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Development of a quantitative PCR assay for the detection of *Mycoplasma hyorhinis* in clinical samples

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Introduction: *Mycoplasma hyorhinis* has recently emerged as an important cause of mortality in nursery pigs.[1] Approximately 50% of the cases with polyserositis received at the Minnesota Veterinary Diagnostic Laboratory have the involvement of this pathogen based on isolation or detection by PCR.[2] Several protocols for detection of *M. hyorhinis* DNA by gel-based PCR have been published, but the majority of them are for the detection of *M. hyorhinis* in cell cultures. There are no quantitative PCR (qPCR) protocols available for this pathogen. Therefore, the objective of this study was to develop a highly sensitive and specific qPCR to detect *M. hyorhinis.*

Materials and Methods: Forward and Reverse primers and a TaqMan probe specific for the amplification and detection of the 16S rRNA were designed using the Primer3 Software. The qPCR conditions were optimized utilizing the *M. hyorhinis* ATCC 17981. This strain was also utilized to evaluate the analytical sensitivity of the qPCR by testing 10-fold dilutions of extracted DNA. The analytical specificity was evaluated by testing 19 unrelated bacterial species frequently isolated from swine, including *M. hyopneumoniae, M. flocculare and M. hyosynoviae*. A total of 45 clinical samples submitted to the Minnesota Veterinary Diagnostic Laboratory for *M. hyorhinis* testing were analyzed with the qPCR. These samples were from animals that presented typical lesions found in *M. hyorhinis* cases such as pericarditis, pleuritis, arthritis and peritonitis. A total of 30 nasal swabs and 30 oropharyngeal swabs from pigs with clinical signs suggestive of *M. hyorhinis* systemic infection (dyspnea, fever higher than 105°F and lameness) were tested with the new qPCR protocol.

Results: The newly developed qPCR test was more sensitive than gel-based PCR, detecting 40 x 10^4 CFU/reaction. The qPCR detected exclusively *M. hyorhinis*. Twenty-one of the 45 clinical samples from pigs with lesions characteristic of *M. hyorhinis* infection were positive by qPCR compared to only 14 positive by the gel-based test. A total of 26 nasal swabs and 20 tonsil swabs were positive for the presence of *M. hyorhinis*, indicating that nasal swabs are more sensitive to detect shedding of this pathogen.

Discussions: We have developed a sensitive and specific qPCR for detection of *M. hyorhinis* in clinical samples. This test can be used to detect *M. hyorhinis* in clinical samples obtained from diseased pigs or to characterize the prevalence of colonization at different ages. Our results indicate that nasal swabs are more sensitive for detection of colonized pigs compared to oropharyngeal swabs. Although nasal swabs were more sensitive for detection of *M. hyorhinis*, the presence of this pathogen in oropharyngeal samples likely allows its detection in saliva (rope testing). Further studies are needed to evaluate the usefulness of rope testing for detection of *M hyorhinis* in endemically infected herds.

References:

1. Leuwerke B. 2009. *Mycoplasma hyorhinis* – Field experiences in diagnosis and control. Allen Leman Swine Conference Proceedings. Saint Paul, MN. 36:89-90**.**

2. Rovira, A. 2009. Review of *Mycoplasma hyorhinis*. Allen Leman Swine Conference Proceedings. Saint Paul, MN. 36:87-88.