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Comparison of short nasal swab and deep nasopharyngeal swab sampling methods to describe BRD-associated viruses and bacteria detected using a metagenomics approach optimized for virus recovery in fall-placed beef calves shortly after feedlot arrival

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ABSTRACT

Short nasal swabs (SNS) have potential advantages of lower costs, collection time and training of personnel than deep nasopharyngeal swabs (DNPS) for detecting bovine respiratory disease (BRD) pathogens. This study examined differences between DNPS and SNS in BRD-associated pathogens detected using a nanopore metagenomic sequencing protocol, optimized for respiratory RNA viruses, collected from 150 calves in six feedlot pens. Short nasal swabs yielded higher viral read counts and prevalence than DNPS for BCoV (mean reads 75 versus 17; OR = 21.4, $P = 0.001$) and IDV (mean reads: 560 versus 192; OR = 2.60, $P = 0.02$). Agreement varied among viruses: IDV (κ=0.57), BRSV (κ=0.43), and BCoV at both ≥1 read (κ=0.35) and ≥30 reads (κ=0.10). No BoHV-1 and BAdV3 were detected. *Mannheimia haemolytica* was detected (≥14 reads) more frequently in SNS than DNPS (mean reads: 169 versus 57; OR = 5.73, $P = 0.001$), as was *Pasteurella multocida* (≥ 1 read) (mean reads: 4.0 versus 1.2; OR = 2.02, $P = 0.02$). *Mesomycoplasma dispar* was less prevalent in SNS (mean reads: 5.2 versus 29; OR = 0.27, $P = 0.001$). Detection of *Histophilus somni, Bibersteinia trehalosi*, and *Mycoplasmopsis bovis* did not differ between swab types. Agreement for detection of *M. haemolytica* (≥14 reads) was moderate (κ = 0.46, $P = 0.001$). For all other bacteria examined in this analysis, kappa values were very low. Short nasal swabs were a sensitive and practical alternative for BRD pathogen surveillance providing evidence of which viruses and bacteria are circulating, potentially informing vaccination and disease management.

1. Introduction

Bovine respiratory disease (BRD) is caused by a complex interplay of viral and bacterial pathogens, including *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, Mycoplasmopsis bovis,* bovine herpesvirus-1, bovine respiratory syncytial virus, bovine viral diarrhea virus, and bovine parainfluenza-3 virus (Chai et al., 2022a; Pardon & Buczinski, 2020). Traditional diagnostic methods such as culture and PCR are limited in scope and sensitivity and can fail to detect coinfections or pathogens not routinely targeted by the laboratory (Chen et al., 2022; Fulton & Confer, 2012). In contrast, metagenomic sequencing offers a culture-independent approach for a comprehensive profile of microbial communities in reduced time (Ciuffreda et al., 2021). The potential to detect both respiratory bacteria and viruses of interest with a single metagenomic protocol has been described previously (Donbraye et al., 2025).

Sampling technique can significantly impact respiratory pathogen detection (Chen et al., 2022). Different approaches like bronchoalveolar lavage fluid (BALF), tracheal washes, and nasopharyngeal swabs yield varying microbial profiles and concentrations, affecting pathogen identification sensitivity (Chai et al., 2022b; Timsit et al., 2018; Zhang et al., 2019). Deep nasopharyngeal swabs are regarded as the benchmark for sampling the upper respiratory tract because they can access the nasopharynx, a common site for the colonization of many commensals and BRD pathogens (Doyle et al., 2017; Godinho et al., 2007). However, the process of collecting deep nasopharyngeal swabs (DNPS)

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is sometimes considered invasive and requires good animal restraint and trained personnel, which can be a concern for use in large-scale surveillance projects or frequent sampling (Doyle et al., 2017). Short nasal swabs (SNS) offer a less invasive and more practical alternative, particularly in commercial feedlot settings (Chai et al., 2022a). While they provide practical advantages for sample collection, the diagnostic efficacy of SNS, especially in terms of different pathogen read counts and the microbial diversity revealed through metagenomics, has not been thoroughly explored (Crosby et al., 2022).

With growing interest in the use of metagenomics for monitoring BRD pathogens (Klima et al., 2019; Mitra et al., 2016; Ng et al., 2015; Zhang et al., 2021; Zhang et al., 2019), an assessment of whether SNS might be a reasonable alternative to DNPS for identifying respiratory pathogens in feedlot calves could potentially make field sample collection more practical and increase opportunities to obtain data for surveillance. In the present study frozen paired samples were available for testing, using an established protocol for the metagenomic detection of bovine respiratory viruses. The objectives of this study were to compare the counts of respiratory virus and bacterial reads, as well as the detection of respiratory viruses and bacteria of interest, between SNS and DNPS from a single test on each sample using an existing metagenomics protocol developed for viral detection. The results of this study will contribute to the optimization of BRD diagnostics and the enhancement of respiratory pathogen surveillance within the beef industry.

2. Materials and methods

The study research protocols and procedures were approved by the University of Saskatchewan Animal Care Committee (AUP 20190069) and adhered to the Canadian Council on Animal Care guidelines for humane animal use (CCAC, 1993).

2.1. Study population and sampling procedure

The study population and sample collection have been previously described in detail (Abi Younes et al., 2024). Recently weaned mixed-breed steer calves were purchased at a local auction market and shipped approximately 50 km to the Livestock and Forage Centre of Excellence (LFCE) research feedlot near Clavet, Saskatchewan. One group of 100 calves was purchased every week for eight weeks, from October to December in 2021. After arrival at the LFCE, these calves were held overnight in a pen and processed the following morning. All animals (n = 800) were processed at 1 day on feed (DOF) following a protocol typical for moderate- to high-risk calves in commercial feedlots that included the placement of a feedlot identification ear tag, verification of castration, and subcutaneous administration of a *M. haemolytica* and a modified live viral vaccine (Pyramid® 5 + Presponse®, Boehringer Ingelheim Animal Health, Burlington, ON, Canada) and a multivalent clostridial vaccine (Ultrachoice® 8, Zoetis Inc., Kirkland, QC, Canada). All calves also received a growth implant (Ralgro®, Merck Animal Health, Kirkland, QC, Canada) and a topical anthelmintic (Solmectin™, Solvet, Calgary, AB, Canada). Half of the cohort (n = 400) received metaphylactic injectable oxytetracycline (Oxyvet®200 LA, Vetoquinol, Lavaltrie, QC, Canada; 20 mg/kg) at arrival processing and the other half (n = 400) received metaphylactic tulathromycin (Draxxin®, Zoetis Inc., Kirkland, QC, Canada; 2.5 mg/kg). Following processing, each group of calves (n=100) were moved to an assigned outdoor dirt floor pen where they remained as a single group for the length of the study.

Each group (n=100) of calves was sampled at arrival processing (1 DOF) and again 12 days later (13 DOF). Samples were collected after calves were restrained in a hydraulic chute with a neck extender to stabilize their head. First, the external nares were wiped clean with a single-use paper towel. A 15 cm, nylon tipped SNS (COPAN Diagnostics, Inc., Murrieta, CA, USA) was then introduced into a nostril to its full length and vigorously rotated 360 degrees against the mucosa for at least 6 s. The short swab was withdrawn and placed into a 1 ml vial of Amies transport medium. Next, a 74.9 cm double-guarded culture swab (DNPS) (Continental Plastic Corp., Delavan, Wisconsin, USA) was directed approximately 20 cm into the ventral meatus of the same nostril. The polyester-tipped swab was advanced through the inner sheath and outer guard and vigorously rotated against the pharyngeal mucosa for 5-6 rotations. The swab was withdrawn into the inner sheath and outer guard prior to removal from the nostril to avoid contamination, and approximately 3 cm of swab tip was cut and placed in a vial containing 1 ml Amies transport medium. Sample collectors changed to new disposable nitrile gloves between each calf. The vials were stored in a polystyrene cooler containing ice packs and immediately transported to the study laboratory at the Western College of Veterinary Medicine (WCVM), University of Saskatchewan, Saskatoon. Swabs and Amies media were transferred into individual 2 ml screw-capped cryovial tubes containing 20 μL bovine calf serum and stored at -80°C until processing.

2.2. Sample selection and preparation for metagenomic sequencing

The samples used in this study were a convenience sample of 150 matching pairs of SNS and DNPS, collected at 13 DOF from the larger project (Abi Younes et al., 2024). The 13 DOF samples were purposively selected given the higher expected prevalence and diversity of detectable targets of interest at 13 DOF as compared to arrival (Abi Younes et al., 2024; Donbraye et al., 2025). Frozen samples were matched as SNS and DNPS pairs prior to analysis and processed together. The chosen samples represented paired SNS and DNPS from 25 calves available per pen of 100 from 4 pens injected with tulathromycin at arrival (100 matched pairs total) and 2 pens injected with oxytetracycline at arrival (50 matched pairs total). No calves included in this analysis were treated for BRD at the time of sampling.

The details of the nucleic acid extraction and amplification protocol were previously described (Donbraye et al., 2025). The samples were thawed in their tubes in a room-temperature water bath and stored on ice during processing. Samples were processed in batches of 30 (15 DNPS and 15 matching SNS) and each batch included an extraction negative control (molecular biology grade water).

As previously described for reverse transcription using SuperScript IV First-Strand Synthesis kit (Applied Biosystems, Waltham, MA, USA) (Donbraye et al., 2025a), 10 uL of extracted nucleic acids from each sample were annealed with 3.5μl of a random primer mastermix (1 μM FR26RV-N primer (5′-GCC GGA GCT CTG CAG ATA TCN NNN NN-3′), 0.5 mM dNTPs, 0.42 μl of DEPC-treated water) and incubated at 65°C for 5 min to anneal the random primers to the template (Allander et al., 2005). Each sample was placed on ice for 1 min to stabilize the nucleic acid-primer complex and then combined with 7 μL of reverse transcription master mix (4 μL Superscript IV 5 × buffer, 1 μL DTT, 1 μL RNase inhibitor, and 1 μL RTase; Invitrogen, Waltham, MA, USA) and incubated at 23°C, 55°C, and 80°C for 10 min at each temperature according to manufacturer's instructions. After incubation, samples were placed on ice for 1 min. To remove the RNA, 1 μL of *E. coli* RNase H (5 U/μl) was added to each reaction and incubated at 37°C for 20 min.

Second-strand synthesis was performed using Sequenase DNA polymerase (Applied Biosystems, Waltham, MA, USA) according to manufacturer's instructions. Samples were cooled to room temperature for 10 min and then combined with 10 μL of Sequenase mastermix (2 μL 5 × Sequenase buffer, 0.3 μL Sequenase enzyme, 7.7 μL RNase-free water; Applied Biosystems, Waltham, MA, USA) and then incubated in a ramped fashion: slow ramp from 10°C to 37°C over 8 min, 37°C for 8 min, 94°C for 2 min, and finally 10°C for 5 min. The reaction was then topped up with an additional 0.3 μL of Sequenase enzyme and 0.9 μL of dilution buffer and incubated under the same reaction conditions.

The resulting samples and extraction negative controls were cleaned-up and the cDNA was size-selected with a ratio of 1:1 AMPure XP bead suspension by volume (Beckman Coulter, Brea, CA, USA) according to

the manufacturer's instructions, with a final elution volume of 10 μL. Eight microlitres of size-selected cDNA was combined with 42 μl of amplification mastermix (1 × Standard Buffer, 100 μM dNTP, 1.5 mM $MgCl_2$, 0.5 μM FR20RV primer (Allander et al., 2005), 0.25 μl polymerase; NEB, Ipswich, MA, USA) and then cycled with the following reaction conditions: denaturation for ten min at 94°C, followed by 40 cycles of 94°C for 1 min, 65°C for 1 min, and 72°C for 3 min, followed by a final extension of 5 min at 72°C.

2.3. Nanopore library preparation and sequencing

The sequencing methodology described here is similar to that previously reported (Donbraye et al., 2025). Amplified cDNA from each sample was purified and size selected with 0.4 × AMPure XP beads (Beckman Coulter, Brea, CA, USA) and normalized to 50 ng total DNA before library preparation. Library preparation was done according to the Oxford Nanopore Technology protocol, "Ligation sequencing gDNA - Native Barcoding kit 96 V14" (SQK-NBD114.96, Oxford Nanopore Technologies, Oxford, UK) in a 96-well plate high-throughput library format with minor modifications to minimize the potential for any misclassification across barcoded samples or background barcode cross-talk (Jurasz et al., 2021; Wick et al., 2018).

Barcode ligation was followed by adding 1 μl of EDTA (Invitrogen, Waltham, MA, USA), a 10 min room temperature incubation and a 10 min 65°C incubation. Barcoded DNA samples were pooled together in groups of 30 samples including 15 matching pairs of SNS and DNPS with two additional water (negative) controls per library to detect and account for any potential contamination or barcode cross-talk that might occur during the library preparation and sequencing process (Sui et al., 2020). Extraction negative controls were submitted as a group on a separate flow cell with additional water controls.

Following library preparation, the samples and water controls were submitted as a group of 32 (15+15+2) for sequencing on the same flow cell. Twenty (20) ng of each prepared library was sequenced, under contract, by the Omics and Precision Agriculture Laboratory (OPAL) (Saskatoon, SK, Canada) using the PromethION24 platform. The ONT R10.4.1 flow cells (FLO-PRO114M, Oxford Nanopore Technologies, Oxford, UK) were loaded according to the manufacturer's recommendations. Sequencing was performed for 48 hours with default run parameters using high-accuracy real-time basecalling with a Q-score cutoff of 9.

2.4. Bioinformatics analysis

The bioinformatics methodology described here is similar to that previously reported (Donbraye et al., 2025a). Data from the fastq_pass folder from MinKNOW was processed with Porechop v0.2.4 (Wick et al., 2017) to remove primers or adapters (using default parameters). NanoFilt (version 2.8.0) was used to discard reads shorter than 200 bp and NanoStat (version 1.6.0) provided statistics about the distribution of read length by total base pairs per sample (De Coster et al., 2018).

The taxonomic classification of reads was achieved by using Kraken 2 (version 2.1.3) with a confidence score threshold of 0.05 ("–confidence 0.05") (Wood et al., 2019). A custom database was used for Kraken 2 classification which included bacterial, viral, and archaeal subsets of the November 2023 RefSeq database (Goldfarb et al., 2025), as well as the *Bos taurus* ARS-UCD1.2_Btau5.0.1Y genome assembly from GenBank, which consists of the ARS-UCD1.2 genome assembly (Rosen et al., 2020) and the Y chromosome sequence from Btau5.0.1 (Elsik et al., 2009). Typically, sequences classified as host would be removed before downstream processing; however, we detected a small population of chimeric *B. taurus*-bacterial reads (<0.1% of all reads). A custom program, kmer_filter.py, was written to retrieve host-classified reads that met a threshold of 25% non-host sequence using Kraken 2 k-mer identity and include these reads for downstream processing. Host-filtered reads (i.e., those not assigned to the *B. taurus* taxid 9913) were extracted using the KrakenTools v1.2 (Lu et al., 2022) utility extract_kraken_reads.py, and these were added to the chimeric reads using a combination of bash utilities and the BBTools v38.86 (Bushnell, 2020) filterbyname.sh script. Bracken v2.7 (Lu et al., 2017) with a minimum read length of 200 bp ("–read-length 200") was used to improve the species-level estimation of abundance reported by Kraken 2. Reads that were classified as host were removed from further consideration. A custom script, report_taxon_read_lengths.py, added additional context to the Bracken results, including the total amount of sequence in base pairs reported for each species (including child taxa) and the fraction of total classified sequence. Further details and all custom scripts can be found at https://github.com/coadunate/ASSETS_2.

For all viral and bacterial taxonomy data and associated statistical analyses in this paper, the read counts for each virus and bacteria of interest were adjusted for the corresponding read counts in the water controls. The average read count for each virus and bacteria in the water controls for each flow cell was subtracted from the observed read counts for each sample to account for any potential misclassification across barcoded samples or barcode cross-talk (Jurasz et al., 2021; Wick et al., 2018).

2.5. Statistical analysis

Initial descriptive statistics were reported for the sequencing data. Subsequent analyses were limited to 10 viruses that met the following inclusion criteria: five BRD viruses for which there are commonly used commercial respiratory vaccines (bovine respiratory syncytial virus (BRSV), bovine herpes virus 1 (BoHV-1), bovine viral diarrhea virus 1 (BVDV-1) and bovine viral diarrhea virus 2 (BVDV-2), bovine parainfluenza virus 3 (BPIV-3)) in North America (Fulton, 2020) as well as five viruses reported to be associated with BRD: influenza D virus (IDV), bovine coronavirus (BCoV), bovine rhinitis B virus (BRBV), bovine adenovirus 3 (BAdV3), and ungulate copiparvovirus (UCPV-1) (Mitra et al., 2016; Ng et al., 2015; Saegerman et al., 2022; Vlasova & Saif, 2021; Zhang et al., 2019).

The read count used as a cutoff (detection threshold) to identify samples positive for BoHV-1, BCoV, BPIV-3, IDV and BRSV was previously determined using Bayesian latent class modelling (BLCM), referencing qPCR results to optimize both sensitivity and specificity, with a minimum threshold of 0.90 for metagenomic specificity (Donbraye et al., 2026). The BLCMs (Donbraye et al., 2026) used JAGS software v4.3.0 (Plummer, 2003) and the runjags package v2.2.0-2 (Denwood, 2016) in R v4.1.0 (R Foundation for Statistical Computing, Vienna, Austria). Samples were classified as positive for BRD-associated viruses based on ≥1 read (BoHV-1, BRSV, IDV) except for BPIV-3 (≥5 reads) and BCoV (≥30 reads). For viruses for which there was no evidence-based read count cutoff available (BVDV-1, BVDV-2, BRBV, BAdV3, UCPV-1) samples were classified as positive based on the detection of ≥1 read per sample.

Similar read count cutoffs (detection thresholds) to identify samples positive for targeted BRD-associated bacteria identified with the same protocol had also previously been determined using BLCMs, referencing culture data for *M. haemolytica, P. multocida* and *H. somni*, or PCR in the case of *M. bovis*, from matching samples (Donbraye et al., 2026). The number of reads to consider a sample as positive was ≥14 reads for the detection of *M. haemolytica,* ≥9 reads for detection of *P. multocida,* ≥8 reads for *H. somni*, and ≥1 read for *M. bovis* (Donbraye et al., 2026). For bacteria for which there was no evidence-based read count cutoff available (*B. trehalosi* and*M. dispar*) the detection of ≥1 read per sample was used to classify samples as positive.

Differences in read counts and detection of target organisms between SNS and DNPS were estimated using generalized linear mixed models (GLMM) with a fixed effect for swab type and a random effect for each flow cell (Stata/SE 18.0 for Windows, StataCorp LLC, College Station, TX, USA). Negative binomial distributions with log link functions were used to analyze read counts and binomial distributions with logit link

functions were used for analyzing the presence and absence of viruses and bacteria. Back transformed means and differences were reported as relative differences or odds ratios with 95% CI. Results were considered significant when $P \leq 0.05$. Exact Poisson regression was used in the two instances where cell counts were too infrequent for GLMM.

A sensitivity analysis was completed to evaluate whether the potential for fecal contamination could account for the differential presence of some viruses that are also found in bovine feces in SNS and DNPS. The number of *E. coli* reads was introduced into the negative binomial models for the viruses as an indicator of fecal contamination as a potential confounder. Given the potential for a non-linear association between *E. coli* and the log of the virus count, a quadratic term was also added to the models and retained where it was significant. The *E. coli* adjusted virus counts and relative differences between SNS and DNPS were also reported to facilitate discussion of the importance of fecal contamination.

Kappa was calculated to estimate agreement above the agreement expected by chance alone in the detection of the primary organisms of interest (Stata/SE 18.0 for Windows, StataCorp LLC, College Station, TX, USA).

3. Results

3.1. Sample population

Matched pairs of SNS and DNPS were analyzed from 150 fall-placed calves (25 calves per pen from 6 different feedlot pens) sampled in October and November 2021 at 13 DOF.

3.2. Summary of metagenomic data

After demultiplexing, trimming, and quality filtering with Porechop, 62.3 million reads (26.2 DNPS, 36.1 SNS) were generated. The average quality score per sample ranged from 12.3 to 14.0 for DNPS (mean across DNPS = 13.3) and 12.6 to 14.7 for SNS (mean across SNS 13.4). The average read length per DNPS sample varied from 385 to 914 bp (mean across samples = 636 bp) and for SNS varied from 360 to 926 bp (mean across samples = 588 bp).

Following the removal of host-related sequences, which made up 72% (18.8 million reads) of total DNPS reads and 66% (23.6 million reads) of total SNS reads, 19.8 million DNPS reads and 12.4 million SNS reads remained. Of the total reads, 0.05% (13,107 DNPS reads) and 0.14% (51,968 SNS reads) were classified as viral and 1.1% (288,918 DNPS reads) and 1.9% (691,278 SNS reads) were classified as bacterial. Also from the total reads, 0.003% (662 DNPS reads) and 0.003% (1,205 SNS reads) were classified as archaeal, while 26% (6.9 million DNPS reads) and 31% (11.3 million SNS reads) were unclassified or classified ambiguously.

3.3. Detection of BRD-associated viruses in short nasal swabs and deep nasopharyngeal swab samples from fall-placed calves

After adjusting read counts for the means of the water controls, 44,076 reads from the 150 SNS and 6,627 reads from the 150 DNPS were associated with the ten respiratory viruses of interest. The total number of reads classified for each virus ranged from 13 (BRSV) to 23,836 reads (BCoV) from SNS and 1 read (BVDV-2, BPIV-3) to 6,058 reads (IDV) from DNPS. For the SNS, median read lengths ranged from 491 bp (UCPV-1) to 824 bp (BCoV). For the DNPS, median read length ranged from 642 bp (BRBV) to 1013 bp (BVDV-2).

From the SNS, there were no BVDV-1, BVDV-2, BoHV-1, or BAdV3 reads detected, while from the DNPS, there were no BoHV-1 or BAdV3 reads detected (Table 1).

The mean number of reads detected in SNS was higher than in DNPS for BCoV, IDV, BPIV-3, and BRBV, whether or not the extent of fecal contamination, as measured by *E. coli* count, was accounted for in the model (Table 1). However, the *E. coli* count was a significant predictor for both BCoV and BRBV in these models and the difference between SNS and DNPS decreased by half after accounting for *E. coli*. The mean *E. coli* read count for the SNS samples (206, 95% CI 27, 386) was 2.34 times (95% CI 1.37, 3.98) higher than the DNPS samples (88, 95% CI 11, 166). The mean number of UCPV-1 reads displayed a different pattern, and was significantly lower in SNS than DNPS, whether or not the extent of fecal contamination was accounted for in the model (Table 1).

Similarly, the likelihood of detecting BCoV (≥30 reads and ≥1 read), IDV, BPIV-3 (≥1 read), and BRBV was higher from SNS than DNPS (Table 2). The proportion of SNS samples where UCPV-1 was detected was significantly lower than DNPS samples.

There was a high level of observed absolute agreement in detection of viruses between DNPS and SNS (Table 3), particularly BRSV (97%), BVDV-1 and BVDV-2 (both 99%), and BPIV-3 (100% at ≥5 reads). However, the kappa values were 0 for four viruses (BPIV-3 (≥5 reads and ≥1 read), BRBV, BVDV-1, BVDV-2), suggesting no agreement above that expected by chance. Moderate agreement beyond chance was observed for IDV (κ=0.57) and BRSV (κ=0.43). Fair agreement for BCoV was based on a cutoff of ≥1 read (κ=0.35), with only slight agreement

Table 1
Differences in counts of reads for BRD-associated viruses of interest identified by metagenomic sequencing in short nasal and deep nasopharyngeal swab samples collected from fall-placed calves at 13 days on feed.

Virus	Short nasal swabs (*n*=150)		Deep nasopharyngeal swabs (*n*=150)		Difference		
	Mean no. of reads	95% CI	Mean no. of reads	95% CI	Relative difference	95% CI	*P*-value
BCoV	75	0, 306	17	0, 67	4.57	2.69, 7.77	**0.001**
IDV	560	0, 2025	192	0, 694	2.94	2.00, 4.25	**0.001**
BRSV	0.88	0, 6.9	0.45	0, 3.3	1.96	0.26, 14.8	0.52
BPIV-3	0.17	0, 0.38	0.01	0, 0.02	22.2	2.72, 181	**0.004**
BoHV-1	*No reads detected*				*Not estimated*		—
BRBV	3.4	0, 20	0.44	0, 2.7	7.60	3.36, 17.2	**0.001**
UCPV-1	0.40	0, 1.5	2.7	0, 11	0.14	0.04, 0.48	**0.002**
BVDV-1	0 reads		1 read & 2 reads		1.35	0.41, ∞	0.25*
BVDV-2	0 reads		1 read		0	0.026, ∞	0.99*
BAdV3	*No reads detected*				*Not estimated*		—
	Adjusted for *E. coli* read count ***						
BCoV**	42	0, 157	18	0, 67	2.18	1.24, 3.82	**0.007**
IDV	409	0, 1424	170	0, 589	2.41	1.65, 3.52	**0.001**
BRSV	0.15	0, 0.50	0.07	0, 0.22	2.09	0.98, 1.09	0.18
BPIV-3	0.15	0, 0.34	0.01	0, 0.02	20.6	2.38, 179	**0.006**
BRBV**	1.6	0, 7.8	0.42	0, 2.1	3.73	1.70, 8.20	**0.001**
UCPV-1	0.27	0, 1.0	2.5	0, 10	0.11	0.03, 0.40	**0.001**

Abbreviations: BCoV: bovine coronavirus; BAdV3: bovine adenovirus 3; BoHV-1: bovine herpes virus 1; BPIV-3: bovine parainfluenza virus 3; BRBV: bovine rhinitis B virus; BRSV: bovine respiratory syncytial virus; BVDV-1: bovine viral diarrhea virus 1; BVDV-2: bovine viral diarrhea virus 2; IDV: influenza D virus; UCPV-1: ungulate copiparvovirus 1; No.: number; CI: confidence interval. * Exact Poisson regression used to generate estimates. ** *E. coli* count and associated quadratic term were significant predictors of the virus count in these models. ***E. coli* read count included in model estimating difference between swab types.

Table 2
Differences in detection of BRD-associated viruses in short nasal and deep nasopharyngeal swab samples collected from fall-placed calves at 13 days on feed.

	Short nasal swabs (n=150)		Deep nasopharyngeal swabs (n=150)		Difference		
Virus	No. pos. samples	Prevalence	No. pos. samples	Prevalence	Odds ratio	95% CI	*P*-value
	Cut-offs informed by BLCM						
BCoV (≥30 reads)	28	19%	3	2.0%	21.4	5.58, 81.7	**0.001**
IDV (≥1 read)	107	71%	94	63%	2.60	1.16, 5.85	**0.02**
BRSV (≥1 read)	6	4.0%	3	2.0%	3.91	0.40, 37.8	0.24
BPIV-3 (≥5 reads)	0	0%	0	0%	*Not estimated*		—
BoHV-1 (≥1 read)	0	0%	0	0%	*Not estimated*		—
	Cut-offs not informed by BLCM						
BRBV*	32	21%	11	7.3%	4.27	1.94, 9.42	**0.001**
UCPV-1*	9	6.0%	23	15%	0.29	0.12, 0.74	**0.009**
BVDV-1*	0	0%	2	1.3%	0.41	0, 5.32	0.50
BVDV-2*	0	0%	1	0.7%	1.00	0, 39	0.99
BAdV3*	0	0%	0	0%	*Not estimated*		—
	Sensitivity analysis						
BCoV (≥1 read)*	77	51%	56	37%	2.51	1.35, 4.68	**0.004**
BPIV-3 (≥1 read)*	14	9.3%	1	0.6%	16.0	2.06, 125	**0.008**

The cut-offs used to determine virus-positive fall-placed calves were based on ≥1 read for all viruses except BPIV-3 (≥5 reads) and BCoV (≥30 reads) for the primary analysis. * Cut-offs not informed by BLCM (≥1 read).
Abbreviations: BCoV: bovine coronavirus; BAdV3: bovine adenovirus 3; BoHV-1: bovine herpes virus 1; BPIV-3: bovine parainfluenza virus 3; BRBV: bovine rhinitis B virus; BRSV: bovine respiratory syncytial virus; BVDV-1: bovine viral diarrhea virus 1; BVDV-2: bovine viral diarrhea virus 2; IDV: influenza D virus; UCPV-1: ungulate copiparvovirus 1; No.: number; Pos.: positive; CI: confidence interval; BLCM: Bayesian latent class models.

Table 3
Agreement in detection of BRD-associated viruses in short nasal and deep nasopharyngeal swab samples collected from fall-placed calves at 13 days on feed (*n*=150 pairs).

Virus	Observed agreement	Expected agreement	Kappa	Standard error	*P*-value
	Cut-offs informed by BLCM				
BCoV (≥30 reads)	82%	80%	0.10	0.04	**0.02**
IDV (≥1 read)	81%	55%	**0.57**	0.08	**0.001**
BRSV (≥1 read)	97%	94%	**0.43**	0.08	**0.001**
BPIV-3 (≥5 reads)	100%	100%	0.0	—	—
BoHV-1 (≥1 read)	*No reads detected*				
	Cut-offs not informed by BLCM				
BRBV*	74%	75%	0.0	0.07	0.60
UCPV-1*	83%	81%	0.11	0.07	0.06
BVDV-1*	99%	99%	0.0	0.00	0.50
BVDV-2*	99%	99%	0.0	—	—
BAdV3*	*No reads detected*				
	Sensitivity analysis				
BCoV (≥1 read)*	67%	50%	**0.35**	0.08	**0.001**
BPIV-3 (≥1 read)*	90%	90%	0.0	0.04	0.63

Interpretation of κ as described by Dohoo et al. (2014): κ ≤ 0.00 – poor, 0.01-0.20 – slight, 0.21-0.40 – fair, 0.41-0.60 – moderate, 0.61-0.80 – substantial, 0.81-1.00 – near perfect.
The cut-offs used to determine virus-positive fall-placed calves were based on ≥1 read for all viruses except BPIV-3 (≥5 reads) and BCoV (≥30 reads) for the primary analysis. *Cutoffs not informed by BLCM (≥1 read).
Abbreviations: BCoV: bovine coronavirus; BAdV3: bovine adenovirus 3; BoHV-1: bovine herpes virus 1; BPIV-3: bovine parainfluenza virus 3; BRBV: bovine rhinitis B virus; BRSV: bovine respiratory syncytial virus; BVDV-1: bovine viral diarrhea virus 1; BVDV-2: bovine viral diarrhea virus 2; IDV: influenza D virus; UCPV-1: ungulate copiparvovirus 1; BLCM: Bayesian latent class models.

reported at ≥30 reads (κ=0.10). Overall, the strength of agreement above that expected by chance varied, with only a few viruses showing meaningful concordance between sampling methods.

3.4. Detection of BRD-associated bacteria by viral metagenomic protocol in short nasal swabs and deep nasopharyngeal swab samples collected from fall-placed calves at 13 days on feed

After adjusting read counts for the means of the water controls, 16,393 reads from the 150 SNS and 8,687 reads from the 150 DNPS were associated with six respiratory bacteria of interest including *M. haemolytica, P. multocida, H. somni, B. trehalosi, M. bovis*, and *M. dispar*. The total number of reads classified for each bacterium ranged from 3 reads (*B. trehalosi*) to 14,616 reads (*M. haemolytica*) from SNS and 7 reads (*B. trehalosi*) to 4741 reads (*M. dispar*) from DNPS. For the DNPS, median read length per swab ranged from 227 bp (*H. somni*) to 908 bp (*M. haemolytica*). For SNS, median read lengths per swab ranged from 219 bp (*H. somni*) to 845 bp (*M. haemolytica*).

The mean number of *M. haemolytica* reads were significantly higher in SNS samples than DNPS samples regardless of whether the analysis was adjusted for *E. coli* (Table 4; $P = 0.003$). While the number of *P. multocida* reads was higher for SNS samples than for DNPS samples in the initial analysis ($P = 0.02$), it was not when the analysis was adjusted for the *E. coli* read count ($P = 0.62$). There was no difference in the read counts for *H. somni* and *B. trehalosi* between SNS and DNPS samples. The number of reads for both *M. bovis* and *M. dispar* were significantly higher in DNPS than in SNS samples, regardless of whether the analysis was adjusted for *E. coli* (Table 4; *P*-values of 0.04 and 0.001 respectively).

M. haemolytica (≥ 14 reads) was detected more frequently in SNS than DNPS (51% compared to 31%, $P = 0.001$) (Table 5). Similarly, *P. multocida* (≥ 1 read) was also detected more frequently in SNS than DNSP (25% versus 15%, $P = 0.02$). Conversely, *M. dispar* was significantly more likely to be reported in DNPS than SNS (75% versus 52%, $P = 0.001$). There were no significant differences in detection of *H. somni, B. trehalosi*, and *M. bovis* between the swab types (Table 5).

Agreement above that expected by chance between SNS and DNPS

Table 4
Differences in counts of reads for respiratory bacteria of interest identified by metagenomic sequencing in short nasal swabs and deep nasopharyngeal swab samples collected from fall-placed calves at 13 days on feed.

	Short nasal swabs(n=150)		Deep nasopharyngeal swabs (n=150)		Difference		
Bacteria	Mean no. of reads	95% CI	Mean no. of reads	95% CI	Relative difference	95% CI	*P*-value
M. haemolytica	169	0, 520	57	0, 177	2.94	1.98, 4.38	**0.001**
P. multocida	4.0	0, 9.0	1.2	0, 2.6	3.49	1.25, 9.75	**0.02**
H. somni	3.9	2.6, 5.2	5.0	3.3, 6.6	0.78	0.56, 1.08	0.14
B. trehalosi	0.06	0, 0.38	0.10	0, 0.65	0.58	0.06, 5.88	0.64
M. bovis	0.03	0, 0.08	0.28	0, 0.60	0.12	0.02, 0.74	**0.02**
M. dispar	5.2	0.70, 9.8	29	4.3, 54	0.18	0.12, 0.28	**0.001**
	Adjusted for *E. coli* read count						
M. haemolytica	128	0, 360	70	0, 199	1.83	1.22, 2.75	**0.003**
P. multocida	3.0	0, 6.1	2.3	0, 5.2	1.29	0.47, 3.53	0.62
H. somni	3.8	2.6, 5.0	5.1	3.4, 6.7	0.75	0.54, 1.03	0.08
B. trehalosi	0.04	0, 0.19	0.06	0, 0.31	0.60	0.06, 5.52	0.65
M. bovis	0.04	0, 0.09	0.26	0, 0.57	0.13	0.02, 0.90	**0.04**
M. dispar	4.9	0.81, 9.0	29	5.4, 53	0.17	0.10, 0.27	**0.001**

Abbreviations: No.: number; CI: confidence interval.

Table 5
Differences in detection of BRD-associated bacteria in short nasal swabs and deep nasopharyngeal swab samples collected from fall-placed calves at 13 days on feed.

	Short nasal swabs (n=150)		Deep nasopharyngeal swabs (n=150)		Difference		
Bacteria	No. pos. samples	Prevalence	No. pos. samples	Prevalence	Odds ratio	95% CI	*P*-value
	Cut-offs informed by BLCM						
M. haemolytica (≥ 14 reads)	76	51%	47	31%	5.73	2.41, 13.6	**0.001**
P. multocida (≥ 9 reads)	9	6.0%	3	2.0%	3.27	0.85, 12.6	0.09
H. somni (≥ 8 reads)	19	13%	29	19%	0.60	0.31, 1.12	0.11
M. bovis	3	2.0%	8	5.3%	0.36	0.09, 1.39	0.14
	Cut-offs not informed by BLCM						
*M. dispar**	78	52%	113	75%	0.27	0.15, 0.50	**0.001**
*B. trehalosi**	3	2.0%	3	2.0%	1.00	0.19, 5.18	0.99
	Sensitivity analysis						
M. haemolytica (≥ 1 read)*	103	69%	96	64%	1.47	0.76, 2.82	0.25
P. multocida (≥ 1 read)*	38	25%	22	15%	2.02	1.18, 3.67	**0.02**
H. somni (≥ 1 read)*	104	69%	105	70%	0.96	0.58, 1.60	0.90

The cut-offs used to determine bacteria-positive fall-placed calves were based on ≥1 read for all bacteria except *M. haemolytica* (≥ 14 reads); *P. multocida* (≥ 9 reads); *H. somni* (≥ 8 reads). * Cut-offs not informed by BLCM (≥1 read). Abbreviations: No.: number; Pos: positive; CI: confidence interval; BLCM: Bayesian latent class models.

for detection of *M. haemolytica* classified based on the BLCM threshold (≥ 14 reads) was moderate (κ = 0.46), whereas agreement between SNS and DNPS for detection of *M. dispar*\ was slight but significant (κ = 0.17) (Table 6). For the other bacteria, including *P. multocida, H. somni, B. trehalosi,*\ and *M. bovis*, kappa values were nearly zero, indicating no meaningful agreement beyond chance (Table 6).

4. Discussion

This study compared the use of SNS and DNPS samples to describe important respiratory viruses and bacteria detected using nanopore metagenomic sequencing in fall-placed feedlot calves. Differences in viral and bacterial read counts, detection, and agreement were compared across sampling methods at 13 DOF. All samples were processed in matched pairs throughout sample processing and sequencing to minimize factors other than swab type that could impact the findings.

Beyond anatomical sampling depth, the material of the swab tip may also influence the differences observed between SNS and DNPS. Short nasal swabs generally feature nylon-flocked tips, which enhance the absorption and release of collected material into transport media. In contrast, deep nasopharyngeal swabs often use polyester tips, which, while less efficient at releasing organisms, offer durability for deep sampling (Doyle et al., 2017; Chen et al., 2022; Crosby et al., 2022; McDaneld et al., 2018). Nylon-flocked swabs have demonstrated improved pathogen yield in respiratory sampling due to their superior elution properties compared to traditional fiber swabs (Chen et al., 2022). These material differences, along with anatomical depth, may partly account for the higher viral and bacterial read counts observed in SNS compared to DNPS in this study (McDaneld et al., 2018).

Viral read counts and the proportion of samples where viruses were detected were significantly higher from SNS than from DNPS for BCoV, IDV and BRBV. Read counts were also higher for BPIV-3. There was no significant difference in these outcomes between swab types for BRSV. The only virus more likely to be detected in DNPS than SNS was UCPV-1. These results suggest that when using nanopore metagenomic sequencing, SNS could offer a sensitive and practical alternative to DNPS for BRD-associated viral surveillance in feedlot settings.

In the present study, there were either no positive swabs for BPIV-3, BoHV-1, BVDV-1, BVDV-2 and BAdV3, or the very low number of positive swabs substantial limited power for comparison of these sampling methods. While the metagenomic workflow summarized across both types of swabs yielded a low proportion of total reads classified as

Table 6
Agreement in detection of BRD-associated bacteria in short nasal and deep nasopharyngeal swab samples collected from fall-placed calves at 13 days on feed (n=150 pairs).

Bacteria	Observed agreement	Expected agreement	Kappa	Standard error	*P*-value
Cut-offs informed by BLCM					
M. haemolytica (≥ 14 reads)	73%	50%	0.46	0.08	**0.001**
P. multocida (≥ 9 reads)	92%	92%	0.00	0.07	0.67
H. somni (≥ 8 reads)	73%	73%	0.02	0.08	0.42
M. bovis	93%	93%	0.00	0.07	0.66
Cut-offs not informed by BLCM					
*B. trehalosi**	96%	96%	0.00	0.08	0.60
*M. dispar**	59%	51%	0.17	0.07	**0.01**
Sensitivity analysis					
M. haemolytica (≥ 1 read)*	64%	64%	0.00	0.00	0.50
P. multocida (≥ 1 read)*	29%	37%	-0.13	0.04	0.99
H. somni (≥ 1 read)*	43%	40%	0.05	0.05	0.16

Interpretation of agreement as described by Dohoo et al. (2014): κ < 0.00 – poor, 0.01-0.20 – slight, 0.21-0.40 – fair, 0.41-0.60 – moderate, 0.61-0.80 – substantial, 0.81-1.00 – near perfect.
*Cut-offs not informed by BLCM (≥1 read). Abbreviations: BLCM: Bayesian latent class models.

viruses (0.1%), the metagenomic sequencing protocol was successful in identifying many BRD-associated viruses previously reported in DNPS from feedlot calves (Mitra et al., 2016; Zhang et al., 2021; Zhang et al., 2019). Overall, the sequencing results provided additional evidence of the utility of untargeted sequencing in complex respiratory microbiomes (Ambrose et al., 2023). However, the high proportion of host (68%) and unidentified reads (29%) suggests opportunities for continued protocol refinement, expansion of viral reference databases and improved classification algorithms (Wright et al., 2023).

In addition to describing viruses, this study compared detection of key BRD-associated bacteria between SNS and DNPS using the same metagenomic sequencing data set. Despite the protocol being optimized for viral targets, six bacterial species of interest were also successfully identified, with *M. haemolytica, H. somni, P. multocida* and*M. dispar* being the most prevalent. *M. haemolytica* was the dominant bacterial species detected, with significantly higher read counts and prevalence in SNS compared to DNPS using a BLCM-informed threshold of >14 reads. This finding aligns with previous reports that *M. haemolytica* is typically abundant in the upper respiratory tract of healthy cattle and cattle with BRD (Klima et al., 2014; Lima et al., 2016). One possible explanation for the abundance of *M. haemolytica* in the upper respiratory tract is the colonization pattern of the bacterium in cattle, where the bacterium has high affinity for the tonsillar crypts under non-pathogenic conditions (Ackermann & Brogden, 2000).

In addition, these observations align with previous findings that nasal swabs can effectively capture upper respiratory tract pathogens (Crosby et al., 2022). Crosby and colleagues (2022), in their study of *M. haemolytica* and other respiratory bacteria, compared their identification from 29.5 inch (74.9 cm) deep-guarded nasopharyngeal swabs, 16 inch (40.6 cm) unguarded proctology swabs, and 6 inch (15.2 cm) unguarded nasal swabs and found that the microbial communities showed a high degree of similarity across samples from the different swab types using culture, qPCR and 16S rRNA sequencing techniques using short-read Illumina MiSeq. However, while the qPCR results were highly concordant among all swabs, the DNPS showed significantly lower amounts of *M. haemolytica* DNA compared to the other swab types. This finding aligns with the results of this study, where more *M. haemolytica* reads were found in SNS compared to DNPS.

Among other prominent BRD-associated bacteria, more *P. multocida* reads were detected in SNS than DNPS and *P. multocida* was more frequently detected in SNS than DNPS when the positive threshold was set at ≥ 1 read. While there was no significant difference for *H. somni* reads detected by SNS and DNPS, the average number of *M. bovis* reads was slightly higher in DNPS. These results suggest a variation in pathogen detection based on swab type, which contrasts with the findings reported by McDaneld and colleagues, which reported that SNS yielded comparable results to DNPS for *Mannheimia* species and *Mycoplasma* species from next-generation sequencing with sample preparation optimized for detection of bacterial DNA using short-read Illumina MiSeq (McDaneld et al., 2018).

Similar to *M. bovis*, more *M. dispar* reads were detected using DNPS than with SNS, and *M. dispar* was also detected in a significantly higher proportion of DNPS samples than SNS samples. These results might suggest a deeper colonization pattern for the Mycoplasma-like organisms but might also reflect that these organisms might have been easier to detect when overall numbers of the other organisms were lower. These differences align with previous reports that certain BRD-associated bacteria could preferentially inhabit distinct anatomical niches, which has implications for sample selection in diagnostic protocols (Holman et al., 2017; McMullen et al., 2020; Timsit et al., 2016; Timsit et al., 2018; Zhang et al., 2019).

This study is the first to use a single metagenomic analysis protocol to compare SNS and DNPS for the antemortem identification of both respiratory viruses and BRD-associated bacteria in feedlot cattle. While this study employed metagenomics to assess swab types and identify both bacteria and viruses associated with BRD in cattle, most previous reports have primarily concentrated on identifying bacteria from different swab types (Crosby et al., 2022; McDaneld et al., 2018; Zeineldin et al., 2017). McDaneld et al. (2018) conducted a comparison of BRD-associated bacteria identified using Illumina MiSeq from SNS and DNPS in calves diagnosed with BRD post-weaning. The study by Zeineldin et al. (2017) characterized the respiratory bacterial communities in healthy feedlot cattle using nasopharyngeal swabs and bronchoalveolar lavage (BAL) fluid collected from healthy feedlot calves (n = 8) also using an Illumina MiSeq protocol optimized for the detection of bacterial DNA. Crosby and colleagues (2022) investigated respiratory bacteria using three swabbing techniques analyzed with culture, qPCR, and 16S rRNA using Illumina MiSeq sequencing. Where metagenomics had previously been reported, the method described was Illumina MiSeq whereas the present study used nanopore metagenomic sequencing.

Similar to this study, Doyle and colleagues (Doyle et al., 2017) conducted research that identified both respiratory viruses and bacteria associated with BRD. In addition to using SNS and DNPS, they also identified pathogens from bronchoalveolar lavage (BAL) and transtracheal wash (TTW), collecting samples from 100 dairy calves with BRD. However, in their study, viral agents were identified using real-time RT-PCR, bacteria through aerobic culture, and *M. bovis* with PCR, whereas this study utilized nanopore metagenomic sequencing for the identification of all pathogens. Additionally, while they investigated two viruses (BCoV and BRSV) and four bacteria species (*M. haemolytica, P. multocida, M. bovis* and*Mycoplasma spp.*), this study examined ten viruses and six bacteria species.

The diagnostic performance of SNS versus DNPS for detecting BRD-associated pathogens was further evaluated through analyzing agreement within sample pairs. For viral targets, while pathogens such as BCoV, IDV and BRSV demonstrated high observed agreement between SNS and DNPS (82%, 81% and 97%, respectively), the corresponding kappa values varied widely (0.10, 0.57 and 0.43, respectively). This discrepancy is an example of what has been described as the "kappa paradox," where high agreement coexists with low kappa due to high expected agreement driven by skewed prevalence or marginal totals (Cicchetti & Feinstein, 1990; Feinstein & Cicchetti, 1990). For instance, BPIV-3, BVDV-1 and BVDV-2 showed near-perfect observed and expected agreement (≥90%), but kappa values were zero, reflecting the

limited variability in test outcomes. In contrast, IDV, which had a balanced distribution of positive and negative results, yielded a higher kappa, indicating more meaningful agreement beyond chance. These findings underscore the limitations of relying solely on kappa in low-prevalence contexts and highlight the importance of interpreting it alongside observed agreement and prevalence data (Bexkens et al., 2018; Byrt et al., 1993).

Similarly, agreement analysis for BRD-associated bacteria revealed variable concordance between SNS and DNPS, influenced by both detection thresholds and pathogen-specific colonization patterns. *M. haemolytica* showed moderate agreement ($\kappa = 0.46$) when using a BLCM-informed threshold (> 14 reads), suggesting that detection of *M. haemolytica* by SNS and DNPS were similar. However, when a non-BLCM threshold (≥ 1 read) was applied, the kappa dropped to 0.00 despite a 64% observed agreement, again illustrating the kappa paradox but in this case associated with very high prevalence. Other bacteria such as *P. multocida* and *H. somni* showed high observed agreement (92% and 73%, respectively) but negligible kappa values, likely due to uniform detection patterns or low prevalence. Interestingly, *M. dispar* showed slight but statistically significant agreement ($\kappa = 0.17$), potentially reflecting its deeper anatomical niche and more variable detection across swab types. These results emphasize the value of SNS for detecting certain pathogens while also highlighting the need for pathogen-specific sampling strategies. Moreover, the use of BLCM-informed thresholds enhances the interpretability of agreement metrics with evidence-informed cut points in the absence of a gold standard, a critical consideration in veterinary diagnostics (Cheung et al., 2021).

The detection of *E. coli* in SNS and DNPS was reported as an indicator of fecal contamination (LeJeune & Wetzel, 2007). While *E. coli* could transiently colonize the upper respiratory tract under stressed conditions, it is not considered as a dominant member of respiratory microbiota in healthy cattle (Holman et al., 2015). In this study, counts of *E. coli* reads were significantly higher in SNS than DNPS. This was expected given DNPS samples were collected from a deeper and more protected region of the upper respiratory tract and with double guarded swabs. The impact of adjusting differences in many of the target viral read counts for *E. coli* in the present study underscores the importance of considering microbial background when interpreting metagenomic data, particularly in surveillance studies (Jurasz et al., 2021). These findings suggest that although SNS can offer greater practicality and scalability, they are also more prone to environmental contamination, including fecal-associated bacteria like *E. coli*, which can complicate pathogen detection and quantification.

Data suggesting fecal contamination in SNS might also suggest the risk of contamination of SNS samples by other organisms of interest, for example other respiratory bacteria and viruses that can be transmitted from calf to calf or through environmental reservoirs, such as water bowls (Kos et al. 2023), without necessarily indicating current infection status. While these findings do support caution in using SNS for diagnostics on individual sick animals, they do not necessarily limit the potential surveillance or risk assessment value of the data for comparing SNS data collected using the same protocols between pens or feedlot and across time within pens or feedlots.

In these models, the *E. coli* count emerged as a significant predictor for both BCoV and BRBV, and the disparity between SNS and DNPS was reduced by half after accounting for *E. coli*. The association between the extent of fecal contamination measured by *E. coli* and the numbers of BCoV reads can be at least explained in part by the expected presence of BCoV in bovine feces. There have been reports of the detection or identification of both bovine enteric coronaviruses and bovine respiratory coronaviruses from feedlot cattle. Cho et al. (2001) found that 22% of fecal samples and 17% of nasal swabs from feedlot cattle were positive for BCoV. Almost half of the cattle shed the virus through both enteric and respiratory routes, with a strong correlation between these shedding patterns, suggesting concurrent infection (Cho et al., 2001). Hasoksuz et al. (2002) reported that 53% of fecal swabs and 48% of nasal swabs from feedlot cattle in Ohio tested positive for BCoV using ELISA. Even higher detection rates were observed with RT-PCR, showing 84% positive in nasal swabs and 96% in fecal swabs. Concurrent shedding through both routes was observed in 38% of the cattle (Hasoksuz et al., 2002). Thomas et al. (2006) found significant differences in BCoV shedding among feedlot calves. New Mexico calves with high antibody titers showed minimal nasal (2%) and fecal (1%) shedding, while Arkansas calves with low antibody titers had high shedding evidenced in 64% of nasal and 65% of fecal samples (Thomas et al., 2006). These findings collectively demonstrate that fecal shedding of BCoV in feedlot cattle is widespread, influenced by factors such as age, immune status, and source of origin, and plays a critical role in the transmission dynamics and establishment of infection within feedlot populations.

The reason why BRBV read counts would be affected after adjusting for *E. coli* remains unknown, as there were no documented reports of BRBV transmission or detection in cattle feces and available literature does not report gastrointestinal replication or shedding (Bhattarai et al., 2022).

The results of this study further underscore the potential of metagenomic sequencing, even when optimized for viral detection, to provide meaningful insights into bacterial coinfections (Donbraye et al., 2026). However, the relatively low read counts for some bacteria and the high proportion of host and unclassified reads highlighted the need for targeted enrichment strategies or dual-purpose protocols to improve bacterial detection sensitivity (Chiu & Miller, 2019; Periyasamy et al., 2025). Despite the relatively low read counts for some of the viruses and bacteria of interest, previous work with similar samples has shown that even with a cutoff of ≥ 1 read the estimated diagnostic specificity of these tests was $> 90\%$ (Donbraye et al. 2026). For example, for BRSV the specificity was 94%, for BoHV-1 it was $>99\%$, and for *M. bovis* it was 98%.

The present study incorporated high throughput nanopore sequencing of hundreds of samples. This technical advancement, together with the use of SNS, is critical for scaling metagenomic surveillance in commercial feedlots, where sample volumes are high and rapid turnaround is essential. This leads to faster identification, targeted interventions, and potentially reduced disease incidence and economic impact.

The variability in the detection between SNS and DNPS swabs reinforces the importance of sample type selection in BRD investigations (Zhang et al., 2019). While SNS offer practical advantages and potentially higher detection rates for several pathogens, DNPS may still be necessary for capturing deeper-residing organisms (Carella et al., 2024). The pen prevalence of pathogens measured using SNS might also be more likely to reflect the potential for cross contamination among animals or from the environment that DNPS. These findings support a complementary sampling approach, particularly in outbreak investigations where comprehensive pathogen profiling is essential.

5. Conclusion

In summary, this study shows that SNS were a practical and effective tool for detection of many BRD organisms in feedlot calves. SNS consistently detected more reads than DNPS of key BRD-associated viruses such as BCoV, IDV, and BPIV-3, as well as the dominant bacterial pathogen *M. haemolytica*, with significantly higher read counts and prevalence. Their ease of collection, reduced invasiveness, and compatibility with high throughput metagenomic sequencing make SNS especially well-suited for large-scale surveillance in commercial feedlot settings. These advantages, combined with the scalability of modern sequencing platforms, position SNS as a cornerstone of efficient, cost-effective, and comprehensive BRD monitoring protocols. However, use of SNS for individual animal diagnostics should be considered with caution given the increased potential for environmental contamination.

Data availability

The genomic data used in this study have been deposited in the Sequence Reach Archive (SRA) within BioProject ID: PRJNA1374179. Details on the bioinformatics and any custom scripts can be accessed at: https://github.com/coadunate/ASSETS_2

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Ethics statement

The study research protocols and procedures were approved by the University of Saskatchewan Animal Care Committee (AUP 20190069) and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

Submission declaration

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CRediT authorship contribution statement

Emmanuel Donbraye: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Lianne McLeod:** Writing – review & editing, Project administration, Methodology, Investigation, Data curation. **Zhijian Chai:** Writing – review & editing, Methodology, Investigation. **Stacey R. Lacoste:** Writing – review & editing, Methodology, Investigation. **E. Luke McCarthy:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Matthew G. Links:** Writing – review & editing, Software, Methodology, Funding acquisition. **Cheryl L. Waldner:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Cheryl Waldner reports financial support was provided by Genome Canada. Cheryl Waldner reports financial support was provided by Beef Cattle Research Council. Cheryl Waldner reports financial support was provided by Genome Prairie. Cheryl Waldner reports financial support was provided by Genome Alberta. Cheryl Waldner reports financial support was provided by Agricultural Development Fund. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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