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Development and validation of a SYBR green real-time PCR for the quantification of porcine circovirus type 2 in serum, buffy coat, feces, and multiple tissues

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Abstract

The emergence of multiple genotypes of PCV2, as demonstrated by phylogenetic analysis of whole genome or capsid sequences, makes it necessary to have quantitative diagnostic assays that perform equally well on all strains. The objectives of this study were to develop and validate a novel real-time polymerase chain reaction (PCR) assay targeting the highly conserved *rep* gene (ORF1) and investigate the effects of diagnostic specimen choice on its performance. The assay was tested in naturally infected conventional pigs, experimentally infected gnotobiotic pigs, and plasmid-spiked negative serum, lung tissue, and feces and found to have a linear detection range of 2.2×10^3 to 2.2×10^{10} copies of PCV2 per mL. The assay successfully detected and quantified PCV2 DNA in serum, buffy coat, feces, and multiple lymphoid (bronchial, mesenteric, and superficial inguinal lymph nodes; thymus; tonsil; ileal Peyer's patches; and spleen), and non-lymphoid (myocardium; lung; kidney; liver; and gluteal muscle) tissues from naturally infected pigs. Across all tissues and sera of naturally infected pigs, the mean PCV2 concentration was 3.0 logs higher in wasting versus non-wasting pigs. PCV2 concentration measured by tissue culture and immunohistochemical staining in homogenized liver samples of experimentally infected gnotobiotic pigs were compared to the concentrations estimated by quantitative PCR. Similar trends were noted with increasing PCV2 concentration detected in subclinically infected to severely PMWS-affected pigs across all assays. Our diagnostic assay was

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developed with a conserved target sequence, and performed efficiently in quantification of PCV2 in a variety of tissues from naturally and experimentally infected pigs.

Keywords: SYBR green real-time PCR; Quantitative PCR (qPCR); Porcine circovirus type 2 (PCV2); PMWS; PCVD; PCVAD

1. Introduction

Non-pathogenic porcine circovirus (PCV) was discovered in 1974, as a picornavirus-like contaminant of a pig kidney tissue culture cell-line (PK15) (Tischer et al., 1982). PCV consists of a single stranded circular DNA genome of 1.7 kb, is non-enveloped, and is approximately 17 nm in diameter. In the mid-1990s, postweaning multisystemic wasting syndrome (PMWS) was recognized and found to affect primarily pigs 6–15 weeks of age (Harding, 1996; Clark, 1997). A genetically (Meehan et al., 1998) and antigenically (Allan et al., 1998) distinct PCV was recovered from a PMWS-affected pig in 1996 (Ellis et al., 1998). The pathogenic PCV was subsequently named porcine circovirus type 2 (PCV2). PMWS is characterized by wasting or unthrifty pigs, jaundice and respiratory distress, accompanied by gross and/or histopathological lesions affecting multiple organs and tissues (Ellis et al., 1998; Allan et al., 1998; Harding et al., 1998). Pathologically, PCV2 is associated with interstitial pneumonia, hepatitis, lymphadenopathy, pancreatitis, myocarditis, nephritis, and enteritis (Allan et al., 1998; Clark, 1997). PCV2 is the etiological agent of PMWS and is found associated with additional disease syndromes including: congenital tremors (Stevenson et al., 2001), respiratory disease complex (Kim et al., 2003), porcine dermatitis and nephropathy syndrome (PDNS) (Allan et al., 2000a), abortion (West et al., 1999), and reproductive disorders (O'Connor et al., 2001). Diseases associated with PCV2 are now collectively termed porcine circovirus diseases (PCVD), or porcine circovirus-associated disease (PCVAD).

PCV2 nucleic acid or antigen associated with lesions in the tissues of affected pigs is the primary target for the diagnosis of PCVD in pigs. An increasing number of herds globally are infected with PCV2; however, the infection remains subclinical in the majority of pigs. Progression to overt disease, or PCVD, requires coinfection with another pathogen (Dorr et al., 2007) such as porcine respiratory and reproductive syndrome virus (Allan et al., 2000b), porcine parvovirus (Krakowka et al., 2000; Ellis et al., 2000), *Mycoplasma hyopneumoniae* (Opriessnig et al., 2004), an immune stimulus (Krakowka et al., 2001, 2007), or cofactor which may include early weaning or inappropriate management practices (Rose et al., 2003).

Recently, viral load has been associated with PCVD clinical severity in pigs (Krakowka et al., 2005) and it has become more desirable to use quantitative methods to determine the magnitude of PCV2 infection. In addition, real-time PCR is generally more rapid, less labour intensive, less expensive, and may be more reliable than conventional PCR. Several previously published assays exist using either a probe-type (Chung et al., 2005; Brunborg et al., 2004; Olvera et al., 2004; Yu et al., 2007), or a SYBR green (Yang et al., 2007; Gilpin et al., 2003) real-time PCR methodology. PCV2 differential diagnostic assays are now available due to the increasing concern regarding the spread of potentially more virulent genotypes of PCV2 (Dupont et al., 2008); however, there is conflicting evidence (Allan et al., 2007; Gagnon et al., 2007).

The objectives of this study were to develop and validate a novel SYBR green real-time polymerase chain reaction (PCR) assay targeting ORF1 (*rep*) exploiting oligonucleotide primer binding sites conserved across 244 characterized PCV2 genomes, and to investigate the effects of diagnostic specimen choice on its performance. The assay was designed to target all genotypes of PCV2 (PCV2a/2b) (Gagnon et al., 2007), or genogroups (PCV2-G1/G2) (Allan et al., 2007), and was tested to determine its versatility and performance in multiple tissues using naturally infected conventional pigs, experimentally infected gnotobiotic pigs, and plasmid-spiked PCV2-negative serum, lung tissue, and feces. Historically, homogenized and serially diluted pig tissues or serum have been applied to PK15 cells to determine the tissue