

SIMULTANEOUS DETECTION OF PORCINE CIRCOVIRUS TYPE 2 AND CLASSICAL SWINE FEVER VIRUS IN PIGS

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Abstract: Three piglets aged four weeks belonging to a private farm in Thrissur district were brought to the department of Veterinary Microbiology, college of Veterinary and Animal sciences, Mannuthy for disease investigation. The owner reported that a total of about 25 animals in the herd experienced severe respiratory disease and wasting since weaning. The clinical signs observed in the pigs were depression, anorexia and severe dyspnea. On postmortem examination, pneumonic lesions on lungs, enlargement of lymph nodes and spleen, necrotic areas on liver and petechiation on kidney were observed. On culturing of lungs, liver, spleen and kidney, no bacteria could be isolated. Lymph nodes, tonsils, spleen, liver and kidneys samples were processed and subjected to polymerase chain reaction (PCR) targeting porcine circovirus 2 (PCV2) and reverse transcriptase polymerase chain reaction (RT-PCR) targeting classical swine fever virus (CSF). Samples revealed amplicon size of 371 bp and 308 bp specific to PCV 2 and CSF respectively.

Keywords: Porcine circovirus 2, Classical Swine Fever virus, piglets, Polymerase Chain Reaction, Reverse transcriptase Polymerase Chain Reaction.

INTRODUCTION

Porcine circovirus 2 (PCV) is an economically devastating emerging pathogen of porcine species (Rajkhowa, 2012). Porcine circovirus 2 infection causes a variety of symptoms called as PCV 2 associated disease syndromes (PCVADs), the most common among them is post weaning multi systemic wasting syndrome (PMWS), mainly affecting the weaned pigs (Harding *et al.*, 1998) between 5-15 weeks of age (Meng, 2012) and is characterized by wasting, dyspnoea and occasionally pallor (Harms *et al.*, 2001).

Classical Swine Fever (CSF) or Hog cholera is an economically important, highly contagious disease of pigs caused by classical swine fever virus (CSFV) (Tamura *et al.*, 2012). The infectious agent responsible for the disease is a virus, CSFV of the genus *pestivirus* in the family *Flaviviridae*. Clinical signs of CSF remain undetected, particularly during infection

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with CSFV strains of low virulence (Risatti *et al.*, 2003). Acute disease is still the prevalent form in younger animals, with subacute and chronic forms often observed in older animals.

The present communication deals with a case report on the simultaneous detection of PCVADs and CSF from an outbreak in a pig farm in Thrissur district.

Materials and methods

There was report of severe respiratory disease and wasting since weaning in a herd of swine belonging to a private farm in Thrissur district. Three piglets were presented with symptoms of depression, anorexia, and severe dyspnoea to the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy. On postmortem, pneumonic lesions on lungs, enlargement of lymph nodes and spleen, necrotic areas on liver and petechiation on kidney were observed. The samples lung, spleen, liver and kidney were inoculated onto Blood agar (BA) and Sabouraud's Dextrose Agar (SDA). Blood agar plates were incubated at 37°C and the duplicate samples on SDA plates were incubated at 37°C and at room temperature for 24 h. For conducting PCR, samples were collected aseptically from lymph nodes, tonsils, spleen, liver and kidney. The tissues were collected in sterile specimen vials containing RNA lysis solution for extraction of RNA and in phosphate buffer saline for DNA extraction. Then the samples were transferred to a sterile mortar and pestle, and 5ml of 0.8% saline was added. The tissues were triturated and further subjected to RNA and DNA extraction. RNA was extracted by Trizol method and DNA using HiPurA (HiMedia) multi sample DNA purification kit.

The extracted DNA was subjected to a PCR with specific primers based on the sequences of ORF1 of PCV2 (Liu *et al.*, 2015). Reverse transcriptase polymerase chain reaction was carried out using specific primers as per Kumar *et al.* (2015). The first strand cDNA was synthesized using Revert Aid First Strand cDNA synthesis kit according to the manufacturer's recommendations (Thermoscientific). The RNA extracted from the CSF vaccine (Lapinised CSF vaccine, Institute of Animal Health & Veterinary Biologicals, Palode) was served as the positive control.

A 12.5 µl reaction mixture was set up for the single PCR reaction consisting of:

10X PCR master mix 6.25µL

Forward Primer 1µL

Reverse Primer 1µL

Template DNA 3µL

Nuclease Free Water 1.25µL

The PCR tubes were placed in a thermal cycler (Eppendorf) and reaction for porcine circo virus was run as per the following protocol

Initial denaturation	95°C for 5 min		
Denaturation	95°C for 30 sec	}	35 cvcles
Annealing	51.8°C for 45 sec		
Elongation	72°C for 1 min		
Final extension	72°C for 10 min		

The reaction for classical swine fever was run as per the following protocol

Initial denaturation	94°C for 4 min		
Denaturation	94°C for 1 min	}	35 cvcles
Annealing	54.4°C for 1 min		
Elongation	72°C for 1min		
Final extension	72°C for 10 min		

Identification of the PCR product was done in a submerged agarose gel electrophoresis system using one per cent agarose stained with ethidium bromide, and Tris Borate EDTA buffer was used as the matrix at a voltage of 50V. The gel was visualized under a UV transilluminator and results were documented on gel documentation system (Biorad).

RESULTS AND DISCUSSION

In the present study, the owner reported severe respiratory distress and wasting in four piglets. On BA, no bacterial organisms of pathogenic significance could be isolated. No growth could be obtained on SDA even after 7 days of incubation at room temperature. The isolation and identification of the virus is time consuming and laborious, whereas, the molecular detection methods like PCR offers a rapid, sensitive and specific technique that can detect PCV2 DNA in infected tissues as well as in several body fluids from live pigs (KraKowka *et al.*, 2000; Shibata *et al.*, 2003). The gold standard diagnosis of CSF performed by virus isolation can be problematic, particularly due to biosecurity risks involved with handling live infectious agents (Dias *et al.*, 2014).

The DNA extracted from clinical samples were subjected to genus specific PCR for PCV2 and CSFV. A positive PCR result was obtained indicated by the presence of a 371 bp and 308 bp fragments specific to PCV and CSF respectively in electrophoresed gel under UV transillumination (Figure 1).

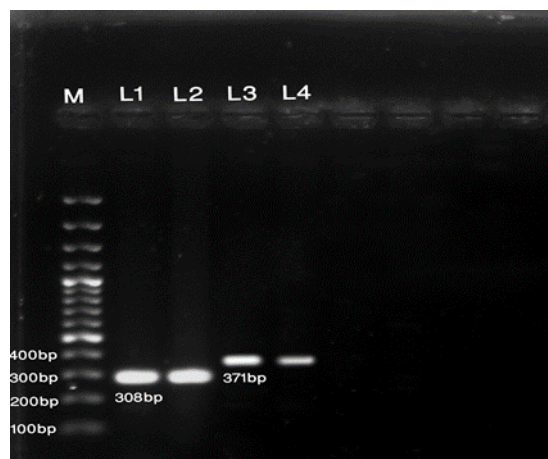


Figure 1: Agarose gel showing polymerase chain reaction amplified product of PCV2 and CSFV

Lane M: Molecular weight marker (100 bp)
Lane 1: CSF control (308 bp)
Lane 2: CSF positive sample (308 bp)
Lane 3: Porcine circovirus control (371 bp)
Lane 4: Porcine circovirus positive sample (371 bp)

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