RESEARCH ABSTRACT

SWINE HEALTH

Title: Development of improved diagnostic PCR assays for *Mycoplasma hyopneumoniae*-**NPB# 01-135**

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Date Received: 7/19/2002

Abstract

The diagnosis of pneumonia caused by *Mycoplasma hyopneumoniae* remains frustrating. To increase our ability to detect *M. hyopneumoniae,* we modified our present *M. hyopneumoniae* PCR assay to enable us to quantify the number of organisms in a sample and to allow the detection of the organism in formalin fixed and paraffin embedded lung tissue samples. An ELISA-based *M. hyopneumoniae* PCR assay utilizing phenylboronic acid- saclicylhydroxamic acid, biotinylated primers and strepavidin-alkaline phosphatase conjugation was used to quantify *M. hyopneumoniae* DNA. Known amounts of *M. hyopneumoniae* DNA was used to develop a standard curve and known and unknown samples of *M. hyopneumoniae* were assayed. In addition, the quantitiy of DNA obtained from cultured organisms was successfully compared to titration of cultured organisms, which is currently considered the gold standard. Detection of *M. hyopneumoniae* by PCR was successful using fixed lung tissues collected at necropsy from pigs experimentally infected with *M. hyopneumoniae* . Lung tissues were placed in formalin for various lengths of time followed by DNA extraction and subjected to PCR. The results correlated to the PCR results obtained from fresh tissues. *M. hyopneumoniae* DNA was detected by PCR in all experimentally infected lung tissues after 168 hours of fixation in formalin. In order to evaluate the PCR assay under field conditions, lung tissue samples from 7 pigs presented to the Iowa State Veterinary Diagnostic Laboratory (ISUVDL) with respiratory disease were also assayed. Three lung samples were *M. hyopneumoniae* PCR positive using fresh samples, and 2 remained positive following 24 hours of formalin fixation. Paraffin-embedded lung tissues from experimentally infected pigs were processed, DNA extracted and subjected to PCR. Preliminary results indicate that DNA for *M. hyopneumoniae* can be successfully extracted from paraffin embedded samples and detected by PCR.