# Is *Mycoplasma bovis* in Bedding Sand Transmissible to Naive Dairy Calves?

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## Introduction

The primary objective was to evaluate the possible transmission of *Mycoplasma bovis* from positive sand bedding to naive dairy calves. *Mycoplasma* spp, most commonly *M. bovis*, can infect calves and adults, causing arthritis, pneumonia, septicemia, or death. Cows may also contract mastitis, metritis, or agalactia.

## Materials and Methods

Estimating 25% sensitivity for nasal/ear swabs, and 50% prevalence if mycoplasma is present, 32 pre-weaned Holstein calves were swabbed for mycoplasma culture on a farm over several weeks; all were negative. Probability of having failed to detect a true-positive herd was (0.875)32=0.013; thus, 99% probability that the herd was mycoplasma-free. Twelve additional calves a few days to two weeks old were obtained for study, also swab-negative for mycoplasma. Recycled sand bedding from a dairy farm repeatedly positive for *M. bovis*, including by confirmatory PCR, was used to bed calves (n=6) in the exposed group. Sand quarry sand was used to bed six control calves. Calves were blocked in pairs by height and weight and randomly allocated. Calves were housed in individual plastic hutches with wire fences nine feet (three meters) apart during the 105-day study; control calves were separated from mycoplasma-exposed calves by 80 feet (25 meters). Sand for both groups was added twice daily on top of straw. Commercial milk replacer, calf starter feed, and free-choice water were fed. Controls were cared for before exposed calves, and biosecurity measures were used to avoid spread of organisms between calves. Blood and nasal/ear swabs were collected weekly, sand bedding was sampled weekly, and tracheal swabs (under anesthesia, guarded culture swabs) were collected every four weeks. Serum was tested with a *M. bovis*-specific antibody ELISA, other samples were mycoplasma cultured. After death or euthanasia at completion of the study, lung, retropharyngeal lymph node, and trachea samples were cultured and PCR tested for multiple *Mycoplasma* spp. A complete necropsy was performed. Probability of failure to detect infection if present four weeks after exposure was estimated using .75 for nasal/ear swabs and .25 for tracheal swabs. E.g., if a calf was tested over 62 days with seven nasal/ear swabs and two tracheal swabs, the probability of failure to detect infection if present was (.75)(.75)(.75)(.25)(.75)(.75)(.75)(.75)(.25)=.008; thus (1 –.008)= 99.2% probability of detection by at least one positive test if truly mycoplasma-infected.

## Results

The exposed group sand was mycoplasma-positive during weeks 1, 5, 6, 7, and 11 of the 15-week study; all other sand samples were negative. Exposed calves' total days bedded on mycoplasma-positive sand were 138; total days following first exposure were 385. All 16 tracheal swabs and 67 nasal/ear swabs from all calves were mycoplasma-negative. Probabilities of detection if calves had become truly infected with mycoplasma four weeks after exposure were between 96.5% and 99.8% for individual calves. Three calves were euthanized, two died before the end of the 105-day study, and the remaining seven were euthanized at the conclusion. None of the 12 calves had any gross lesions indicating infection with *Mycoplasma* spp. All 72 postmortem culture (n=36) or PCR (n=36) tests on all 12 calves' lung, retropharyngeal lymph node, and trachea samples were mycoplasma-negative.

## Significance

Dairy producers whose farms have concurrently had mycoplasma mastitis and mycoplasma detected in bedding samples have asked the practical question regarding infectivity of recycled bedding. Bedding sand positive for *Mycoplasma bovis* showed no evidence of being a source of infection to naive calves, despite exposure over a period of several weeks. Further studies regarding transmission of mycoplasma mastitis from contaminated bedding are still needed in lactating cows because of the possibility of infection through the teat canals causing mastitis. However, mycoplasma-positive bedding is apparently not a source of infection to non-lactating animals, including pre-weaned dairy calves.