Proceedings of the

23rd International Pig Veterinary Society (IPVS) Congress

VOLUME II

23rd IPVS CONGRESS
MEXICO 2014

June 8 – 11, 2014
Cancun, Quintana Roo, Mexico

Proceedings of the 23rd International Pig Veterinary Society (IPVS) Congress

Volume 2

Poster presentations

June 8 – 11, 2014
Cancun, Quintana Roo, Mexico

Development of a multiplex PCR assay for simultaneous detection of five single-stranded DNA viruses in pig lungs

D Gava[1]; R Schaefer[1]; AMG Ibelli[1]; MC da Silva[2]; JR Ciacci-Zanella[1]
[1] *Embrapa Swine and Poultry Research Center, Animal Health Laboratory, Concórdia, Brazil.* [2] *Diagnostic Center for Animal Health (CEDISA), Concórdia, Brazil.* danielle.gava@embrapa.br

Introduction

Porcine parvovirus (PPV1) and porcine circovirus type 2 (PCV2) cause clinical disease in swine (1, 3). Porcine parvovirus 4 (PPV4), torque-teno sus virus (TTSuV1 and TTSUV2) have been implicated as co-factors for PCV2 disease development, but their epidemiology and pathology remains unclear (1, 2). In order to simultaneously detect these five DNA viruses (PCV2, PPV1, PPV4, TTSuV1 and TTSuV2), a multiplex polymerase chain reaction (mPCR) was designed, optimized and tested in pig lungs.

Materials and Methods

Primer design: Multiple sequences of each virus retrieved from GenBank database were aligned using MEGA 5.2. The primer set was designed using Primer3Plus software targeting a conserved region of each virus.

Positive controls: Amplicons of TTSuV1 (101bp), PCV2 (284bp), TTSuV2 (341bp), PPV4 (440bp) and PPV1 (561bp) were cloned into pCR4 plasmid (TOPO TA Cloning Kit, Invitrogen) and purified using the PureLink Quick Plasmid Miniprep Kit (Invitrogen). All cloned amplicons were verified by sequencing and quantified using a ND-1000 spectrophotometer.

Sensitivity and specificity: A 10-fold serial dilution of 1.2×10^{10} up to 1.2×10^{0} DNA copies/µL was carried out to evaluate the sensitivity of the mPCR. The specificity was tested using influenza A virus and PCV1.

Multiplex PCR: The mPCR was performed in a 30µL reaction and contained 4mM $MgCl_2$, 1.5× PCR Buffer, 0.4mM dNTP, 0.05µM TTSuV1, TTSuV2 and PCV2 primers, 0.16µM PPV1 primer, 0.4µM PPV4 primer and 1.8U Platinum *Taq* DNA Polymerase (Invitrogen). The PCR cycling conditions consisted of an initial denaturing at 95°C for 30s followed by 35 cycles at 95°C for 1 min, 60°C for 1 min and 72°C for 1 min followed by a final extension at 72°C for 10 min. The amplicons were analyzed by electrophoresis in a 2% agarose-TBE gel and stained with ethidium bromide.

Clinical samples: Seventy-five lung samples were collected from 15-180 day-old pigs, from 2009 to 2013 in Southern Brazil. Viral DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen) and the mPCR was performed as described above.

Results

The sensitivity of the mPCR was 1.2×10^{3} DNA copies/µL and no amplification was observed with other pathogens. All clinical samples were positive for at least one virus. Sixty-eight (90.7%) samples were positive for PPV4, 38.7% for TTSuV1, 37.3% for TTSuV2, 17.3% for PCV2 and 16% for PPV1. Among the 75 samples, PPV4 was present as the only virus in 29.3% of the tested samples. Co-infection by PPV4 and TTSuV1 was observed in 14.7% samples, co-infection by PPV4 and TTSuV2 occurred in 12% and triple co-infection by PPV4, TTSuV1 and TTSuV2 was detected in 10.7%. A single infection by PCV2 or PPV1 was not observed.

Conclusions and Discussion

A rapid, sensitive, specific and cost-effective mPCR assay is described and applied for simultaneously and differential detection of TTSuV1, PCV2, TTSuV2, PPV4 and PPV1 in pig lungs. In current intensive swine production, pigs can be infected at the same time with two or more viral pathogens (1, 2, 3). The five viruses included here are involved in multifactorial diseases that cause significant economic losses in swine production worldwide (1, 2). A rapid and reliable detection of these viruses is important for herd management and the prevention of disease spread. Moreover, the detection of viral co-infections that have been shown to enhance the severity of PCV2 infection would allow the implementation of control measures directed against these possible 'trigger' factors.

Acknowledgements

This work has been founded by Embrapa/proc. 02.11.10600-03). JRC Zanella is a fellow of the National Council for Scientific and Technological Development (CNPq).

References

1. Blomstrom et al.:2010, Virus Res 152:59-64.
2. Ellis et al.:2008, Am J Vet Res 69:1608-14.
3. Segales et al.:2013, Vet Microbiol 165:13-20.