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Is *Mycoplasma bovis* in Sand Bedding Infectious to Dairy Calves?

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Introduction

Mycoplasmas are unusual bacteria that can infect all ages of cattle, and can cause arthritis, pneumonia, and death. Dairy cows may also contract mastitis, metritis, or virtually cease milk production. The most common mycoplasma affecting cattle is *M. bovis*; there are several other *Mycoplasma* spp. as well (Pfutzner et al., 1996; González and Wilson, 1997). Because standard microbial culture methods do not isolate *Mycoplasma* spp., special laboratory methods are needed for diagnosis (González and Wilson, 1997; González et al., 1995). Outbreaks of mycoplasma mastitis and pneumonia often follow the introduction of new and presumably infected (sometimes confirmed by pre-purchase testing that was ignored) animals into previously mycoplasma-negative dairy herds. Mycoplasma spreads by inhalation and respiratory secretions and also at milking time via contaminated inflations in milking units (González et al., 1993; Jasper, 1977; Wilson et al., 2007).

It is important to make clear that "preventing mycoplasma in calves" is something that we do not currently have the ability to do. The vast majority of dairy herds, even if closed to any purchased animals for many years, have pre-weaned calves infected with mycoplasma; often 30% to 50% of baby calves test positive. Fortunately, most calves never develop severe clinical signs, and nearly all do not develop mastitis or other infections with mycoplasma as adult cows (Virtala et al., 1996; Wilson et al., 2007). However, *Mycoplasma* spp. has also been detected in straw, sand, recycled manure, and other bedding, often in association with cows with mycoplasma mastitis lying on that bedding (Bray et al., 1997; Justice-Allen et al., 2010). Therefore, producers often ask about mycoplasma-positive recycled bedding's risk to baby calves. There is understandable interest in the question of whether recycled bedding on a farm where the herd currently has a mycoplasma mastitis outbreak will infect calves if they are bedded with it. Despite finding mycoplasma in straw, recycled manure, and some other environments, including

hospital pens where cows sometimes calve as well, we have found it much more likely to be found in sand than other bedding types, all on farms with cows already mycoplasma-positive (Justice-Allen et al., 2010). Therefore we investigated whether mycoplasma-positive sand bedding infected naïve dairy calves.

Mycoplasma-Positive Bedding Sand and Naïve Calves

Large piles of recycled sand bedding positive for *M. bovis* were found on a dairy farm with infected cows. 8,000 pounds of the sand was transported to Utah State University. Mycoplasma-negative sand was obtained from a sand quarry. Sand bedding samples of identical size were collected weekly from standard depths within the two sand piles. Sand samples were cultured at dilutions of 1,000, $1{:}10^4$, $1{:}10^5$, $1{:}10^6$. Mycoplasma culture was performed on modified Hayflick medium incubated at 37 ºC with 10% CO_2 using standard methods (Justice-Allen et al., 2010). Results were the mean of colony forming units (cfu/gm) of *Mycoplasma* spp. that were counted in all dilutions.

Baby calves from each of several dairy farms were sampled using nasal and ear swabs to screen for mycoplasma; as expected most had several positive calves. However, one farm was found with 44

calves tested, all mycoplasma-negative, resulting in 99% probability that the herd was truly free of mycoplasma. Calves (n = 12) were studied, blocked in pairs with similar weight and height and randomly assigned as 6 unexposed controls or 6 exposed. Calves were housed in individual plastic hutches with wire fences during the 105 day study. Control calves were separated from mycoplasma-exposed calves by 80 feet. Calves were bedded at least twice every day with the appropriate sand on top of straw. The straw was added as needed for moisture absorption and comfort, more frequently in cold or wet weather. Calves were fed a commercial all-milk milk replacer and a calf starter feed with free choice water.

Laundered barn clothes were worn to the calf housing area at every visit. Care always began with the unexposed group and moved to the exposed group. Boots and disposable gloves were washed and disinfected between every calf; gloves were changed after handling any visibly sick calf and between the control and exposed groups. Blood samples and nasal and ear swabs were collected weekly, except when tracheal swabs were collected every 4 weeks. For tracheal swab collection, calves were anesthetized and a laryngoscope was used to pass a sterile catheter swab through the larynx into the trachea for sampling. Anesthesia was reversed with a reversing drug so calves could stand immediately following. Nasal, ear and tracheal samples were cultured for mycoplasma as described above for bedding. Serum was tested for *M. bovis*-specific antibody using an ELISA test.

Necropsy

Culture for mycoplasma and a PCR differentiating multiple *Mycoplasma* spp. were performed on samples of lung, retropharyngeal lymph node, and trachea from each calf following humane euthanasia. The PCR and a complete necropsy to test for other diseases or pathology were both performed at the Utah Veterinary Diagnostic Laboratory (UVDL) (Justice-Allen et al., 2010). The study protocol was approved by the Utah State University Institutional Animal Care and Use Committee.

Mycoplasma Transmission Risk Results

The exposed group sand cultured positive for *Mycoplasma* spp. during weeks 1, 4, 5, 6, 7, 11 of the 15 week study, with concentrations between 200 and 14,200 colony forming units per gram. Control group sand weekly samples were all negative for mycoplasma. Exposed group calves had 385 days of combined exposure to mycoplasma-positive bedding. Calves always rooted in the sand when it was added to their hutches, with sand clinging to their muzzles and visible in the bottom of their water buckets when water was changed at least twice daily.

All 94 serum samples had no antibody against *M. bovis*, suggesting no infections. Allowing for 4 weeks of incubation following first exposure to mycoplasma in bedding, there were 16 tracheal swabs (8 from controls, 8 from exposed calves) and 60 nasal and ear swabs (30 from controls, 30 from exposed), all tested negative for mycoplasma. Testing of control calves was to evaluate the possibility that they may have unintentionally become exposed to mycoplasma despite precautions. The 76 negative tests were used to calculate the probability that calves were truly never infected. Three calves died or were euthanized in the first 25 days of life because of infection with common calfhood diarrhea pathogens. The remaining 9 calves had probabilities of detection between 96.5% and 99.8% of being detected positive at least once if they had become infected with mycoplasma; they were almost certainly never infected.

At necropsy, none of the 12 calves had any gross lesions or positive culture or PCR results from the lung, lymph node, or trachea samples indicating infection with *Mycoplasma* spp. All 72 post-mortem culture (n = 36) or PCR (n= 36) tests on all 12 calves were negative for *Mycoplasma* spp.

Summary

Dairy producers whose herds have been found with mycoplasma mastitis, with resultant detection of mycoplasma in bedding have asked the question regarding infectivity of the bedding, including its use underneath calves. There was no evidence of *Mycoplasma bovis* infection in naive calves despite exposure over a period of several weeks to positive bedding sand. This has practical implications considering that mycoplasma has been found in bedding of dairy herds, including recycled bedding following a manure separation process in association with mycoplasma mastitis (Justice-Allen et al., 2010). Not being able to use recycled bedding under calves can cost $1 to $2 million per year depending on the size of the dairy herd. We can't be sure why the bedding was not infectious, but physical contact is not the same disease transmission process as inhalation. It is likely that inhalation of aerosolized organisms is necessary for infection via the oral and nasal route, and despite the fact that all calves regularly rooted in the sand and covered their muzzles with it, this is not a sufficient vehicle for infection of calves with mycoplasma.

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