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ORIGINAL ARTICLE

Generation of *E. coli*-derived virus-like particles of porcine circovirus type 2 and their use in an indirect IgG enzyme-linked immunosorbent assay

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Abstract Porcine circovirus type 2 (PCV2) causes increased mortality and poor growth or weight loss in apparently healthy swine. Therefore, methods to detect PCV2-specific antibodies in swine serum are important for prevention, diagnosis, and control of PCV2-associated diseases (PCVAD). In this study, PCV2 virus-like particles (VLPs) were used to develop a rapid, simple and economical indirect enzyme-linked immunosorbent assay to detect (with high sensitivity) PCV2-specific antibodies in swine serum. The PCV2 capsid protein (Cap) was overexpressed in *E. coli* after optimizing the *cap* gene. Subsequently, the soluble Cap was rapidly purified in one step by automated fast protein liquid chromatography (FPLC). The purified PCV2 Cap was shown by transmission electron microscopy and gel filtration chromatography to be capable of self-assembling into VLPs *in vitro*. Using the purified VLPs as antigens, optimal operating conditions for the VLP ELISA were determined. The concentration of PCV2 VLPs was 1 μg/ml per well, and the dilution factors for swine serum and horseradish peroxidase (HRP)-labeled goat anti-pig antibody were 1:150 and 1:4000, respectively. Out of 241 serum samples tested with this assay, 83.4 % were found to be positive. Importantly, the VLP ELISA had a total coincidence rate of 97.4 % (74/76) compared to an Ingezim PCV2 ELISA IgG assay. In summary, this rapid, inexpensive VLP ELISA has the potential to greatly facilitate large-scale investigations of PCV2-associated serotypes.

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Introduction

Porcine circovirus type 2 (PCV2), a member of the genus *Circovirus* in the family *Circoviridae*, causes various syndromes and diseases in swine, collectively designated porcine circovirus-associated diseases (PCVAD) [1]. Furthermore, PCV2, together with other pathogens, has also been associated with PCV2 subclinical infection (PCV2-SI) [2]. That PCV2 causes increased mortality and poor growth or weight loss in apparently healthy swine, resulting in substantial economic losses, makes it an important pathogen. Therefore, methods to detect PCV2-specific antibodies are important for monitoring infection caused by this virus and vaccination efficacy.

PCV2 is a small, non-enveloped animal virus with a circular single-stranded DNA genome (1.76 kb) that encodes a structural capsid protein (Cap), two replicase proteins (Rep and Rep') required for viral replication, and a protein that induces apoptosis of host cells. PCV2 virus-like particles (VLPs), assembled from 60 monomers of the Cap, elicit serum neutralizing antibody (SNAb) in animals and have been used as the active component of commercial vaccines against PCV2 infection [3–5]. Although monomeric Cap and truncated Cap have been shown to induce antibodies in swine, those antibodies lacked neutralizing activity and did not protect herds from PCV2 infection. Monomeric Cap elicited antibodies predominantly against a peptide (169-STIDYFQPNNKR-180) in the C-terminal region of Cap; those antibodies were extensively detected in diseased pigs [6]. Therefore, this peptide is regarded as a decoy epitope that apparently helps the virus evade host immune defenses [6]. In other studies, PCV2 VLPs harvested from *Spodoptera frugiperda* (sf21) insect cells and yeast have also been used as antigens to detect PCV2-specific antibodies in an indirect enzyme-linked