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Development of Nested Polymerase Chain Reaction for the Detection of *Mycoplasma hyopneumoniae* in Formalin-fixed Paraffin-embedded Lung Tissues

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Introduction

Mycoplasma hyopneumoniae is among the most prevalent and important infectious agents associated with porcine respiratory disease complex. The airway dagame caused by *M. hyopneumoniae* adversely affect the pulmonary host defense mechanisms and may lead to secondary bacterial infections. Culture is considered to be the "gold standard" for diagnosis but this is a very slow and labor-intensive procedure. Isolation of *M. hyopneumoniae* is complicated by its fastidious nature and extremely slow growth. Thirty days of incubation may be necessary to detect the organism in primary broth cultures. The purposes of the study were (i) to develop nested PCR for the detection of *M. hyopneumoniae* for the detection of *M. hyopneumoniae* DNA in the formalin-fixed, paraffin-embedded lung tissues from experimentally and naturally infected pigs and (ii) to compare the utility of nested PCR with in situ hybridization.

Materials and Methods

Fifty colostrum-deprived pigs aged 13 days were randomly allocated either to infected or control groups. A tissue homogenate containing a derivative of *M. hyopneumoniae* strain SNU98703 (10^5 color changing units per ml) was administered intratracheally to 25 pigs in the infected group at a dilution of 1:100 in 10 ml of mycoplasmal Friis medium. Twenty five control pigs were exposed in the same manner to Friis medium. DNA was extracted from formalin-fixed, paraffin-embedded lung tissues as previously described [1]. In situ hybridization was performed as previously described [2].

Results

The sensitivity was determined by dividing the number of nested PCR positive lungs by the number of *M. hyopneumoniae*-inoculated pigs; 25/25 = 100%. The specificity was determined by dividing the number of control samples that were negative for nested PCR by the number of mock-infected control pigs: 25/25 = 100%.

Hybridization signals for *M. hyopneumoniae* DNA were detected in pigs inoculated with *M. hyopneumoniae*. Positive cells had dark brown or black reaction product, and there was no background staining. A strong hybridization signal was detected mainly in the luminal surface of bronchial and bronchiolar lining epithelial cells whereas no hybridization signal was seen in the cytoplasm of bronchial and bronchiolar lining epithelial cells. The rate of agreement between nested PCR and in situ hybridization was 100% for the detection of *M. hyopneumoniae* in formalin-fixed, paraffin-embedded lung tissues.

Discussion

Formalin fixation is the standard method for tissue preservation for microscopic evaluation and this material forms the major source of tissues for many studies. Formalin-fixed, paraffin-embedded tissue is often the only sample available in pathology laboratory archives because fresh tissue and serum from suspect cases are rarely saved for extended periods. The large amount of paraffin embedded material existing in the pathology archives of diagnostic laboratories is an important resource for retrospective studies of infectious diseases. However, formalin-fixed, paraffin-embedded tissues are regarded commonly as poor sources of DNA due to supposed damages occurred during the fixation and embedding processes. The recovery of DNA from from formalin-fixed, paraffin-embedded tissues and the development of the PCR method have vastly expanded the opportunity for molecular analysis of paraffin-embedded tissues.

References

1. **Kim, J. and Chae, C.** J. Virol. Methods 2001. **92,** 105-111.
2. **Kwon D. and Chae C.,** Vet. Pathol. 1999. **36,** 308-313.