Toward a better understanding of upper respiratory tract bacterial colonization in neonatal beef calves

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Introduction
Bovine respiratory disease (BRD) is one of the most costly disease complexes to the beef industry. Bacterial pathogens associated with BRD include Mannheimia haemolytica (MH), Histophilus somni (HS), Mycoplasma bovis (MB) and Pasteurella multocida (PM). However, the source and timing of when these bacterial pathogens colonize in the upper respiratory tract of neonates is poorly understood. The objective of this study was to evaluate the bacteria and viruses present in the upper respiratory tract of neonatal beef calves immediately following parturition and for the first 24 hours of life.

Materials and methods
Twenty-six late-gestation beef cows were transported from Mount Pleasant, Wis. to a ranch near Clayton, N.M. Calving dates were estimated via rectal ultrasound by a veterinarian 24h post-arrival. Two weeks prior to the earliest estimated calving date, cows were transported to the Clayton Livestock Research Center in Clayton, N.M. where they were housed in individual pens (20.5 x 7.5 ft; 6.25 x 2.29 m). Cows were fed a mixed ration once daily at 2% BW (DM basis) and had ad libitum access to water. Immediately following parturition (0h), each dam was separated from her calf and moved to a hydraulic chute where left (L) and right (R) nasal swabs (NS) and a vaginal swab (VS) sample were collected using a nylon-flocked swab and placed in liquid Amies medium. For calves, L and R NS samples were collected at 0h, and 6, 12 and 24h after parturition. Dams were removed from calves, calves were sampled, and immediately reunited with their dam after each sampling time. Samples were stored at 4°C (40°F) and shipped overnight to the University of Nebraska Veterinary Diagnostic Center for bacterial culture, isolation and identification of BRD bacteria via MALDI. Additionally, samples from 10 cow-calf pairs were selected for RT-PCR to identify BRD-associated viruses (infectious bovine rhinotracheitis virus, bovine viral diarrhea virus, bovine coronavirus and bovine respiratory syncytial virus) and bacteria based on their having the shortest time between sampling and time of culture.

Results
All cow-calf pairs had complete sample sets except for 1 calf with missing 24h NS samples. We were unable to culture BRD-related bacterial pathogens in any swab samples. Overall, via RT-PCR we isolated MH from 0/20 dam NS, 1/10 VS, and 1/78 calf NS; HS from 3/20 dam NS, 1/10 VS, and 3/78 calf NS; MB from 4/20 dam NS, 0/10 of VS, and 3/78 calf NS; PM from 2/20 dam NS, 0/10 of VS, and 0/78 of calf NS. RT-PCR analysis revealed Cow 28 had detectable levels of HS (R ct = 35.29), MB (L ct = 35.23; R ct = 38.75), and PM (L ct = 35.14; R ct = 36.70) in NS samples at the time of parturition, but MH was not detected. In Calf 28, HS was only detected in both L and R NS at 6h (ct = 31.94 and 37.48, respectively). Inconsistent detection of MB was observed in the VS, as well as both NS of Cow 13, and the L NS of Calf 13 at 0h (ct = 27.56, 35.10, 35.40, 36.38, respectively). However, MB was detected (ct = 37.29) only in the R NS of Calf 13 at 12h and was undetected in subsequent samples. All other pathogens were only detected at a single time point. Viral detection via RT-PCR was limited. Infectious bovine rhinotracheitis was detected in only one L NS (ct = 37.84; Cow 13). Bovine respiratory syncytial virus was detected in one dam L NS (ct = 39.65; Cow 13) and one calf R NS at 6h (ct = 39.74; Calf 6).

Significance
RT-PCR was more sensitive than culture for detecting BRD-related bacteria in dams and neonatal beef calves, but overall, relatively few pathogens were identified in this group of cow-calf pairs. Unfortunately, a clear source and timing of upper respiratory tract colonization of BRD-associated bacteria was not identified in this study. Further research with more sensitive methods such as full length 16S rRNA sequencing may be needed.