Prevalence of Oral, Pharyngeal and Respiratory Infection in *Mycoplasma bovis* Experimentally Infected Neonatal Calves

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Introduction

Mycoplasma bovis is an important etiologic agent of neonatal calf disease such as pneumonia, arthritis, otitis media, and conjunctivitis. Oral colonization of calves with *M. bovis* may be an important risk factor in the development of these disease conditions. Many pharmaceuticals used to treat calfhood infectious disease are often ineffective against *M. bovis*, due to the unique physiologic characteristics of the pathogen. Establishing effective animal model systems to study the pathophysiology and treatment of mycoplasmosis is important. The objective of this study was to establish oral, pharyngeal, and respiratory system colonization of *M. bovis* in milk-fed calves using a virulent, outbreak, wild type strain in experimentally infected neonatal bull calves. We hypothesized pathogenic wild type strains of *M. bovis* would be more effective in establishing respiratory colonization than previous ATCC strains tested in our model system.

Materials and Methods

Twenty-four age-matched calves (<24 hours of age) were acquired from a commercial dairy. Calves were examined by a veterinarian, housed in individual polydome hutches separated by approximately two feet, and fed two quarts of commercially available 20% crude protein, 20% crude fat milk replacer twice daily. A medical record was maintained on each calf, including subjective health evaluations and temperatures. After a seven day acclimation period, each calf was fed *M. bovis*-inoculated calf replacer twice daily in a modified bottle system for three days. This wild strain was obtained from a large western calf ranch with an outbreak of otitis media and respiratory disease. Each inoculation carried a minimum *M. bovis* concentration of 10^8 CFU in 2mL of phosphate buffered saline. Oral pharynx swab samples were taken from each calf on post-dosing days 7, 14, and 21. All samples were enriched and plated using National Mastitis Council techniques and incubated for four days in 10% CO_2 at 98.6°F (37°C). On day 21 post-exposure, surviving calves (n=20) were humanely euthanized with sodium pentobarbital (IV, 0.66mg/kg of body weight) and submitted to the Washington Animal Disease Diagnostic Laboratory for diagnostic necropsy and postmortem sampling. Systems showing evidence of disease were evaluated for etiologic agent determination. Postmortem swab samples were taken from the eustachian tube, tympanic bullae, tracheal bifurcation, and lung. *Mycoplasma* agar plates were examined for growth evidenced by colony morphology at 2, 4, and 10 days of culture. Positive cultures were sent to the University of Minnesota Veterinary Diagnostic Lab. DNA from each sample (vspA locus) was amplified using the polymerase chain reaction method (PCR) and sequenced. Resulting sequences were compared to the original strain fed to each calf. If any sequences did not match, that individual was removed from the study.

Results

No febrile response or clinical signs of respiratory disease or otitis media were observed in calves during the trial. All calves exhibited moderate malabsorptive diarrhea immediately post-inoculation in this experiment. Neither temperatures, nor subjective daily observations, were useful in detecting antemortem disease. Antemortem oral pharyngeal culture prevalence of *M. bovis* was 88%, 100%, and 86% for post-dosing days 7, 14, and 21, respectively. Postmortem culture prevalence was 100% for eustachian tube, 95% for tympanic bullae, 62% for tracheal bifurcation, and 37% for lung samples. Sequenced DNA from positive cultures were identical to the sequence of the original strain used for inoculation.

Significance

Prevalence of *M. bovis* colonization in this study indicates a robust model for inducing colonization of the oral pharynx and tympanic bullae by day 21 post-exposure in calves. Previous studies from our lab, using a similar model system and a different strain of *M. bovis* bacteria (ATCC 27368), resulted in postmortem colonization rates of 56% (oral pharynx) and 81% (tympanic bulla) (Stebbins JC, Schneider CS: Effects of feeding *Mycoplasma bovis* to neonatal bull calves. *Proc Am Assoc Bov Pract Conf* 41:278, 2008). We conclude that the use of a virulent, wild type strain of *M. bovis* was proportionally similar in establishing experimental infections in the model system. This model, where infection rates are approaching 100%, could be a useful tool for future studies investigating the pathophysiology and treatment of mycoplasmosis in calves. These studies indicate that contamination of calf feed with pathogenic *M. bovis* bacteria may be a risk factor for calves developing oral and respiratory colonization. In this study, rectal temperature and clinical signs associated with otitis media and pneumonia were not effective in identifying calves that had *Mycoplasma* colonization at privileged locations (tympanic bulla and lung parenchyma). This may be important as veterinarians consider treatment options in the face of *Mycoplasma* outbreaks in neonatal calf raising systems.