

Development and validation of a quantitative real-time PCR for the detection of *Actinobacillus suis*: The miracle tool?

Maria J. Clavijo¹; Simone Oliveira², DVM, MS, PhD

¹ Universidad Central de Venezuela; ² University of Minnesota, St. Paul, Minnesota

Statement of the problem

Actinobacillus suis is an emerging pathogen considered to be a new threat for swine production. The isolation of this pathogen from tissues submitted to the University of Minnesota Veterinary Diagnostic Laboratory (MVDL) increased considerably in the past three years. In 2006, a 16S rRNA gel-based PCR was developed and since then requests for *A suis* PCR testing have raised significantly.¹ This tool has been highly employed in protocols attempting to eradicate *A suis*. However, complete elimination of the pathogen has not been achieved.^{2,3} Limitations of this gel-based PCR include the non-specific amplification of *A equuli* and the lack of bacterial quantification. Therefore, a more sensitive and specific quantitative diagnostic tool is needed to improve eradication protocols.

Objective

To develop and validate a sensitive and specific quantitative real-time PCR assay for the detection of *Actinobacillus suis* in clinical samples.

Materials and methods

Forward and Reverse primers and a TaqMan probe specific for the amplification and detection of an *A. suis* housekeeping gene involved in thiophene oxidation (*Thdf*) were designed using the *Primer 3 Software* (<http://frodo.wi.mit.edu/primer3/input.htm>). Real-time PCR conditions were optimized utilizing an ATCC *A suis* reference strain. This strain was also utilized to evaluate the analytical sensitivity of the real-time PCR by testing 10-fold dilutions of extracted DNA. The analytical specificity was evaluated by testing 28 unrelated bacterial species frequently isolated from swine clinical samples, including *Erysipelothrix rhusiopathiae*, *Streptococcus suis*, *Staphylococcus aureus*, *Haemophilus parasuis*, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* (15 reference strains), *Actinomyces pyogenes*, *Actinobacillus porcini*, *Actinobacillus minor*,

Actinobacillus indolicus, *Salmonella sp.* and *Escherichia coli*. In addition, DNA extracted from *A equuli*, which is a mainly an equine pathogen but is occasionally isolated from swine, was tested. The stability of the targeted gene sequence among *A suis* field isolates was also evaluated by testing 70 different isolates representing 12 genotypes circulating among US swine herds.

Significant results

The newly developed real-time PCR detected a minimum of 3.5×10^1 CFU/ml, compared to 7.2×10^2 CFU/ml detected by the 16S rRNA gel-based PCR test. The *Thdf* real-time PCR was highly specific, detecting exclusively *A suis* isolates. There was no amplification of *A equuli* DNA, as previously observed with the gel-based PCR. The new real-time PCR amplified the *Thdf* gene from all 70 *A suis* field isolates tested, indicating that this housekeeping gene is highly conserved among different strains circulating in US swine herds.

Discussion and field applications

We have developed a more sensitive and specific quantitative real-time PCR for detection of *A suis* in clinical samples. This new test will allow swine veterinarians to perform a more comprehensive comparison of different control and eradication protocols. All attempts to eradicate *A suis* from endemically infected populations have failed so far. In most of these cases, *A suis* was not detected at weaning following intensive antibiotic treatments, but was detected at the end of the nursery using the gel-based PCR. The new real-time PCR, which is more sensitive and specific than the previous gel-based PCR, will allow a better characterization of prevalence at weaning. Quantification of *A suis* in tonsil swabs will also provide a more detailed characterization of treatment effect, and will allow swine veterinarians to evaluate the cost-benefit of each protocol. We expect the new *A suis* real-time PCR to be an important aid in *A suis* control and eradication.

References

1. Oliveira S., Gayle R., Tomaszewski J., Collins J. (2007) Development of a duplex PCR for the simultaneous detection of *Actinobacillus suis* and *Actinobacillus pleuropneumoniae* in clinical samples from swine. Proceedings of the 50th American Association of Veterinary Laboratory Diagnosticians Annual Meeting, Reno, p. 135.
2. Geiger J., Engle M., Oliveira S. (2007) *Actinobacillus suis* eradication: is it possible? Proceedings of the Swine Disease Eradication Center Annual Meeting, Minneapolis, MN p. 14.
3. Miller S.P., Stanford, S, Oliveira, S. (2008) Case study – *Actinobacillus suis*: elimination or reduction? Proceedings of the 2008 Carlos Pijoan International Symposium: New solutions to old problems. In: Allen Leman Swine Conference Proceedings, saint Paul MN, p. 29–32.

