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# Porcine circovirus 2 (PCV2) increases the expression of endothelial adhesion/junction molecules

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ABSTRACT

Porcine circovirus type 2 (PCV2) is the primary causative agent of porcine circovirus disease, a complex multisystem syndrome in domestic pigs. Despite the significant economic losses caused by porcine circovirus disease, the mechanisms of pathogenesis underlying the clinical findings remain largely unclear. As various reports have highlighted the potential key role of vascular lesions in the pathogenesis of porcine circovirus disease, the aim of this work was to investigate effects of PCV2 infection on vascular endothelial cells, focusing on cell viability and expression of adhesion/junction molecules. PCV2 infection reduced endothelial cell viability, while viral infection did not affected the viability of several other classical cell lines. Also, PCV2 infection in endothelial cells displayed a dual/biphasic effect: initially, infection increased ICAM-1 expression, which can favor leukocyte recruitment and emigration to tissues and possibly inducing characteristic porcine circovirus disease inflammatory lesions; then, secondarily, infection caused an increase in zonula occludens 1 tight junction protein (ZO-1) expression, which in turn can result in difficulties for cell traffic across the endothelium and a potential impairment the immune response in peripheral tissues. These virus-induced endothelial changes could directly impact the inflammatory process of porcine circovirus disease and associated vascular/immune system disturbances. Data suggest that, among the wide range of effects induced by PCV2 on the host, endothelial modulation can be a pivotal process which can help to explain PCV2 pathogenesis in some porcine circovirus disease presentations.



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## Introduction

Porcine circovirus type 2 (PCV2) is a small non-enveloped virus, with a single-stranded circular DNA genome[1] and is the primary causative agent of a complex syndrome in domestic pigs called porcine circovirus disease (PCVD).[2,3] Several clinical presentation forms of PCVD have been reported worldwide, all exhibiting multisystem clinical manifestations, such as reduced weight gain in piglets as well as respiratory, enteric, renal, vascular and dermatological disorders.[3,4] The pathogenic mechanisms underlying PCV2 infection and the clinical findings for PCVD remain unclear.

In addition to the effects of PCV2 on the immune system, endothelial cell alterations and vascular system disturbances could be (at least partially) implicated in the pathogenesis of PCVD,[5] particularly in cases of pneumonia, dermatitis and necrotizing lymphadenitis. This hypothesis is supported by several previous pathological findings[6–9] that proved the importance and high frequency of vasculitis cases in swine with PCVD. Recently, some seminal reports highlight the involvement of vascular lesions/alterations in the pathogenesis of some PCVD presentations.[10–12]

It is important to consider that even a non-cytopathic virus such as PCV2 can disrupt several organ systems and cause severe lesions as a result of slight alterations (caused by virus-induced cell changes) to the finely-balanced physiological processes. This becomes more important when the virus-altered endothelial cells and play an essential role in several key physiological processes (e.g. immune migration and cell nutrition).[13–15]

It has been previously demonstrated that PCV2-infected endothelial cells display an activated and prothrombotic phenotype, leading to vascular leakage, leukocyte migration, tissue inflammation and necrosis associated with PCVD clinical findings.[5] These processes are finely regulated through several endothelial cell signaling systems and mediators such surface molecules, as adhesins and junction proteins, which in turn modulate cell-to-cell signaling, and consequently cell migration, fluid leakage and chemotaxys.[14,16,17] In this sense, studies that address the phenomena induced by PCV2 infection of endothelial cells should improve the understanding of viral pathogenesis. Therefore, the aim of this study was to investigate the effects of PCV2 infection on endothelial cells, focusing on cell viability and the expression of adhesion/junction molecules.

## Material and methods

### *Virus strain*

The PCV2b strain used in this work was isolated in 2006 from kidneys of naturally infected piglets from Rio Grande do Sul state (Southern Brazil) before the introduction of PCV2 vaccines in Brazil.[5] This strain was isolated from animals showing the classical triad of clinical findings indicative of PCVD[18]: clinical signs – wasting, reduced weight gain, diarrhea, dermatitis; characteristic lesions – lymph node atrophy with lymphoid depletion and histiocytic replacement of follicles in lymphoid tissues, dermatitis and vasculitis, and pale kidney with diffuse cortical white foci; PCV2 presence confirmed by immunohistochemistry and PCR. Additionally, the isolated viral inoculums were negative for other pathogens, such as pestivirus, swine parvovirus, influenza A virus, Torque-Teno virus, porcine reproductive and respiratory syndrome virus (PRRSV) and *Mycoplasma* spp.[5]

### *Molecular analysis*

Total DNA was extracted from viral inoculum and cells using size-fractionated silica particles according to a previously described protocol.[19] TaqMan™ qPCR was conducted to confirm and quantify PCV2 infection according to a protocol previously described,[20] using the ABI Prism 7300 detection system and sequence detection software (Applied Biosystems, Forster City, CA, USA). The quantification was expressed as virus copies.

### *Cell cultures*

Primary cell cultures of swine testicle (ST) cells were generated as previously described.[21] Briefly, testicles from healthy piglets were aseptically removed and further processed in a laminar flow hood where the testicles were minced into small pieces, washed and digested with 0.25% trypsin. The resulting cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum (FCS), penicillin (200 U/mL) and streptomycin (200 mg/L), and maintained at 37 °C and 5% $CO_2$.

EAhy926 cell line (derived from human endothelial cells) was used as a model to study the effects of PCV2 on endothelial cells (as previously reported).[5] The cell lines PK-15 (porcine kidney cells), Vero (African green monkey kidney cells) and HEK293 (human embryonic kidney cells) were also used in this work. Cells were cultivated in DMEM containing 10% FCS, penicillin (200 U/mL) and streptomycin (200 mg/L) at 37 °C in a 5% $CO_2$ atmosphere. Medium for EAhy926 cells was supplemented with 100 mM hypoxanthine, 0.4 mM aminopterin and 10 mM thymidine (HAT) as previously described.[22] All cells used in this work were free of PCV1 contamination, as confirmed by routine molecular analysis.

### *PCV2 infection in cultured cells*

Cells were seeded into 25-$cm^2$ cell culture flasks and after a 24 h incubation period at 37 °C in 5% $CO_2$ atmosphere, the culture medium was discarded and the cells (≈80% confluence) were inoculated with PCV2 (2 $\log_{10}$ virus copies, as determined by qPCR) or an equal volume of DMEM (control cultures). After 1 h, the supernatant was discarded and DMEM containing 3% FCS was added.

PCV2 infection and replication in cultured cells was confirmed and quantified by qPCR analysis. At 72 h after infection of EAhy926 cells, PCV2 viral load (DNA copies) was more than 15 times higher than initial viral load (1 h after infection) in all set of experiments used in this work, which confirmed PCV2 replication in endothelial cells. As a rule, for other cell lines and ST cells, PCV2 viral load also increase at least three times