Full Length Article

# ADAPTATION AND ANALYSIS OF PORCINE CIRCOVIRUS 2 IN PORCINE KIDNEY (PK-15) CELLS CULTURED WITH LOW SERUM SUPPLEMENTATION

**A.Kalaimathi[1], S.Parthiban[2]*, P.Raja[3], J.Vijay Anand[4] and M.Parthiban[5]**

*Department of Animal Biotechnology,*
*Faculty of Basic Sciences,*
*Madras Veterinary College,*
*Tamil Nadu Veterinary and Animal Sciences University*
*Chennai- 600 007*

## ABSTRACT

*This study aimed to cultivate PK-15 cells under serum-deprived conditions and subsequently adapt a field-isolated PCV2b genotype in these adapted cells to investigate both cellular and viral changes. PK-15 cells were grown in three different serum-supplemented media: 0%, 2%, and 10%. The cells adapted to these varying serum conditions were then infected with genotyped field PCV2b and propagated for up to three blind passages. Haematoxylin and eosin (H&E) staining of PK-15 cells grown in 0% and 2% serum-supplemented media, with and without PCV2b infection, revealed cytoplasmic granularity compared to cells grown in 10% serum. MTT assay results indicated that PCV2b infection had minimal impact on cell viability, suggesting efficient viral propagation in serum-deprived conditions with minimal cytopathic effects. PCR analysis of cell lysates from all three 0%, 2%, and 10% serum conditions confirmed the presence of the PCV2b genome, demonstrating its adaptability grown to PK-15 cells under serum-deprived conditions. Molecular analysis of the major epidemiological marker gene, ORF2, from PCV2b grown in both 0% and 10% serum conditions showed identical nucleotide (702 bp) and amino acid (234 aa) compositions. This study highlights the feasibility of growing PK-15 cells under serum-deprived conditions and the adaptability of PCV2b in these cells.*

**Keywords:** PK-15 cell, Serum, deprivation PCV2, adaptation, genetic analysis

Received : 28.03.2025 Revised : 18.05.2025 Accepted : 24.06.2025

[1]M.Sc Scholar, Department of Biotechnology, School of Biological Sciences, CMS College of Science and Commerce, Coimbatore
[2]Assistant Professor, *Corresponding author Email: parthis17@gmail.com
[3] Assistant Professor
[4] Assistant Professor
[5]Professor and Head

## INTRODUCTION

Porcine circovirus (PCV) was first identified as a non-cytopathic contaminant in the PK15 continuous cell line by Tischer *et al.*, (1982). To date, four species of PCV have been documented globally: PCV1, PCV2, PCV3, and PCV4 (Maity *et al.*, 2023). Among these, PCV-2 is the small

non-enveloped, non-cytopathic globally reported emerging viral pathogen in swine responsible for the post weaning multi-systemic wasting syndrome (PMWS), porcine circovirus associated diseases (PCVAD) including reproductive failure, porcine respiratory disease complex, porcine dermatitis and nephropathy syndrome, proliferative necrotizing pneumonia, and congenital tremor (CT) resulting in huge economic loss in swine production (Xu *et al.,* 2022). Indian swine husbandry also documented positivity of PCV2 associated multiple clinical and sub-clinical infections with multiple genotypes (Parthiban *et al.,* 2022; D'silva *et al*., 2023).

The isolation and characterization of PCV2 associated with clinical infections in various regions are essential for the development of effective vaccines and diagnostic tools. The porcine kidney (PK-15) cell line has been widely utilized for PCV2 virus isolation, diagnostic purposes, vaccine development, and antiviral studies. Due to the non-cytopathic nature of PCV2, the indirect immunofluorescence assay (IFA) is commonly employed for confirming viral replication and determining virus titers (Allan and Ellis 2000). Fetal bovine serum (FBS) used in cell culture systems may be contaminated with mycoplasma and non-cytopathic (ncp) viruses, which are often difficult to detect. These contaminants can cause silent infections in cell cultures, potentially compromising research and diagnostic outcomes (Pecora *et al.,* 2020). Therefore, developing alternative virus adaptation approaches with minimal growth supplementation is a critical need. This study aims to adapt and propagate PCV2 in PK-15 cells under serum-deprived conditions to explore its adaptability and growth. Further, the study focuses on analyzing any changes in the host cell response as well as alterations in the viral genome. Understanding the viral growth under these conditions will provide critical insights into its adaptation mechanisms and potential implications for future vaccine and therapeutic development.

## MATERIALS AND METHODS

PCV2 is generally considered non-cytopathic in nature replicates slowly and does not cause visible damage to PK-15, allowing infected cells to remain viable. Additionally, it subtly modulates host pathways without triggering strong apoptotic or inflammatory responses in vitro, resulting in minimal or no cytopathic effects. PK-15 cells were cultivated in growth media supplemented with three different serum levels: 0%, 2%, and 10%. To avoid possibility of PCVs contamination from commercial swine origin trypsin an recombinant enzyme TrypLE™ Express (Gibco by Life Technologies, Thermo Fisher Scientific, USA, Cat No: 12605010) were used for dissociating adherent PK-15 cells used in this study. After three consecutive subcultures, the PK-15 cells adapted to each serum condition were infected with a genotyped field strain of PCV2b at a multiplicity of infection (MOI) of 0.1, with viral titer of $10^6$ $TCID_{50}$/ml. The virus was propagated through three blind passages under each growth condition (Farnham *et al.,* 2003). Both 0 and 10 % serum supplemented PCV2b uninfected and infected PK-15 cells were monitored for

cytopathic changes such as cell rounding, clumping, detachment, inclusions, cell shrinkage and cell death in both unstained preparations and haematoxylin and eosin (H&E) stained cells (Fischer *et al.*, 2008). Furthermore, cell viability in PCV2b-infected PK-15 cells grown under serum-deprived conditions was evaluated using the MTT assay to assess cell viability, proliferation, and cytotoxicity (Kumar *et al*., 2018). Further real time based quantification done to confirm virus propagation in both 0 and 10% serum supplemented conditions.

Molecular confirmation and sequence analysis of the major epidemiological marker gene (ORF2) of PCV2

DNA was extracted from Passage 3 PCV2b-infected PK-15 cells grown under each serum condition (0%, 2%, and 10% serum supplementation). The extracted DNA was subjected to molecular detection and confirmation of PCV2b using ORF2 gene-specific PCR, which amplifies an 802bp fragment of the epidemiological marker gene of PCV2. The resulting amplicons were sequenced and analyzed at both the nucleotide and amino acid levels to assess genetic diversity within the PCV2 genome (Parthiban *et al.*, 2022).

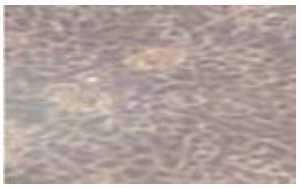
## RESULTS AND DISCUSSION

PK-15 cells were able to grow in serum-supplemented media, with adaptability and monolayer formation occurring in 5 days, 4 days, and 3 days under 0%, 2%, and 10% serum-supplemented conditions, respectively, with minimal morphological alterations. H&E staining of PK-15 cells grown in 0% and 2% serum-supplemented media, both with and without PCV2b infection, revealed cytoplasmic granularity compared to PK-15 cells cultured with 10% serum supplementation (Figure 1). Serum is an essential component of cell culture media; however, reducing or eliminating serum is often desirable to lower costs. H&E staining revealed cytoplasmic granularity in PK-15 cells grown under 0% and 2% serum conditions, both with and without PCV2b infection, compared to those cultured with 10% serum supplementation. This observation suggests that serum deprivation induces cellular stress, potentially affecting cell morphology and metabolism. The granularity observed in the cytoplasm may be attributed to altered protein synthesis, accumulation of stress granules, or changes in organelle structure due to reduced serum availability (White *et al.,* 2020). Despite these morphological differences, the absence of significant cytopathic effects in PCV2b-infected cells indicates that viral replication occurs efficiently without severely compromising cell integrity. Real-time PCR–based quantification confirmed virus propagation in both 0% and 10% serum-supplemented conditions, with Ct values decreasing two fold and four fold, respectively. Although in situ confirmation of virus propagation by immunofluorescence assay would be ideal, this study relied on real-time PCR–based analysis. This finding suggests that PK-15 cells can adapt to serum-deprived conditions while supporting PCV2b propagation.

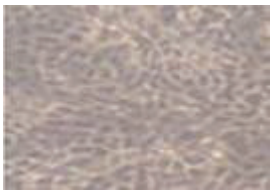
Whereas, Hosono *et al.* (2019) demonstrated that continuous Porcine

Kidney non-serum (CPK-NS) cell line has been shown to grow in serum-free culture medium. A single passage of cells infected with PCV2 at a concentration of $\geq 10^{4.5}$ $TCID_{50}$/ml evidenced clear cytopathic effects (CPE) making it detection and titration easier. MTT assay results showed that the viability of uninfected PK-15 cells grown in 0%, 2%, and 10% serum-supplemented media was 90.44%, 94.86% and 99.47%, respectively. In PCV2b-infected PK-15 cells, viability was slightly reduced, with 88.25% in 0% serum and 94.66% in 2% serum conditions, compared to 99.27% in cells grown with 10% serum supplementation. These findings indicate that serum deprivation has minimal impact on cellular response and viability upon PCV2b infection. This aligns with previous reports that PCV2 is non-cytopathic in PK-15 cells and does not induce significant morphological changes (Segalés, 2012). Furthermore, the minimal difference in cell viability across different serum concentrations suggests that PCV2b infection does not significantly affect cellular metabolism or survival in a serum-deprived environment. However since it's a preliminary study, this study needs to be extended for more passages with prevalent PCV2 genotypes.

Cell lysates from all three passages of PK-15 cells grown in 0%, 2% and 10% serum-supplemented media with PCV2b infection tested positive for the PCV2b genome, as confirmed by a specific PCR amplicon of 802 bp (Figure 2). This indicates the successful adaptation of PCV2b in PK-15 cells under serum-deprived conditions. Sequence analysis of the major epidemiological marker gene, ORF2, from PCV2b grown in both 0% and 10% serum conditions revealed identical nucleotide sequences (702bp) and amino acid compositions (234 aa) (Figure 3). Furthermore, molecular detection of PCV2b up to passage 3 in 0% and 10% serum conditions showed consistent positive results, confirming the virus's adaptability to serum-deprived conditions. The detection of the PCV2b genome across all passages in PK-15 cells under serum-deprived conditions confirms its ability to adapt and replicate efficiently in lower serum concentrations. Serum reduction does not drive significant mutations or alter viral genome.



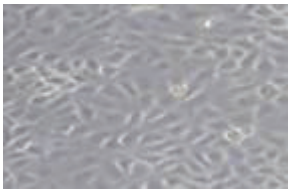
A.PK-15 grown with 0% serum uninfected revealed s(40x), unstained



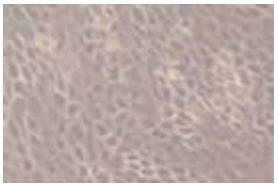
B.PK-15 grown with 0% serum infected with PCV2b-P3 revealed no cytopathic changes (40x), Unstained

C.PK-15 grown with 0% serum uninfected revealed cytoplasmic granularity (40x), H & E stained

D.PK-15 grown with 0% serum infected with PCV2b-P3 revealed cytoplasmic granularity (40x), H & E stained



E.PK-15 grown with 10% serum uninfected (40x), Unstained

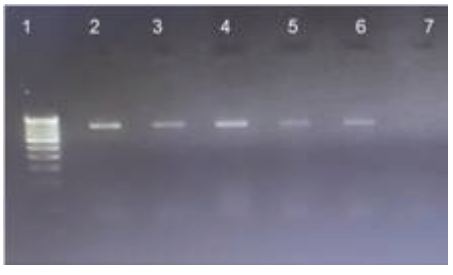


F.PK-15 grown with 10% serum infected with PCV2b-P3 revealed no cytopathic changes (40x), Unstained

G.PK-15 grown with 10% serum uninfected (40x), H & E stained

H.PK-15 grown with 10% serum infected with PCV2b-P3 (40x), H & E stained

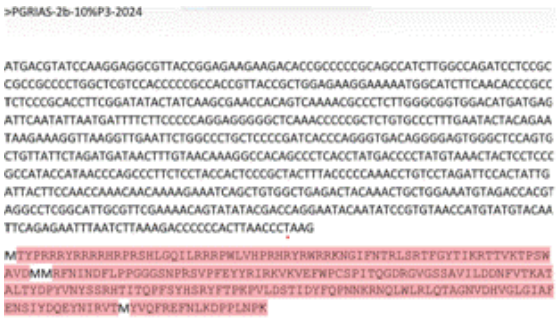
**Fig.1. Cytopathic changes of PCV2 grown in serum deprived PK-15 cells**



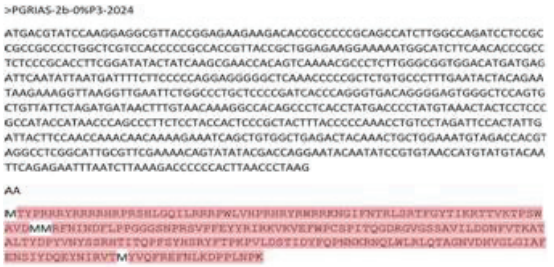

802 bp

Lane 1- 100 bp Ladder
Lane 2- PCV2b adapted in PK15 with 10% serum P2
Lane 3- PCV2b adapted in PK15 with 10% serum P3
Lane 4- PCV2b adapted in PK15 with 0% serum P2
Lane 5- PCV2b adapted in PK15 with 0% serum P3
Lane 6- PCV2b positive control
Lane 7- Non-Template control

**Fig.2. Molecular confirmation of PCV2b adaptation in PK-15**




a. **PCV2b adapted and grown in 0% serum supplemented media**




b. **PCV2b adapted and grown in 10% serum supplemented media**

**Fig.3. Nucleotides and amino acid sequences of ORF2 gene of PCV2b adapted to grow in varying serum supplemented PK-15 cells**

## CONCLUSION

PCV2b successfully adapted to growth in PK-15 cells under reduced serum conditions, with no significant morphological or cytopathic changes observed in infected cells. MTT assay results indicated minimal impact on cell viability upon infection, suggesting efficient viral propagation in serum-deprived media. Molecular sequencing confirmed no genetic variations in PCV2b grown in 0% and 10% serum conditions. These findings highlight a cost-effective approach for culturing PCV2b, supporting virology research, viral isolation, vaccine development, and diagnostics. This study demonstrates the adaptability of PCV2b in PK-15 cells under serum-deprived conditions, offering potential for future research applications. However, further passages of PCV2b in serum-deprived conditions, along with a detailed analysis of cellular changes, immune gene expression, and complete viral genome characterization, could provide deeper insights into PCV2 adaptation and host-virus interactions.

## REFERENCES

Allan, G.M. and Ellis, J.A. (2000). Porcine circoviruses: a review. *Journal of Veterinary Diagnostic Investigation*, **12(**1): 3–14. https://doi.org/10.1177/104063870001200102.

D'silva, A.L., Bharali, A., Buragohain, L., Pathak, D.C., Ramamurthy, N., Batheja, R., Mariappan, A.K., Gogoi, S.M., Barman, N.N., Dey, S. and Chellappa, M.M. (2023). Molecular characterization of porcine circovirus 2 circulating in Assam and Arunachal Pradesh of India. *Animal Biotechnology*, **34**(2): 462–466. https://doi.org/10.1080/10495398.2021.1955700.

Farnham, M.W., Choi, Y.K., Goyal, S.M. and Joo, H.S. (2003). Isolation and characterization of porcine circovirus type-2 from sera of stillborn fetuses. *Canadian Journal of Veterinary Research = Revue canadienne de recherche veterinaire*, **67**(2):108–113.

Fischer, A.H., Jacobson, K.A., Rose, J. and Zeller, R. (2008). Hematoxylin and eosin staining of tissue and cell sections. CSH protocols, 2008, https://doi.org/10.1101/pdb.prot4986

Hosono, S., Shiokawa, M., Kobayashi, T., Fukusho, A., and Aoki, H. (2019). Porcine circovirus type 2 induces a strong cytopathic effect in the serum-free culture cell line CPK-NS. *Journal of Virological Methods,* **273**: 113706.https://doi.org/10.1016/j.jviromet.2019.113706.

Kumar, P., Nagarajan, A. and Uchil, P.D. (2018). Analysis of cell viability by the MTT assay. *Cold Spring Harbor Protocols*, 2018(6), https://doi.org/10.1101/pdb.prot095505.

Maity, H., Samanta, K., Deb, R. and Gupta, V. (2023). Revisiting porcine circovirus infection: recent insights and its significance in the piggery sector. *Vaccines*, **11**(8): 1308. https://doi.org/10.3390/vaccines11081308.

Parthiban, S., Ramesh, A., Karuppannan, A.K., Dhinakar Raj, G., Hemalatha, S., Parthiban, M., Senthilkumar, K., Balasubramaniyam, D., Sumanth Kumar, R., Ranganatha, S. and Ravishankar, C. (2022). Isolation and genetic analysis of porcine circovirus 2 in southern India evidences high circulation of porcine circovirus 2d genotype. *Molecular Biology Reports*, **49**(12), 11829–11846. https://doi.org/10.1007/s11033-022-07946-5.

Pecora, A., Pérez López, J., Jordán, M.J., Franco, L.N., Politzki, R., Ruiz, V. and Alvarez, I. (2020). Analysis of irradiated Argentinean fetal bovine serum for adventitious agents. *Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*, **32**(6), 892–897.

Segalés J. (2012). Porcine circovirus type 2 (PCV2) infections: clinical signs, pathology and laboratory diagnosis. *Virus Research*, **164**(1-2), 10–19. https://doi.org/10.1016/j.virusres.2011.10.007.

Tischer, I., Gelderblom, H., Vettermann, W. and Koch, M. A. (1982). A very small porcine virus with circular single-stranded DNA. *Nature*, **295**(5844): 64–66.

White, E.Z., Pennant, N.M., Carter, J. R., Hawsawi, O., Odero-Marah, V. and Hinton, C.V. (2020). Serum deprivation initiates adaptation and survival to oxidative stress in prostate cancer cells. *Scientific reports*, **10**(1), 12505. https://doi.org/10.1038/s41598-020-68668-x.

Xu, T., You, D., Wu, F., Zhu, L., Sun, X. G., Lai, S.Y., Ai, Y.R., Zhou, Y.C. and Xu, Z.W. (2022). First molecular detection and genetic analysis of porcine circovirus 4 in the southwest of China during 2021-2022. *Frontiers in microbiology*, **13**:1052533.