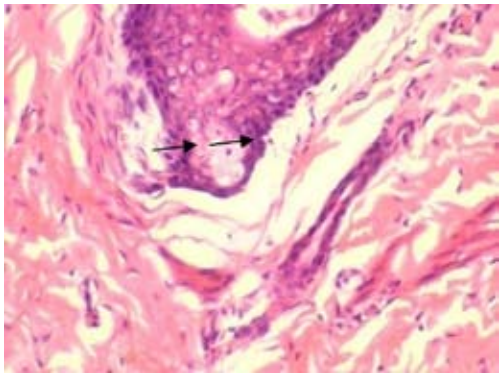

Fig. 7. Luminal thrombosis, spongiosis (arrow), vasculitis and infiltration of mononuclear cells and neutrophils in skin (H&E X 400)

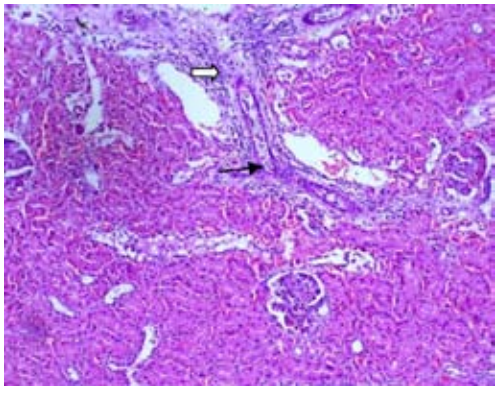

Fig. 8. Vasculitis in the renal pelvis (black arrow), multifocal haemorrhages, congestion, disruption of tubules and severe infiltration of mononuclear cells (white arrow) in kidney (H&E X200)

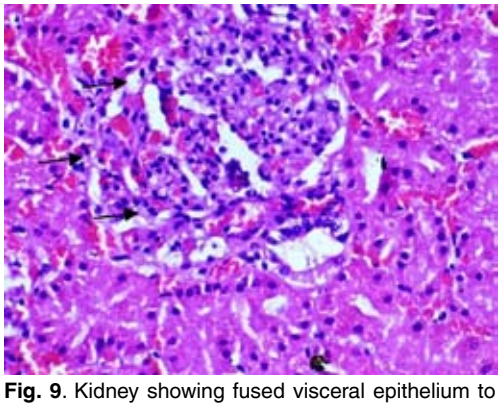

Fig. 9. Kidney showing fused visceral epithelium to Bowman's capsule occasionally (synechia) (arrow). Disruption of glomerular tuftsand glomerular nephritis, congestion and haemorrhages with occasional macrophages (H&E X 400)

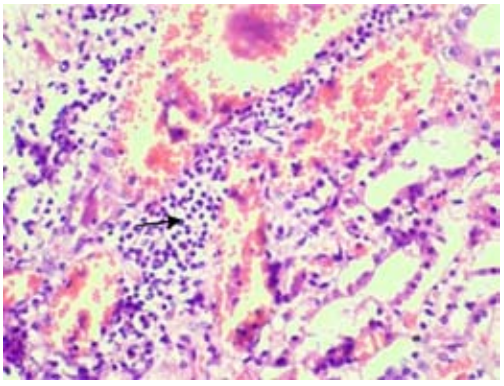

Fig. 10. Kidney showing severe congestion and haemorrhages and lympho-histiocytic infiltration (arrow) in the cortex. Multifocally tubules were degenerative as well as necrotic with sloughed cellular debris (H&E X 400)

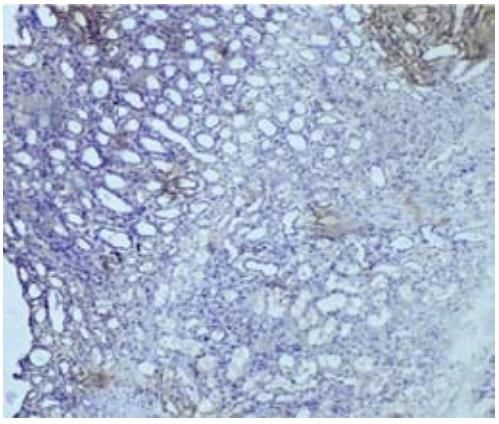

Fig. 11. PCV-2 antigen positive signals (brown) in kidney (IHC X 100)

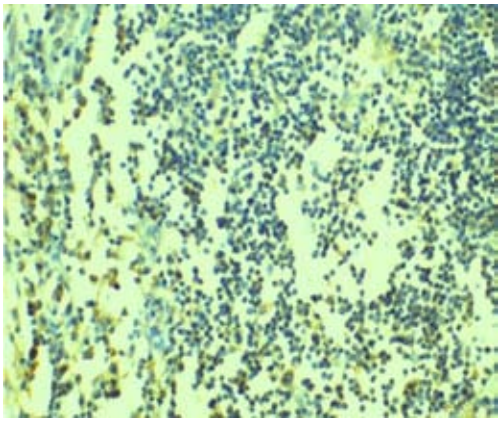

Fig. 12. PCV-2 antigen positive signals (brown) in lymph node (IHC X 400)

may indirectly depend on the genotype of the virus that are circulating in the pig population. *Pasteurella multocida* was ruled out in these cases based on cultural examinations as a possible contributing etiological agent. In this study, PCV-2 has been identified in association

with history, histological lesions, PCR and IHC of marked lymphoid depletion in PDNS, but the role of PCV-2 in this disease manifestation should be further studied. The major limitation of the present study is the smaller samples as well as absence of sequenced data. The PDNS has emerged as a significant menace to swine farmers globally (Sahoo *et al*., 2022). Hence, further studies are necessary to estimate the true prevalence of PDNS and associated genotypes in our swine population.

Conclusion

Based on concurrent clinical and histological data along with PCR and immunohistochemical findings, PDNS was suggested in these cases. Future studies are warranted to understand the pathogenesis and genotypes of the isolates which induce PDNS.

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Conflict of interest

The authors declare no conflict of interest.

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