The Pharma Innovation Journal 2019; 8(6): 485-488

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(6): 485-488

www.thepharmajournal.com
Received: 12-04-2019
Accepted: 13-05-2019

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Studies on naturally infected cases of post weaning multi systemic wasting syndrome (PMWS) in piglets

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Abstract
Porcine circovirus-2 (PCV-2) is one of the important viral disease causing severe economic losses to swine production globally. The PCV2 has been associated with various clinical manifestations like PCV2 systemic disease (PMWS), enteric disease, lung disease, porcine dermatitis, nephropathy syndrome and reproductive failure, they are collectively called as porcine circoviral associated diseases (PCVAD). The disease can be encountered as sporadic affecting few animals or herd problem affecting large number of animals. The present research work was designed with the objectives of studying the gross and histopathological changes of PCV2 in piglets, detection of PCV2 from tissues by Polymerase Chain Reaction (PCR). In the present study, fifty two samples were screened for the detection of PCV2 by PCR. Total of eight piglets were found to be positive for PCV2 by PCR.

Keywords: Porcine circovirus-2, Polymerase chain reaction, Nucleocapsid gene, lymphoid depletion, Immunosuppression, Piglets

1. Introduction
Porcine circovirus 2 (PCV2) is an emerging viral pathogen causing multisystemic diseases characterized by wasting, immunosuppression and it has a great economic impact among pigs worldwide. The PCV2 was first described in 1996 as an etiology of post weaning multisystemic wasting syndrome (PMWS) in specific pathogen free swine herds in Western Canada (Chae, 2004) [3]. The PMWS was a wasting disease of pigs mainly affecting post weaned pigs and it characterized by progressive weight loss and respiratory symptoms. The hallmark of PCV2 infection was enlargement of lymph node or lymphadenopathy (Allan and Ellis, 2000) [1]. Therefore this investigation was planned to ascertain piglet mortality caused by PCV2 by detection of viral nucleic acids in tissues of dead pigs in Kerala.

2. Materials and methods
The porcine carcasses submitted for postmortem examination to the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy formed the study material. Fifty six pig carcasses belonging to the age group ranging from 3-8 weeks with lesions suggestive of porcine circovirus were investigated. Samples such as lymph nodes mainly bronchial, mesenteric and inguinal lymph nodes and visceral organs like lung, liver, kidney and heart were collected from carcasses during necropsy examination in phosphate buffer saline (PBS) and 10 per cent neutral buffered formalin. Until DNA extraction, the samples which collected in PBS were kept at -20° C. The gross lesions were recorded and the collected tissue samples were subjected to PCR and histopathology.

2.1 Polymerase chain reaction (PCR)
Deoxyribonucleic acid was extracted from the pooled organ samples collected from the lesions bearing carcasses suggestive of PCV2 infection by using kit Nucleospin (Genomic DNA extraction from tissues), as per the procedure provided in the kit. PCR primers designed from the nucleocapsid antigen gene of PCV was used for the precise detection of circovirus .The primers allowed the amplification of 481bp fragment specific to circoviruses (Ellis *et al.*, 1999) [5].
The sets of primer used to detect the PCV2 was
Forward primer: 5'- CGG ATA TTG TAG TCC TGG TCG-3'
Reverse primer: 5'- ACT GTC AAG GCT ACC ACA GTC A- 3'
Reaction conditions at 94° C at 1 min for one cycle followed by 35 cycles at 94° C for 1 min, 55° C for 1 min and 72° C for 1 min and then final cycle at 72° C for 10 min.

The procedure was repeated for 35 cycles to amplify 481-bp products.

2.2 Histopathology
The samples were subjected to routine histopathological examination as described by Bancroft and Cook, (1995). Then these stained sections were mounted with DPX mountant for histopathological examination.

3. Results and Discussion
Out of 56 samples which were screened by conventional PCR, 29 animals were suckling piglets in the age group of 0-4 weeks while 27 animals were nursery piglets between 4-12 weeks of age. Most of the affected animals were suckling and nursery piglets showing signs of debility, wasting, lethargy, dyspnea while some of the piglets showed purplish red discolouration of the skin mainly extremities of legs, ears and thigh regions. The visible mucous membranes appeared pale anaemic to severely congested.

3.1 Detection of Porcine circovirus-2 by PCR
The PCR has become an important diagnostic tool for veterinary biological.In the current study, nucleocapsid gene specific PCR amplicons of 481 bp was used to detect porcine circovirus. In the present study, fifty six samples were screened for the detection of PCV2 by PCR. Total of eight piglets, comprising of one suckling and seven nursery piglets were found to be positive for PCV2. The occurrence of PCV2 was 14.28 per cent (8/56)

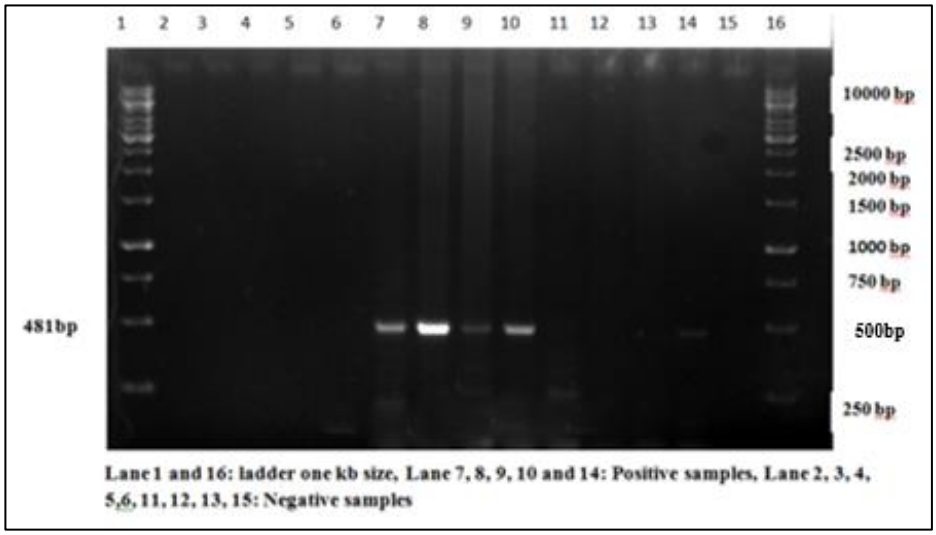

Fig 1: Agarose gel electrophoresis of PCR product showing 481 bp nucleocapsid gene of PCV2 from pooled organ samples.

3.2 Gross lesions
During the present study, the highest mortality was noted among the piglets of 4-12 weeks of age. Most of the piglets brought for the post mortem examination revealed weak, poor body condition, decreased back fat thickness, rough hair coat and visible bony prominence. The significant gross lesions observed in the piglets were tan-mottled lungs with edema and varying degrees of congestion (Sibila et al., 2004) [12]. Many animals had enlarged, congested or haemorrhagic lymph nodes. Few cases showed congestion and consolidation of the dorsal lobes of the lungs and petechial to ecchymotic haemorrhages. Enlarged, congested friable and icteric liver was noted in many cases.

Systemic lymphadenopathy was observed in most of the cases. The bronchial lymph nodes were enlarged and mild to severely congested. Enlarged, severely congested mesenteric lymph nodes along with greatly engorged mesenteric vessels were also recorded in some cases. Mild to moderately enlarged and firm spleen bearing non-congested cut surface could also be noticed. Multiple petechial hemorrhages randomly distributed white necrotic foci and degenerative changes were noticed in the kidney cortices. Few case of piglets revealed, haemorrhagic gastritis with or without enteritis.

3.3 Microscopic lesions
A detailed histopathological study of the lymph nodes, spleen and affected organs was carried out in eight piglets tested positive for PCV2 by PCR. In PCV2 positive cases, histopathological examination of lymph nodes revealed varying degrees of lymphocyte depletion and clear demarcation between follicular and para follicular areas were absent (Chae, 2015) [4]. Apoptotic changes could be noticed in the lymphocytes of the bronchial, inguinal and mesenteric lymph node. Another characteristic finding was the presence of amorphous, single or multiple small, basophilic intracytoplasmic (Botyroid bodies) inclusion bodies in the cytoplasm of macrophages, apoptotic changes and histiocytic replacement could be noticed in bronchial, mesenteric an ingunal lymph nodes (Segales et al., 2000) [9]. Granulomatous inflammatory changes were observed in the lymphoid tissues and other non-lymphoid tissues as well. In some cases, necrotizing lymphadenitis characterized by areas of necrosis in the follicular areas of enlarged inguinal, bronchial and mesenteric lymph nodes was observed. Congestion and severe depletion of B-cell areas were remarkable in the spleen. Mild to marked depletion of lymphocytes in periarteriolar lymphoid sheaths of spleen could be observed.

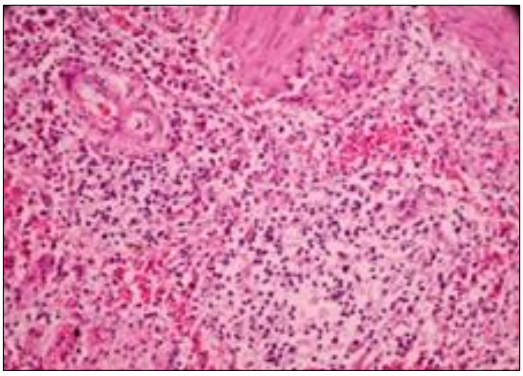

Fig 2: Lymph node showing severe lymphoid depletion with congestion, H&E, 400X

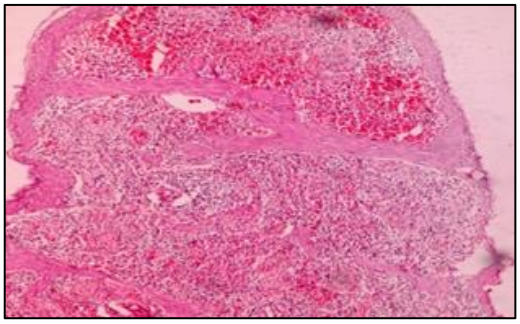

Fig 3: Spleen showing marked depletion of lymphocyte in white pulp areas, H&E, 100X

In the lungs, interstitial pneumonia along with congestion overwhelming areas of alveolar collapse and emphysema were appreciable. In six cases, the lungs revealed, bronchointerstitial pneumonia which is characterized by necrosis, metaplasia and hyperplasia of bronchiolar epithelium associated with the infiltration of neutrophils and macrophages in the pulmonary interstitium (Segales et al, 2004) [11]. Partial or complete desquamation of bronchial epithelium with sub mucosal or mucosal replacement with fibrous tissue was observed. A number of bronchi/ bronchioles and alveoli contained necrotic epithelial cells and inflammatory cells. Moderate amount of cytoplasmic vacuolation, varying degrees of sinusoidal congestion, lymphohistiocytic infiltration and single cell necrosis were observed in the liver. In some cases, focal infiltration of inflammatory cells, formation of micro granuloma and portal fibrosis (arterioles were surrounded by fibrous tissue) and bile duct hyperplasia in portal areas were observed as well. In the kidneys, vacuolation and fatty changes of the renal tubular epithelial cells and renal congestion along with focal granulomatous changes were observed.

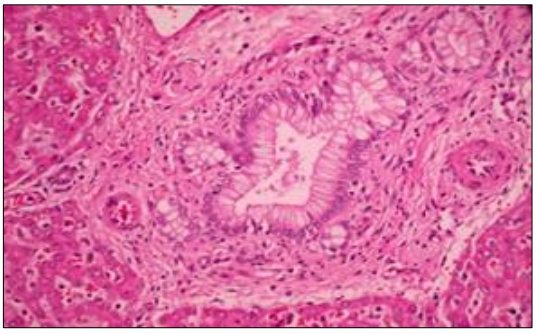

Fig 4: Liver showing bile duct hyperplasia and hyperplasia of the fibroblasts around the bile duct, H&E, 400X

4. Conclusions

The main thrust of the study was to detect the presence of PCV2 in pooled organ samples by PCR which targets the nucleocapsid gene with 481 bp. The positive samples revealed the PCV2 infection present in the suckling and nursery piglets grouped into the PCV2-systemic disease (PCV2-SD- also known as PMWS). The present study confirms the presence of PCV2 infection among piglets in Thrissur district of Kerala. As this virus could cause immunosuppression in the piglets the roles of other co-infectious agent or opportunistic pathogens in the pathogenesis of PCV2-SD infection need to be studied. The major cell types that support the viral replication is still not fully understood so extensive studies on the molecular pathogenesis of the virus and difference in the virulence among different PCV2 isolates need to be carried out. Good management practices are to be followed in order to reduce the secondary opportunistic pathogen in the herd. Management practices that will be cost effective in controlling PCV2 infection at the farm level need to be identified.

5. Acknowledgments

The research reported here was supported by the State plan project 2016-17 entitled as “Viral etio-pathological studies in porcine necropsy cases with special reference to porcine circovirus-2” Kerala

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