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DEVELOPMENT AND VALIDATION OF DIRECT PCR AND REAL-TIME QUANTITATIVE PCR ASSAYS TO DETECT PORCINE CIRCOVIRUS 3 (PCV-3) GENOME

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Introduction

Since the identification of porcine circovirus 2, the *Circovirus* genus has become of major relevance, especially for its impact in the swine industry. Recently, a new species (*Porcine circovirus 3*, PCV-3) has been detected in healthy and diseased pigs. Consequently, there is an urgent need for reliable and widely accessible diagnostic tools for both routine diagnosis and research purposes.

Material & Methods

Two assays, a direct PCR (requiring no DNA extraction) and a quantitative real-time PCR (qPCR) targeting the conserved Rep gene were developed to detect the PCV-3 genome. The full genome of PCV-3 was chemically synthesized and cloned in a pUC57-Kan plasmid. Ten-fold plasmid dilutions were performed on different matrices (i.e. swine lung homogenate, oral fluid and serum) and used to validate the sensitivity and repeatability of the assays. Assay specificity was evaluated using a panel of several swine DNA pathogens. Additionally, a total of 120 field samples were used to perform the diagnostic validation step.

Results

Both methods were proven to be extremely sensitive (detection up to 10 viral genome/µL), specific, and repeatable, independently of the considered matrix. The high reproducibility of quantitative results, combined with the implementation of an internal control, demonstrated the reliability of the qPCR assay in viral titer quantification. Diagnostic performance evaluation demonstrated the substantially perfect agreement between the two assays, strongly supporting their high sensitivity and specificity.

Discussion & Conclusion

The present study describes the development and validation of two assays for the sensitive, specific, and repeatable detection of PCV-3, which can be reliably applied to the most commonly used diagnostic matrices. The low cost and short processing time features of the direct PCR protocol, together with the reliable quantitative results guaranteed by qPCR, can cover a broad requirement spectrum and favor the establishment of common diagnostic guidelines.