Effects of Feeding *Mycoplasma bovis* to Neonatal Bull Calves

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Introduction

Mycoplasma bovis has been identified as the etiologic agent in a variety of bovine diseases. Transmission of *M. bovis* may occur by direct contact, environmental exposure, vertical transmission at parturition, and often involves fomites. The objective of this study was to evaluate the effects of feeding viable *M. bovis* as manifest through body site colonization, clinical disease and animal health. The hypothesis of this study was feeding *M. bovis* in milk replacer would result in body site colonization and decreased animal health.

Materials and Methods

An experiment designed with three replicas (n = 18, 20, 22) examined the effects of feeding *M. bovis* to colostrally-supplemented neonatal Jersey bull calves. Age-matched calves were acquired from a commercial dairy at <24 hours of age. Calves were examined by a veterinarian, housed in individual hutches, separated by approximately two feet and fed two quarts of commercially available 20/20 milk replacer 2x/day. A medical record was maintained on each calf including subjective health evaluations and daily temperature measurements. On day 0, calves were randomly assigned to treatment groups and were sampled with polyester swabs from accessible body sites: external ear, eye, nose and mouth. All samples were enriched and plated using National Mastitis Council (NMC) techniques and incubated for four days in 10% CO_2 at 37°C. Treatment #1(Tx1) was milk replacer containing approximately 1 x 10^6 colony forming units (CFU) of *M. bovis* in 10 mL of phosphate buffered saline, while treatment #2 (Tx2) was milk replacer containing 10 mL of phosphate buffered saline vehicle (control). Replica 1 calves were fed 1 x 10^6 CFU *M. bovis* in milk replacer 2x daily for three days; replica 2 calves were fed 1 x 10^6 CFU *M. bovis* 2x daily for two days; replica 3 calves were fed 2 x 10^8 CFU *M. bovis* 2x daily for three days. On days 7 and 14, accessible body sites were swab sampled. On day 21, calves were euthanized with sodium pentobarbital (IV, 0.30 mg/lb [0.66 mg/kg] of bodyweight). Necropsies were performed by pathologists at the Washington Animal Disease Diagnostic Laboratory. Postmortem swab samples were taken from eyes, nose, prepuce, oral pharynx, auditory tube, tympanic bulla and tracheal bifurcation. Systems showing evidence of disease were evaluated for etiologic agent determination. Mycoplasma agar plates were examined for growth evidenced by colony morphology at four and 10 days of culture. SAS (SAS, 2007) was used to analyze the proportion of samples positive using Proc GLM programming, assuming a binomial distribution.

Results

Prior to necropsy, 36% (11/30) of Tx1 calves were *Mycoplasma* spp culture positive at one or multiple accessible body sites. At postmortem sampling, 53% (16/30) of Tx1 calves were *Mycoplasma* spp culture positive. The percentage of sites positive in Tx1 calves were eyes 13% (2/16),) deep nares 19% (3/16), oral pharynx 56% (9/16), tracheal bifurcation 43% (7/16), auditory tube 43% (7/16) and tympanic bulla 81% (13/16). The distribution of positive calves for Tx1 by replica was 44%, 30% and 82%, respectively. In Tx2, 10% (3/30) of calves were positive. On postmortem *Mycoplasma* spp sampling, there was a difference between Tx1 (53%; 16/30) and Tx2 (10%; 3/30) ($P < 0.001$). Neither temperatures nor subjective daily observations were useful in detecting premortem disease in calves.

Significance

Results of this study indicate that feeding *M. bovis* to neonatal calves in milk replacer can cause colonization of body sites. There was a high percentage (53%; 16/30) of the *M. bovis* treated calves that were positive on standard Mycoplasma culture of the oral pharynx and associated tissues. These findings support the recommendation of pasteurization of waste milk fed to dairy calves. Perhaps due to the short duration of the study, group level diarrhea and described *M. bovis* disease syndromes were not observed. Establishing a reliable experimental model for colonization of the upper respiratory tract and auditory system of calves has important experimental implications for evaluation of treatment modalities.