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An assessment of the duration of *Mycoplasma hyopneumoniae* infection in an experimentally infected population of pigs

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ABSTRACT

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is the primary pathogen of enzootic pneumonia (EP), a highly prevalent respiratory disease that affects pigs worldwide. Previous studies have demonstrated that *M. hyopneumoniae* infection can be longer than 185 days; however, the total duration of infection has not been determined yet. Therefore, the objective of this study was to determine the duration of *M. hyopneumoniae* infection in asymptomatic carriers.

To achieve our goal, 60 pigs were inoculated with *M. hyopneumoniae* strain 232 and the persistence of *M. hyopneumoniae* in the respiratory tract was assessed by detection of the bacterial DNA in bronchial swabs and the ability of the infected pigs to transmit the pathogen to sentinels.

Infection of the inoculated animals was demonstrated by the detection of *M. hyopneumoniae* DNA in nasal swabs, seroconversion to the bacteria and the onset of dry coughing. Experimentally infected pigs shed *M. hyopneumoniae* prior to and after the cough was observed. *M. hyopneumoniae* DNA was detected in 100% of experimentally infected pigs at 94 days post infection (dpi), 61% at 214 dpi and 0% at 254 dpi. Experimentally infected pigs transmitted the bacteria to sentinels at 80 and 200 dpi.

Results of this study have demonstrated that *M. hyopneumoniae* infected pigs can be incubatory as well as convalescent carriers of the pathogen and that convalescent carriers can remain infectious for up to 200 days. Total clearance of *M. hyopneumoniae* in the group was evidenced, demonstrating that duration of *M. hyopneumoniae* infection lasts less than 254 days.

1. Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is the primary pathogen of enzootic pneumonia (EP) (Mare and Switzer, 1965; Goodwin et al., 1965), a highly prevalent respiratory condition that affects pigs worldwide (Ross, 1999). Economic losses associated to EP are related to pig growth retardation, reduced gain to feed ratio and average daily gain, and the cost associated with antibiotic treatment (Maes et al., 2008). In addition, *M. hyopneumoniae* facilitates the invasion of the respiratory tract by other pathogens, such as *Pasteurella multocida* and porcine respiratory and reproductive syndrome virus, thus playing a central role in the development of swine pneumonia (Thacker, 2006). The main clinical sign associated with *M. hyopneumoniae* infection is a chronic non-productive cough that appears between 10 and 16 days post infection (dpi) (Ross, 1999), and ceases 6–8 weeks after the onset. Once clinical signs are

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not apparent, pigs may remain infected for long periods of time, becoming asymptomatic carriers, capable to infect susceptible pigs (Fano et al., 2005).

The duration of *M. hyopneumoniae* infection has been studied in experimental settings (Underdahl et al., 1980; Kobisch et al., 1993; Sorensen et al., 1997; Fano et al., 2005). Microscopic lesions associated with *M. hyopneumoniae* infection were identified in experimentally infected gnotobiotic pigs for up to 16 weeks after inoculation (Underdahl et al., 1980). The presence of *M. hyopneumoniae* in the respiratory tract of pigs was demonstrated up to 85 dpi by the combination of four diagnostic assays including polymerase chain reaction (PCR; Sorensen et al., 1997). Recently, *M. hyopneumoniae* has been detected by nested-PCR from bronchial swabs up to 185 dpi (Fano et al., 2005). However, the previously referenced studies have not extended time points later than 185 dpi, suggesting that the duration of *M. hyopneumoniae* in the respiratory tract of the pig is longer than previously reported. To evaluate the persistence of *M. hyopneumoniae* in carrier animals constitutes a central issue for swine health management; having effect on factors such as introduction of replacement pigs, transmission dynamics within herds, and establishment of *M. hyopneumoniae* eradication programs. The objective of this study was to determine the duration of *M. hyopneumoniae* infection in experimentally infected asymptomatic carriers.

2. Materials and methods

2.1. Animals, housing, and experimental groups

A total of 108 pigs were included in the study. All animals were obtained from a source known to be negative to *M. hyopneumoniae* and porcine respiratory and reproductive syndrome virus. Pigs were declared negative to *M. hyopneumoniae* based on serology, lack of clinical signs and farm history of more than 5 years. Pigs were housed for 255 days at the Swine Disease Eradication Center Research Farm, in west central Minnesota, USA. No other swine operations were located in a 16-km radius of the Research Farm facilities. All animals were cared for following the standard of the Institutional Animal Care and Use Committee, University of Minnesota. Pigs were distributed in four experimental groups, as follows: (1) *Negative controls*: ($n = 3$) 3-week-old pigs that served as environmental sentinels for external *M. hyopneumoniae* infections; (2) *Principals*: ($n = 60$) 15-week-old female pigs that were experimentally infected with *M. hyopneumoniae* and served as the source population; (3) *Monitor pigs* ($n = 10$) were randomly selected from the group of principals for sampling during the acute phase of infection; and (4) *Sentinels*: ($n = 45$) age-matched with the experimentally infected animals, that served as sentinels for transmission of *M. hyopneumoniae*. All pigs were individually identified.

2.2. Experimental design

The experimental design for evaluating the duration of *M. hyopneumoniae* infection is detailed in Fig. 1. Negative controls served as environmental sentinels for assessing the introduction of *M. hyopneumoniae* to the facilities. Principals were infected with a reference strain of *M. hyopneumoniae*. Subsets of 18 principals were evaluated for detection of *M. hyopneumoniae* at 94, 214 and 254 dpi. The times at which detection of *M. hyopneumoniae* was assessed were chosen to be after coughing was no longer noticed in the group of animals (94 dpi) and after previous reports of *M. hyopneumoniae* persistence (beyond 185 dpi), in order to observe persistence of *M. hyopneumoniae* in asymptomatic carriers.

Monitor pigs were evaluated during the acute phase of infection in order to confirm a successful experimental infection.

Shedding of *M. hyopneumoniae* from principals to susceptible pigs was assessed by direct contact of principals and sentinels. Each contact group consisted of 3 physically separated pens, 3 principals and 5 sentinels per pen, for a total of 9 principals and 15 sentinels. Contact of animals was allowed for 2 weeks at days 80–94, 200–214 and 240–254 of the study.

2.3. Inoculum and experimental infection

M. hyopneumoniae strain 232 was used for infection in this study, this strain have been widely employed in experimental studies in the United States (Minion et al.,

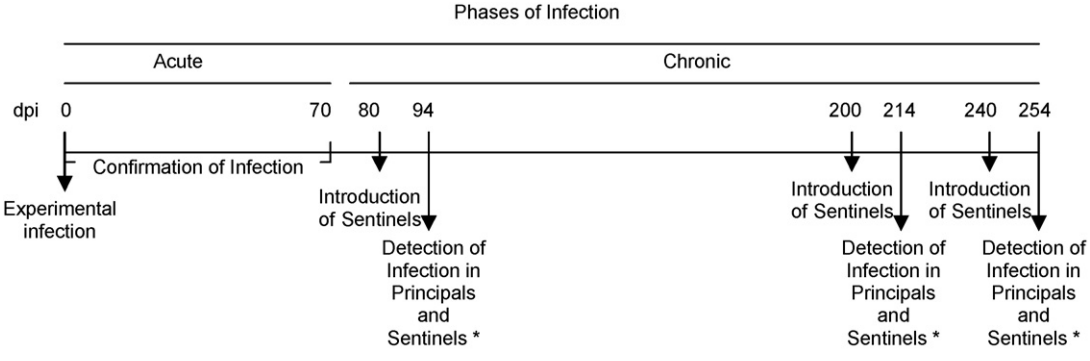

Fig. 1. Experimental design for the assessment of the duration of *M. hyopneumoniae* infection in an experimentally infected population of pigs. *Principals ($n = 18$) and sentinels ($n = 15$) at each time point were humanely sacrificed, *M. hyopneumoniae* DNA and antibodies detected, and lung lesions evaluated.