Sponsors

University of Minnesota
College of Veterinary Medicine

College of Food, Agricultural and Natural Resource Sciences

Extension Service

Swine Center

Thank you to **IDEXX Laboratories** for their financial support to reproduce conference proceedings

Production Assistants
Steven Claas
Michael Klatt

Layout and CD-ROM
David Brown

Logo Design
Ruth Cronje, and Jan Swanson;
based on the original design by Dr. Robert Dunlop

Detection pattern of *Mycoplasma hyopneumoniae* DNA in experimentally infected pigs

Maria Pieters and Carlos Pijoan
Swine Disease Eradication Center, University of Minnesota

Introduction

Enzootic Pneumonia, caused by *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*), is one of the most important contributors to disease-associated loss in swine production and is present in almost every country where pigs are raised.

The ti me at which *M. hyopneumoniae* spreads from one pig to another is not well established. It has been demonstrated that a pig can transmit *M. hyopneumoniae* to another host even when the animal is not showing any clinical signs. A reevaluation of epidemiologic parameters, using the newest diagnostic techniques seems to be an important step to better understand the behavior of this central pathogen.

Molecular based tests have been developed for the identification of this microorganism. Samples from tissues as well as samples from live animals (i.e. nasal swabs, bronchoalveolar lavage fluids) can be qualitatively tested.

Determining if *M. hyopneumoniae* can be transmitted from an apparently healthy pig (before the onset of clinical sings) to a naïve host could be done by establishing the latent period of the microorganism, which is the time interval from infection to shedding and this period can be shorter than the incubation period, which would mean that pigs can be infectious before showing any clinical signs.

We propose that the detection of *M. hyopneumoniae* in the pigs' nasal cavity following experimental infection can be assessed as an indirect measure of shedding. The objectives of this study were to determine the detection pattern of *M. hyopneumoniae* DNA from nasal swabs of experimentally infected pigs, from 0 to 34 days post infection, and to determine the incubation and latent periods, by onset of clinical signs and shedding.

Materials and Methods

The study length was 34 days and all samples were taken *in vivo*.

Three five-week old pigs served as negative control pigs and they were housed in a nursery room of the Swine Disease Eradication Center Research Farm. Sixty, fifteen-week old *M. hyopneumoniae* negative pigs were housed in a different barn.

Blood samples and nasal swabs were taken on day 0 to ensure that all pigs were negative to *M. hyopneumoniae.*

Sixty pigs (as part of a larger transmission study) were experimentally infected, this consisted of an intratracheal inoculation of 20mL per pig of a standardized lung suspension inoculum, produced with a culture of *M. hyopneumoniae* strain 232 resuspended to a final dose of $1x10^5$ colour-changing units (CCU) per mL.

Successful infection was evaluated by clinical signs assessment, n-PCR from nasal swabs and seroconvertion to *M. hyopneumoniae* 34 days after inoculation.

A subset of 10 pigs was randomly chosen as index pigs, which were ear tagged and remained with the whole group.

Index pigs were nasally swabbed on days 0, 4, 7, 11, 13, 18, 22, 25 and 34 postinoculation.

To assess clinical signs, each group of pigs was observed during 30 minutes on the same day that pigs were sampled. The clinical observation was performed before swabbing, in order to do it before the pigs were stressed due to the sampling procedure, which involved animal restrain and manipulation.

The Dako-ELISA kit was used to determine antibodies against *M. hyopneumoniae* and nasal swabs were processed for DNA extraction, then a nested PCR (n-PCR) for *M. hyopneumoniae* was run.

Results

The onset of clinical signs associated with *M. hyopneumoniae* was recorded at day 13 after experimental infection.

Figure 1 summarizes the n-PCR results of the study.

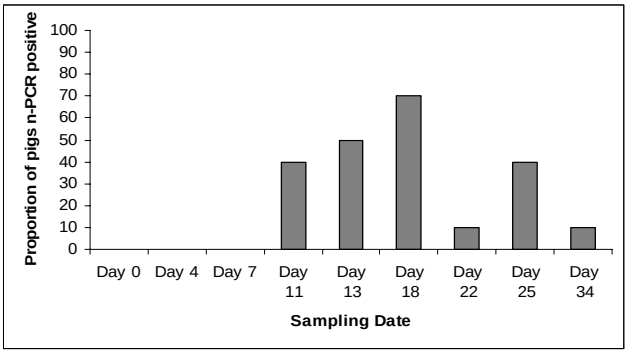

Figure 1. Proportion of pigs n-PCR positive from nasal samples at the different sampling dates of the study.

Discussion

Under the conditions of this study, *M. hyopneumoniae* DNA was detected from nasal swabs samples as early as 11 days post inoculation, but the subsequent detection was inconsistent. These results are in accordance with previous data by Calsamiglia *et al* (1999) and Otagiri *et al* (2005).

These results suggest than the latent period for Enzootic Pneumonia is shorter than the incubation period, meaning that infected animals can shed the organism even before the onset of clinical signs and detection of antibodies against *M. hyopneumoniae*. However, the infectivity of the *M. hyopneumoniae* DNA detected from nasal swabs to susceptible pigs was not assessed.

n-PCR from nasal swabs was a useful diagnostic tool in detecting the agent at early stages of infection.

References

Calsamiglia M. *et al.* (1999). J Vet Diag 11:246-251.

Fano E. *et al.* (2005). Can J Vet Res. 2005 Jul;69(3):223-8.

Fano E. *et al.* (2004) Proceedings IPVS 2004. Vol 1. 186.

Otagiri Y. *et al.* (2005) J. Vet. Med. Sci. 67 (8):801-805.