Evaluating bacterial and viral colonization of the upper respiratory tract of neonatal beef calves during the first 24 hours of life

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Abstract

Little is known regarding the timing of initial establishment of bovine respiratory disease complex (BRDC)-associated bacterial and viral pathogens. The objective was to evaluate the presence of BRDC-associated bacteria and viruses in the upper respiratory tract of newborn calves within the first 24h of life. Commercial beef cows (n = 26) were allocated to individual pens prior to calving. A left nasal swab (LNS), right nasal swab (RNS) and a vaginal swab were collected from each cow immediately following parturition (0h). Calf (n = 26) LNS and RNS sampling occurred at 0h, 6h, 12h and 24h post-calving. Samples were submitted to a diagnostic lab for bacterial culture (n = 26 pairs) and real-time PCR (rtPCR; n = 10 pairs) to isolate and identify bacteria as well as detect bacterial and viral nucleic acid. While we were unable to isolate bacteria of interest using culture, rtPCR yielded some success. At birth, *Histophilus somni* (*Hs*) was detected in 4/30 dam swabs and 1/20 calf swabs, *Mannheimia haemolytica* (*Mh*) in 1/30 dam swabs and 1/20 calf swabs, *Mycoplasma bovis* (*Mb*) in 4/30 dam swabs and 1/20 calf swabs, *Pasteurella multocida* (*Pm*) in 2/30 dam swabs, bovine herpes virus type-1 (BHV1) in 1/30 dam swabs, and bovine respiratory syncytial virus (BRSV) in 1/30 dam swabs. In 6h calf swabs, *Hs* was detected in 3/20, *Mb* in 1/20, and BRSV in 1/20. Associations of bacteria and viruses between dam and offspring remain unclear as establishment of the neonatal microbiome appears complex and more sensitive methods may be needed.

Key words: bacterial culture, beef, neonatal, rtPCR, viruses

Introduction

Bovine respiratory disease complex (BRDC) affects 96.9% of feedlots in the United States, costing the cattle industry upward of one billion dollars every year.[1] In 2015, the annual costs of BRDC in the U.S. cow-calf industry in preweaned calves were estimated to be $165 million.[2] Primary bacterial pathogens attributed to BRDC are *Mannheimia haemolytica* (*Mh*), *Pasteurella multocida* (*Pm*), *Histophilus somni* (*Hs*) and *Mycoplasma bovis* (*Mb*). Viruses associated with BRDC include bovine herpes virus type-1 (BHV1), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea virus (BVDV), bovine parainfluenza-3 virus (PI-3) and bovine coronavirus (BCV). *Mannheimia haemolytica* is considered to be the bacterial species of greatest economic importance in beef cattle. These primary bacteria associated with BRDC can be commensal in nature as they are present in nasal cavities of clinically healthy cattle.[3] However, current marketing systems for beef calves induce chronic stress, leading to immune dysfunction and the subsequent migration of bacteria to the lower respiratory tract, followed by bacterial proliferation and toxin secretion.[4] Sources of stress may include transportation, weaning, dehydration, changes of diet, changes in weather or commingling.[5-7,8,9] Many stressors are commonplace in both the feedlot and cow-calf sectors of the beef industry and are often unavoidable. Immune dysfunction can also negatively alter the structure of the microbial community in the upper respiratory tract (URT) as the abundance and diversity of bacteria is changed.[10] Differences in the microbial communities between cattle diagnosed with BRD and clinically healthy cattle have been observed with BRD-diagnosed cattle having greater abundance of *Proteobacteria* and *Firmicutes*, phyla for *Mh* and *Mb*.[7,10,11] There is a breadth of data focusing on causative bacterial agents related to BRDC in calves as young as 6 months (i.e., weaning), but little is known about bacterial characterization and colonization and virus presence in the URT in beef calves prior to that age.[10,12-14]

Characterizing bacterial colonization in the early stages of life may improve our understanding of BRDC and potentially allow for earlier intervention or development of disease prevention/mitigation strategies. Specifically, there is little information on the timing of URT bacterial colonization in beef calves. The objective of this study was to evaluate the presence of BRDC-associated bacteria and viruses in the URT of newborn calves within the first 24h of life and compare that to bacteria and viruses within the dam's URT and reproductive tract at parturition.

Materials and methods

Animal methods and procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee (21-020). Twenty-six Hereford-cross beef cows with an average body weight of 1,436 lb (651 kg) were transported 1,125 miles (1,810 km) from Mount Pleasant, Wis. to a ranch near Clayton, N.M. 3 weeks prior to the onset of calving. Cattle were purchased through a video auction market and received directly from the seller. Vaccination history of cows was unknown. Cows were selected for study enrollment based on estimated time of calving, length of calving period, and temperament. Upon arrival, cows were unloaded in a pen with

ad libitum access to wheat hay and water in a stock tank. The following day, pregnancy diagnosis via ultrasound and estimation of calving date were completed. One week later, cows were transported 24 miles (39 km) from the ranch to the Clayton Livestock Research Center in Clayton, N.M. Cows were placed in adjoining individual pens separated by galvanized fence panels measuring 20.5 × 7.5 ft (6.25 × 2.29 m). Each pen had access to an automatic drinker[a] and a 7.5 ft (2.29 m) feed bunk. The diet consisted of 44.88% dry matter (DM) wet corn gluten feed[b], 52.67% DM wheat hay, and 2.45% DM of a premix containing calcium carbonate, salt, a trace mineral mix and mineral oil fed once daily at 2% BW on a DM basis. Cows were observed hourly during daylight hours and at 2h intervals at night by trained personnel to monitor for signs of calving such as emergence of the chorioallantoic sac or the feet of the calf. Once feet or a water bag were observed, cows were monitored in 15-minute intervals until natural parturition was completed. All cows delivered their calves unassisted.

Immediately following parturition (0h), cows were briefly separated from their calves to facilitate sample collections (approximately less than 5 minutes). Calves remained in their birthing pen where samples were obtained from left and right anterior nasal cavities using ultrafine swabs[c] that were rotated against the mucosa 3 times then placed in liquid Amies transport medium. Calf noses were wiped with a clean paper towel prior to sampling if covered in debris or dirt from the pen and left nasal swab (NS) and right NS were kept separate throughout the study. Following initial sampling, calves were also individually identified with ear tags matching their dam's identification number. After parturition, cows were briefly moved to an indoor working facility for collection of samples including vaginal swabs (VS) and NS of the left and right anterior nasal cavities. The vulva was wiped using a clean paper towel prior to sampling. Vaginal samples were retrieved by opening the vulva with a gloved hand to limit swab contamination from the vulvar surface. Each dam was then reunited with her calf. Additional NS were collected from calves at 6h, 12h and 24h. All swabs were stored in a refrigerator at 4 °C/40 °F until shipment. Swabs were packaged in insulated kits along with ice packs and shipped via overnight parcel service to an American Association of Veterinary Laboratory Diagnosticians accredited laboratory (Nebraska Veterinary Diagnostic Center; NVDC) for bacterial culture, isolation and identification of BRDC-associated pathogens via MALDI-TOF mass spectrometry as described elsewhere.[15-17] Samples were shipped daily as they were collected. Samples collected on weekends were kept refrigerated and shipped the following Monday. Pairs were observed multiple times a day to monitor for any signs of disruption of bonding between cow and calf. Cow-calf pairs were transported back to pasture 1 day after completion of sampling at 24h.

Ten cow-calf pair sample sets were selected for real-time PCR (rtPCR) analysis to identify BRD-associated viruses and bacteria.[18] Sample sets utilized for rtPCR had the shortest timespan from sample collection to parcel delivery to the veterinary diagnostic center. BRDC-associated bacterial and viral pathogens evaluated by rtPCR were *Mh, Hs, Pm, Mb,* BVDV, BCV, BRSV and BHV1. Real-time PCR was performed using validated assays at NVDC. In brief, liquid transport media tubes were vortexed briefly and 300 uL of liquid was extracted using total RNA/DNA kits[d] in 96 well plates on a Kingfisher flex instrument[e] using the manufacturer's instructions for high volume liquid protocol 4462359HV. Extracted nucleic acid was utilized in rtPCR using an automated thermal cycler[f] as described by Loy et al. (2023).[16] Samples were considered detected if target signal crossed the threshold at 40 cycles or less.

Results

Bacterial culture

Complete sample sets were obtained for all cow-calf pairs except for missing the 24h NS of 1 calf due to sampling error. Bacterial culture did isolate what was identified as *Mannheimia spp.* Angen Cluster V from both LNS and RNS of 1 cow (Cow 28) at 0h. However, it was not isolated in any subsequent samples from that cow, nor was it isolated in any samples from her calf. It is important to note that this was not *Mh*. Additionally, we were unable to culture bacterial respiratory pathogens of interest from any of the remaining 26 pairs. Significant growth of other bacterial species, likely either environmental or non-pathogenic commensals, was observed.

Real-time PCR

Utilizing rtPCR, we were able to detect some BRDC-associated bacterial pathogens (Table 1) and viruses (Table 2) in the subset of 10 cow-calf pairs. Analysis revealed detectable levels of multiple pathogens in NS samples from Cow 28 such as *Hs* (RNS Ct = 35.29*), Mb* (LNS Ct = 35.23; RNS Ct = 38.75), and *Pm* (LNS Ct = 35.14; RNS Ct = 36.70), but *Mh* was not detected. In Calf 28, *Hs* was only detected in both LNS and RNS at 6h (Ct = 31.94 and 37.48, respectively). *Histophilus somni* was detected in a single RNS at 6h in Calf 11 (Ct = 36.4) and a single RNS of Calf 35 at 0h (Ct = 34.59). At 0h, *Hs* was detected in Cow 2 (LNS Ct = 30.53, VS Ct = 36.76) and Cow 7 (RNS Ct = 35.86) but was not found in either of their calves at any timepoint. *Mannheimia haemolytica* was detected in the VS of Cow 20 (Ct = 35.28) and 0h RNS of Calf 35 (Ct = 37.62). Inconsistent detection of *Mb* was observed in the VS, as well as both NS of Cow 13, and the LNS of Calf 13 at 0h and 6h (Ct = 27.56, 35.10, 35.40, 36.38, 37.15, respectively). However, at 12h, *Mb* was detected (Ct = 37.29) only in the RNS of Calf 13 and was not detected in subsequent samples. Viral detection via rtPCR was infrequent. Bovine herpes virus type-1 and BRSV were detected in the same swab from Cow 13 (LNS Ct = 37.84, 39.65, respectively). Bovine respiratory syncytial virus was also found in a calf RNS at 6h (Ct = 39.74; Calf 6). Bovine coronavirus and BVDV were not detected in any cow or calf samples at any timepoint. Table 3 summarizes rtPCR results by sample location.

Discussion

Bovine respiratory disease complex is a multi-faceted disease for which the complete pathogenesis is unknown. Recent studies regarding the respiratory microbial population of calves have focused on the URT or nasal cavity post-weaning or following diagnosis of BRDC using multiple techniques.[10,12-14,18-20] Based on these prior studies, we understand that the composition and beta diversity of the URT microbiome of beef calves prior to weaning is likely an ever-evolving system affected by sample timepoint, sample year, cohort animals, and animal location (pasture or ranch).[13,20] However, timing of initial colonization of BRDC-associated bacterial and viral pathogens in calves is unknown.[13] Previous studies have most commonly followed calves starting at 21 to 40 days of age through the preconditioning phase, feedlot entry, and even 40 days post-feedlot arrival.[10,12-14,20] Distinct changes in

Table 1: Bovine respiratory disease complex-related bacteria detected via rtPCR in swabs from 10 beef cows and their newborn calves during the first 24h of life. Bacteria evaluated included: *Histophilus somni* (*Hs*), *Mycoplasma bovis* (*Mb*), *Mannheimia haemolytica* (*Mh*) and *Pasteurella multocida* (*Pm*).

	Cow			Calf							
	0h			0h		6h		12h		24h	
Item	**LNS[a]**	**RNS**	**VS**	**LNS**	**RNS**	**LNS**	**RNS**	**LNS**	**RNS**	**LNS**	**RNS**
Pair 2	*Hs*	–	*Hs*	–	–	–	–	–	–	–	–
Pair 6	–	*Hs*	–	–	–	–	–	–	–	x	x
Pair 7	–	–	–	–	–	–	*Hs*	–	–	–	–
Pair 11	–	–	–	–	–	–	–	–	–	–	–
Pair 13	–	*Mb*	–	*Mb*	–	*Mb*	–	–	*Mb*	–	–
Pair 20	–	–	*Mh*	–	–	–	–	–	–	–	–
Pair 28	*Mb; Pm*	*Hs; Mb; Pm*	–	–	–	*Hs*	*Hs*	–	–	–	–
Pair 31	–	–	–	–	–	–	–	–	–	–	–
Pair 34	–	–	–	–	–	–	–	–	–	–	–
Pair 35	–	–	–	–	*Hs*; *Mh*	-	–	–	–	–	–

[a] Sample location: LNS = left nasal swab; RNS = right nasal swab; VS = vaginal swab

– = not detected

x = missed sample

Table 2: Bovine respiratory disease complex-related viruses detected via rtPCR in swabs from 10 beef cows and their newborn calves during the first 24h of life. Viruses evaluated included: bovine herpes virus type-1 (BHV1), bovine respiratory syncytial virus (BRSV), bovine coronavirus (BCV) and bovine viral diarrhea virus (BVDV).

	Cow			Calf							
	0h			0h		6h		12h		24h	
Item	**LNS[a]**	**RNS**	**VS**	**LNS**	**RNS**	**LNS**	**RNS**	**LNS**	**RNS**	**LNS**	**RNS**
Pair 2	–	–	–	–	–	–	–	–	–	–	–
Pair 6	–	–	–	–	–	–	BRSV	–	–	x	x
Pair 7	–	–	–	–	–	–	–	–	–	–	–
Pair 11	–	–	–	–	–	–	–	–	–	–	–
Pair 13	BHV1; BRSV	–	–	–	–	–	–	–	–	–	–
Pair 20	–	–	–	–	–	–	–	–	–	–	–
Pair 28	–	–	–	–	–	–	–	–	–	–	–
Pair 31	–	–	–	–	–	–	–	–	–	–	–
Pair 34	–	–	–	–	–	–	–	–	–	–	–
Pair 35	–	–	–	–	–	-	–	–	–	–	–

[a] Sample location: LNS = left nasal swab; RNS = right nasal swab; VS = vaginal swab

– = not detected

x = missed sample

Table 3: Summary of detection of bovine respiratory disease complex-associated bacteria and viruses via rtPCR in vaginal and left and right nasal swabs of 10 beef cows and their newborn calves within the first 24h of life. Bacteria and viruses evaluated included: *Histophilus somni* (*Hs*), *Mycoplasma bovis* (*Mb*), *Mannheimia haemolytica* (*Mh*), *Pasteurella multocida* (*Pm*), bovine herpes virus type-1 (BHV1), bovine respiratory syncytial virus (BRSV), bovine coronavirus (BCV) and bovine viral diarrhea virus BVDV.

	Bacteria of interest				Viruses of interest			
Item	**Hs**	**Mh**	**Mb**	**Pm**	**BHV1**	**BVDV**	**BCV**	**BRSV**
Cows								
Vaginal	10.0% (1/10)	10.0% (1/10)	10.0% (1/10)	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/10)
Nasal								
Left	10.0% (1/10)	0% (0/10)	20.0% (2/10)	10.0% (1/10)	10.0% (1/10)	0% (0/10)	0% (0/10)	10.00% (1/10)
Right	20.0% (2/10)	0% (0/10)	20.0% (2/10)	10.0% (1/10)	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/10)
Calves								
Nasal								
Left	2.6% (1/39)	0% (0/39)	5.1% (2/39)	0% (0/39)	0% (0/39)	0% (0/39)	0% (0/39)	0% (0/39)
Right	7.7% (3/39)	2.6% (1/39)	2.6% (1/39)	0% (0/39)	0% (0/39)	0% (0/39)	0% (0/39)	2.6% (1/39)

the microbiome communities have been noted as time progressed from initial samples to samples taken post-arrival at the feedlot.[12,14,20] In one study, abundance of *Mycoplasma spp.* was found to increase as time progressed with each sample point.[20] Another study evaluated the URT microbiome in dairy calves with the earliest sampling at 3 days of age, and it appeared that the microbiome was already established at this point in time.[19] Current literature on human newborn URT microbial niche development reveals an existing microbiome immediately post-partum with sampling ranging from within 5 minutes to 24h after birth.[21,22] Within 24 to 35h after birth, bacteria with maternal fecal and vaginal origins were detected in the URT of children born via vaginal delivery. Children born via cesarean section have had bacteria detected in the URT linked to the skin of their mother and, in some cases, bacteria of vaginal origin.[21,22] Additionally, the human microbiota reportedly changes the most within the first 2 months of life.[21] Therefore, it may be important to explore very early timepoints in a calf's life to determine when and from where bacterial transmission and microbiome establishment occurs.

There are 2 commonly used sampling strategies for the URT in calves. First is an anterior NS and the second is a deep nasopharyngeal swab (DNS). Sampling with an anterior NS is less invasive and more cost-effective. However, this type of swab is more susceptible to collection of contaminants, thus adding more difficulty to the interpretation of bacterial culture results. Long double-guarded swabs, used for cattle DNS sampling, are primarily used for mare uterine culture; therefore, they tend to be longer than what is necessary for use in calves.[23] The nasopharynx in cattle is approximately at the level of the eye, so this type of sampling is more invasive and more uncomfortable for the calf, as firm restraint, technical skill, and knowledge of anatomy are necessary. However, the double guard allows for the nasal cavity and rostral airways to be bypassed, lowering the potential of sample contamination.[24] There are similarities between the ability of anterior NS and DNS to capture bacteria such as *Mh, Pm* and *Mb* in dairy calves when compared to transtracheal wash as the gold standard.[25] A separate study also found NS and DNS to be similar in their agreement in detecting *M. haemolytica* in beef steers.[24] In this study, the nasal cavity was selected as a sampling location to facilitate quick sampling and limit calf discomfort thereby minimizing any interference with maternal bonding after birth. Refraining from or limiting newborn calf handling immediately following birth as to not disturb the mechanisms of maternal bonding has become a common practice in cattle husbandry. However, there is limited to no research to support the hypothesis that handling immediately following birth negatively impacts maternal bonding. A study found that the maternal bond could be disrupted when dams were separated from offspring for 3 days, after which, maternal responsiveness to her calf upon reuniting was decreased. Interestingly, dam separation and human handling of neonatal calves has been reported to reduce avoidance responses to humans.[26] Additionally, greater responsiveness was observed following separation in multiparous cows compared to primiparous heifers.[27] We did not observe any issues with maternal bonding following the brief separation (approximately less than 5 minutes) for sample collection in our study.

Overall, we were not successful in culturing the respiratory bacteria of interest at 0h, 6h, 12h and 24h following birth. In this study, the diagnostic lab reported large amounts of growth of environmental or non-pathogenic bacteria on the culture plates. Thus, we speculate that our ability to detect the respiratory disease-associated bacteria of interest was adversely affected by bacterial overgrowth. Unfortunately, we cannot differentiate whether this was due to the real-world delay of shipping the samples to the lab or due to the true

composition of the samples. Additionally, due to the inherent limitations of culture, we cannot distinguish if bacteria of interest were truly not present or if they were simply not captured on our swab.[28] Previous work has noted variability in detection of *Mh* and irregularities in isolation using a culture swab with the bacteria present on nasal mucosa.[29]

To address the known sensitivity and specificity issues with bacterial culture, we also performed rtPCR on 108 samples. Advantages of rtPCR compared to bacterial culture include a shorter turnaround time, higher specificity and sensitivity, better reproducibility, and reduced risk of carry-over contamination.[30,31] While rtPCR improved our detection of the pathogens of interest in our samples, there are several key limitations that complicate the interpretation of our results. Although rtPCR has higher specificity and sensitivity than culture plating, the assay does not distinguish between live and dead pathogens, has higher cost for utilization, and variation in assay performance can increase with cycle number and after transformation to linear values.[30,32] Additionally, there is a risk of false negative results due to variability in viral load in the specimen, which may approach the assay limits of detection.[32] It is also possible that bacterial and viral pathogens can be present, but not in concentrations that are consistently detectable via rtPCR. Some Ct values for this study indicated that we were near the limits of detection, which for many of these pathogens, is estimated at one to 10 colony forming units. While we were able to identify some bacterial and viral pathogens of interest via rtPCR, we did observe variability in detection between the left and right side of the anterior nasal cavity. In some cases, pathogens were detected in an LNS or RNS, but rarely in both. When evaluating agreement using culture, previous work has indicated that paired swab samples collected from the left and the right nasopharyngeal regions could yield different culture results.[33] Research is limited evaluating laterality between nasal cavities using rtPCR. Additionally, when looking at pathogen detection over time, we found there was considerable variability with some samples being positive at certain time points but not others, and in shifting anatomic locations at different time points. Similar variability was observed by Capik et al. (2015) in *Mh* culture status of beef calves on-arrival and over the next 3 days.[28] Other studies have also described inconsistencies in detection of bacteria using culture across sample methods, sample location and time points.[29,33,34] Differences in detection were discussed by Capik et al. (2017) where organisms were isolated via culture in nasopharyngeal swabs but were negative in bronchoalveolar lavage samples, indicating that presence of an organism in the URT does not guarantee its presence in the lower respiratory tract.[35] An alternative method for detection and characterization of pathogens would be 16S rRNA gene-based microarray, which is a culture-independent technique that has been successful in characterizing the nasopharyngeal microbiome of BRD-morbid and healthy feedlot cattle.[10]

One limitation of the study was that given the number of pens available, pairs had to share fences on one or 2 sides of pens, allowing for possible nose-to-nose contact with other cows or calves. Thus, neighboring animals may have served as potential outside sources of bacterial or viral pathogens. Additionally, although gloves were changed between sampling each individual, clothing was not, and cows were sampled in a working facility that was also utilized for other cattle in the feedlot. Further, multiple calves were born on some days and the timing of sampling sometimes overlapped. Therefore, while we did house each pair separately, there were opportunities for sharing of pathogens between animals during the study period. To maximize the likelihood viruses would be detected, the study also examined viral pathogens that are included in the BRDC virus diagnostic panel at NVDC, which includes the 4 most frequently detected viruses that NVDC identifies: BRSV, BVDV, BHV1 and BCV. Therefore, one limitation of this study is that samples were not tested for other viruses that may be less frequently encountered such as PI-3.

This study suggests that bacteria and pathogens of interest are present in the anterior nasal cavity of newborn calves within the initial 24h of life. In some cases, bacteria were detected immediately following parturition (0h). The source of bacteria and viruses could be the dam of the calf, or later in time, possibly the pen environment in which they were born. Maternal instinct after parturition of mother cows encourages licking and grooming her calf to stimulate the suckling reflex, nursing and rising to stand. In a production setting, multiple cows and calves could serve as a source of bacteria as they come to inspect a newborn calf. Because of these normal behaviors following calving, we chose to separate pairs into individual pens to limit contact with other cattle as well as for ease of sample collection. The settings in which conventional cow-calf and dairy operations manage calves are vastly different and thus, the establishment and composition of the URT microbiome may differ under other prodution systems. Beef calves are typically born and raised in pastures and are at their dam's side until weaning, while calves born in a dairy setting are managed differently from the time of birth. Depending on how quickly a calf is removed from its dam in a dairy setting, the opportunity for cows to lick their calf is limited or impossible. Dairy calves often do not have extensive contact with their dams following birth; therefore, the dam would not further influence bacteria and viruses present in the URT of their calves in the same way. Including additional sampling time points, such as 48 and 72h post-parturition, would be beneficial in ascertaining the development of neonatal URT bacteria and viruses. Further research regarding the sources(s) of microbial community establishment within a calf's respiratory tract is needed.

Conclusions

Bovine respiratory disease complex-related bacterial pathogens of interest were not identified through culture of the anterior nasal cavity of beef calves within the first 24h of life. Issues regarding shipping to the diagnostic lab, overgrowth of bacteria on culture plates, or failure to capture bacteria of interest on swabs could serve as potential reasons. Low levels of some bacterial and viral pathogens of interest were detected using rtPCR in different samples over time, but with no clear pattern or association within cow-calf pairs. Based on our findings, we are unable to determine whether there is an association between the bacteria or viruses present in the dam's URT and vagina soon after parturition and those found in her offspring within the first 24h of life. Further research is warranted to gain a better understanding of the respiratory microbiome of neonatal beef calves. A more detailed and holistic methodology such as 16S may be beneficial to explore whether

URT colonization of different types of bacteria occurs within the first 24 hours of life.

Endnotes

[a] Watermatic 150, Ritchie Industries Inc., Conrad, IA

[b] Sweet bran, Cargill, Dalhart, TX

[c] Opti-Swab, Puritan, Guiford, ME

[d] MagMAX™ Pathogen RNA/DNA Isolation Kit, ThermoFisher Scientific, Waltham, MA

[e] KingFisher™ Flex Purification System, ThermoFisher Scientific, Waltham, MA

[f] Rotor-Gene Q Thermocycler, Qiagen, Hilden, Germany

Acknowledgements

The authors would like to thank Dr. Dustin Loy and his team members at the NVDC for analyzing samples.

Funding

This work was supported by internal Texas A&M University System funding and, in part, by Hatch (accession number 7006739), from the U.S. Department of Agriculture's National Institute of Food and Agriculture. Cattle use was provided by Sowers Cattle Company.

Conflicts of interest

The authors declare no conflict of interest related to the research reported in this manuscript.

Author contributions

CAS, SFC, JTR, and GCD were involved in the conception and design of the study. MMS and CAS were involved in data acquisition. JDL was involved in analysis of samples. CAS and SFC analyzed the data and drafted the manuscript. CAS, SFC, JDL, JTR, GCD, MMS all edited the manuscript and approved the final version.

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