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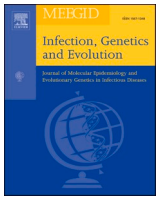

High prevalence of Enterovirus E, Bovine Kobuvirus, and Astrovirus revealed by viral metagenomics in fecal samples from cattle in Central Colombia

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ABSTRACT

Livestock plays a crucial role in ensuring food security and driving the global economy. However, viral infections can have far-reaching consequences beyond economic productivity, affecting the health of cattle, as well as posing risks to human health and other animals. Identifying viruses present in fecal samples, a primary route of pathogen transmission, is essential for developing effective prevention, control, and surveillance strategies. Viral metagenomic approaches offer a broader perspective and hold great potential for detecting previously unknown viruses or uncovering previously undescribed agents. Ubaté Province is Colombia's dairy capital and a key center for livestock production in the country. Therefore, the purpose of this study was to characterize viral communities in fecal samples from cattle in this region. A total of 42 samples were collected from three municipalities in Ubaté Province, located in central Colombia, using a convenient non-probabilistic sampling method. We utilized metagenomic sequencing with Oxford Nanopore Technologies (ONT), combined with diversity and phylogenetic analysis. The findings revealed a consistent and stable viral composition across the municipalities, primarily comprising members of the *Picornaviridae* family. At the species level, the most frequent viruses were Enterovirus E (EVE) and Bovine Astrovirus (BoAstV). Significantly, this study reported, for the first time in Colombia, the presence of viruses with veterinary importance occurring at notable frequencies: EVE (59%), Bovine Kobuvirus (BKV) (52%), and BoAstV (19%). Additionally, the study confirmed the existence of Circular replicase-encoding single-stranded (CRESS) Virus in animal feces. These sequences were phylogenetically grouped with samples obtained from Asia and Latin America, underscoring the importance of having adequate representation across the continent. The virome of bovine feces in Ubaté Province is characterized by the predominance of potentially pathogenic viruses such as BoAstV and EVE that have been reported with substantial frequency and quantities. Several of these viruses were identified in Colombia for the first time. This study showcases the utility of using metagenomic sequencing techniques in epidemiological surveillance. It also paves the way for further research on the influence of these agents on bovine health and their frecuency across the country.

1. Introduction

The domestication of cattle is vital for global food security and the economy, with a significant number of livestock worldwide and a substantial contribution to Gross Domestic Product and employment (Herrero et al., 2013; Tona, 2021). In Colombia, the Ubaté Province in Cundinamarca is known as the "Dairy Capital of Colombia" due to its high milk production and concentration of cattle (Instituto Colombiano

Abbreviations: BoAstV, Bovine Astrovirus; BKV, Bovine Kobuvirus; EVE, Enterovirus E; mNGS, Metagenomic Next-generation sequencing; ONT, Oxforford Nanopore Technologies.

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Agropecuario - ICA, 2022). However, cattle are susceptible to various infectious agents, with viral infections being surprisingly understudied despite their substantial impact on cattle productivity and overall health. Notably, infections like Bovine Viral Diarrhea Virus have caused considerable economic losses in livestock production globally, including Colombia (Richter et al., 2017; Vargas et al., 2012). These infections can lead to reproductive problems and affect the quality and quantity of cattle-derived products.

Viral infections affecting the digestive tract can pose significant risks to calves, especially during their initial month of life, leading to dysentery and diarrhea (Castells and Colina, 2021). Cattle can also act as reservoirs, transmitting viral pathogens to other animals and wildlife, raising concerns about potential "spillover" events. This is particularly worrisome considering ecosystem alterations and the possible introduction of viral diseases to wildlife populations (Ijaz et al., 2022). Notably, many pathogens capable of infecting both cattle and humans are viral agents, highlighting the critical need for surveillance and prevention of viral zoonoses (McDaniel et al., 2014). In the Colombian context, livestock farming practices, deforestation, and close contact with animals heighten the risk of viral disease transmission to humans (Meurens et al., 2021). It is crucial to implement effective measures to prevent the spread of viral diseases and protect the health of both animals and humans.

Early detection of viral agents is crucial for controlling the spread of infections (Al-Hemoud et al., 2021). Serological tests provide rapid results but have limitations in sensitivity and specificity (Soltan et al., 2016). Molecular tests, although targeting specific viral groups, may not detect all viruses contributing to animal pathology (Zhang et al., 2020). Metagenomic Next-generation sequencing techniques (mNGS), particularly viral metagenomics, have significantly advanced our understanding of infectious diseases. Viral metagenomics holds great promise in improving our ability to diagnose and monitor infections. It is an approach that evaluates the entire genetic material in a sample, including non-cultivable agents and viruses that may not be identifiable through specific tests (Delwart, 2012; Simmonds et al., 2017). In previous studies utilizing (mNGS) on livestock samples, a substantial prevalence of viruses from bacteria, archaea, and plants has been demonstrated. However, concerning vertebrate viruses, characteristic viruses indicating exposure have also been identified. Families such as *Picornaviridae* and *Astroviridae* are commonly described and associated with diseases providing insights into the composition of the bovine fecal virome (Kwok et al., 2020a). Metagenomic techniques have detected a range of viruses in bovine samples, influencing the health of cattle. For instance, a study conducted in a Chinese province characterized the virome of dairy cattle's genital tract, identifying viruses from the *Herpesviridae*, *Circoviridae*, *Polyomaviridae*, and *Papillomaviridae* families (Ling et al., 2019). In the United States and Mexico, researchers identified 21 viruses associated with bovine respiratory disease in both symptomatic and asymptomatic cattle (Mitra et al., 2016). Similarly, investigations of fecal samples from healthy and sick cattle found viruses such as Bovine Enterovirus, Bovine Kobuvirus (BKV) and Nebovirus (Guo et al., 2018).

The integrity of the microbiome is essential for various physiological processes, and a disruption of this homeostasis is correlated with a variety of pathological conditions. Viral infections, in particular, can impact the composition of the microbiome in cattle, leading to the development or exacerbation of diseases (Yildiz et al., 2018). Metagenomic analysis can unveil new viruses or genotypes that may indicate the possibility of viral outbreaks. For instance, in Schmallenberg, Germany, the analysis of blood samples from symptomatic cattle led to the discovery of the Schmallenberg Virus, which was responsible for an epidemic of abortions and congenital deformities in calves across Europe (Collins et al., 2019). Newly identified viruses or genotypes may differ in their pathogenic potential and virulence compared to closely related ones. For example, the BPV22 genotype of bovine papillomavirus has been linked to severe symptoms and potential neoplasia (Bauermann et al., 2017). In Bovine Leukosis Virus, variations in the envelope gene could enhance transmissibility and evade host defenses (Polat et al., 2017). Possible epizootic outbreaks can be associated with the discovery of unclassified viruses due to the implications of their variations, as evidenced by the detection of different picornaviruses in cattle feces (Guo et al., 2018).

In Colombia, various molecular techniques have been utilized to identify viral pathogens in cattle. For instance, studies have detected Bovine Herpesvirus 1 in cattle from Montería using PCR-RFLP, found Bovine Leukosis Virus in raw meat and milk from cattle across different departments using nested PCR, and identified the circulation of Bovine Viral Diarrhea Virus 2 for the first time in the country using RT-PCR (Olaya-Galán et al., 2022; Villamil et al., 2018; Zapata et al., 1996). However, there's a lack of reports employing metagenomic techniques for viral characterization in Colombian cattle. It is essential to comprehend the presence and diversity of viral communities affecting cattle health to surveil and control infectious diseases, including zoonotic events. Prompt identification of intestinal pathogens significantly impacts cattle outcomes, transmission, subsequent treatment, morbidity, and potential isolation (King et al., 2021). Fecal samples are particularly valuable for understanding an individual's gastrointestinal environment and serve as a conduit for transmitting viruses that are of veterinary significance. The Ubaté Province stands out as a significant center for livestock farming, with dairy production being a major commercial activity. Its substantial economic influence on the region and the high concentration of animals make it an exemplary setting in this domain. Therefore, this study aimed to elucidate the viral communities present in fecal samples obtained from cattle across different municipalities within the Ubaté Province, Cundinamarca, Colombia.

2. Materials and methods

2.1. Ethical statements

In favor of animal welfare, the project underwent ethical review by the research ethics committee of Universidad Pedagógica y Tecnológica de Colombia (UPTC), and it was granted approval for the collection of samples used in this study. The fecal samples were collected from cattle with the explicit consent of their owners to participate in the study. The privacy and confidentiality of participant information were strictly upheld in accordance with National Law 25,326 on Data Protection, ensuring the anonymity of owners of potentially diseased animals. The personnel responsible for sample collection underwent specialized training in veterinary medicine and animal science and possessed the required expertise to perform the procedures. All activities were carried out in the laboratories of UPTC and Universidad del Rosario, adhering to established biosafety protocols.

2.2. Study area and sample collection

The Ubaté Province, located in the northern part of the Cundinamarca department (5°18′26″ N, 73°48′52″ W), is recognized for its dense cattle population and as a prominent milk-producing region in the country. Most cattle farms in this area are small-scale operations, typically managed by farmers with fewer than 50 heads of cattle. For this study, fecal samples were obtained from farms owned by small-scale producers in three municipalities within the Ubaté Province: Cucunuba, Simijaca, and Ubaté. The selected samples were collected from adult female bovines of Holstein breed or Normando-Holstein crossbreeds, with a defined zootechnical purpose. The sampling approach used was a non-probabilistic convenience method, resulting in a total of 42 collected samples, with 14 samples obtained from each municipality (Ubaté, Simijaca, and Cucunuba) (Fig. 1).

To collect the samples, a lubricant-free palpation sleeve was used. The sleeve was gently inserted into the rectum, and the first fecal round was disregarded to ensure accurate sampling. Once an adequate sample

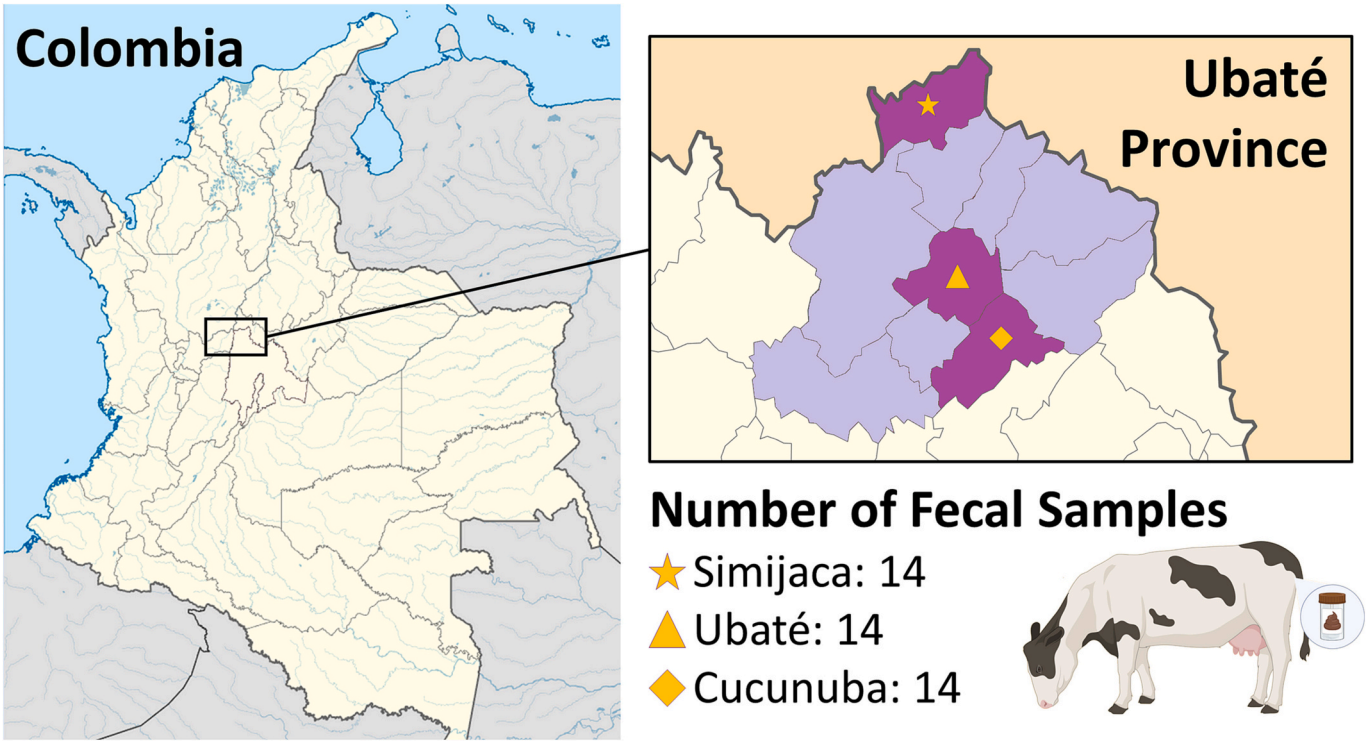

Fig. 1. Location map of the municipalities where fecal samples were collected.
It provides an overview of the national, departmental, and Ubaté Province-level locations.

was obtained, the hand was closed, and the sleeve was carefully inverted to prevent trapping any air. The collected samples were promptly frozen at −4 °C and transported to the laboratory. Upon arrival, they were stored at −20 °C until further analysis .

2.3. Laboratory processes

2.3.1. Sample preparation and extraction of genetic material

To enhance the proportion of viral sequences and enable efficient virome analysis, some fecal sample preprocessing guidelines described previously were followed (Conceição-Neto et al., 2015). Approximately 20 mg of fecal matter were combined with ceramic disruption beads, 250 µL of PBS, and 250 µL of viral lysis buffer in tubes. The samples were then homogenized for 5 min at 30 Hz using a TissueLyser II disruptor. Subsequently, a centrifugation step at 14,000*g* for 3 min was performed to separate cells and cellular debris from the viral particles, which remained in the solution. Afterward, 400 µL of the supernatant were collected, and 25 µL of proteinase K were added to facilitate protein digestion and inactivate nucleases that could degrade genetic material. Viral nucleic acids were extracted using the dual PureLink Viral RNA/DNA Mini Kit (Invitrogen) following the manufacturer's instructions. The resulting elutions were stored at −80 °C until further analysis. The integrity and quality of the extracted nucleic acids were assessed using a 1.5% agarose gel electrophoresis and NanoDrop One spectrophotometry.

2.3.2. Viral enrichment

To enhance the sensitivity of viral detection, we employed a viral enrichment method due to the relatively low abundance of viral genetic material compared to host-derived nucleic acids. Specifically, we utilized the Rapid Switching Mechanism at the 5′ End of the RNA Template (Rapid SMRT-9), which has shown promising results in clinical samples and is compatible with ONT (Claro et al., 2023).

We followed the same concentrations described in the work of (Claro et al., 2023). During the process, we annealed an initial volume of 5 µL of DNA/RNA using RLB-RT-9 N primer (TTTTTCGTGCGCCGCTT CAACNNNNNNNNN) and dNTPs In a 1:1 ratio between each other and a 10:1 ratio with the genetic material. At the same time, cDNA synthesis was performed utilizing Super Script IV (Invitrogen) and the TSO primer (GCTAATCATTGCTTTTTCGTGCGCCGCTTCAACATrGrGrG). Subsequently, 1,25 µL of the generated cDNA was subjected to amplification using LongAmp. To evaluate the method's efficacy, we measured the quantity of genetic material before and after enrichment utilizing the Qubit dsDNA High Sensitivity Assay on the Qubit 3.0 instrument (Life Technologies), following the manufacturer's instructions. This assessment enabled us to gauge the impact of enrichment on the viral genetic material within the samples, offering valuable insights into the sensitivity of our approach. However, additional measures such as filtration and/or the use of nucleases or chloroform treatments could have been employed. Nevertheless, any enrichment procedure carries a bias toward certain viral genera or families, and we aimed to minimize this probability as much as possible (Conceição-Neto et al., 2015; Thurber et al., 2009).

2.3.3. Library preparation and sequencing

To prepare the samples for sequencing, we conducted the End Prep procedure using the appropriate FFPE DNA Repair Mix and Buffer, as well as Ultra II End Prep Reaction Buffer and Ultra II End Prep Enzyme Mix from New England Biolabs. For barcode ligation, we utilized Ultra II Ligation Master Mix and Ultra Ligation Enhancer from New England Biolabs. Adapter ligation was performed using a ligation buffer, Adapter Mix, and Next Quick T4 DNA Ligase from New England Biolabs. To ensure the removal of unligated barcodes and adapters, we carried out a cleanup step using AMPure XP paramagnetic beads from Beckman Coulter (Theuns et al., 2018).

We utilized third-generation sequencing technology for our study. The prepared libraries were loaded onto a FLO-MIN106D flow cell containing 512 nanopore channels within the Oxford Nanopore Technologies MinION device. Here, we utilize a 9.4.1 chemistry in the detection process and subsequent interpretation of the signals generated during sequencing. The sequencing process adhered to the standard 72-h

script using MinKNOW 1.15.1 software, ensuring precise and effective data generation. This sequencing approach significantly aided in analyzing genetic material and yielded valuable insights into the viral communities present in the samples. The obtained sequences have been deposited in GenBank under the BioProject PRJNA1035769.

2.4. Bioinformatic analysis

2.4.1. Quality control and decontamination

To analyze the sequencing data, we first conducted real-time base-calling using Guppy 2.2.7 software to convert the raw electrical signal data generated during nanopore sequencing. Subsequently, we utilized the same software to perform demultiplexing, where we filtered out low-quality reads with a quality score below 7. The obtained set of long reads was then subjected to statistical analysis using the NanoStat V 1.1.2 tool (https://github.com/wdecoster/nanostat) to determine average length and quality scores (De Coster et al., 2018). To focus on viral genetic material and remove non-viral reads, we utilized Minimap 2.24 software (https://github.com/lh3/minimap2), which is specifically designed for aligning long genomic reads obtained from Oxford Nanopore sequencing. Using this software and this command "minimap2 -ax map-ont", we aligned the sequencing reads against two reference databases. For removing cattle-related sequences, we aligned the reads against the reference genome of the host species, *Bos taurus* (GenBank: GCA_002263795.3). To discard bacterial sequences, we performed alignment against the SILVA_138.1 prokaryotic database (https://www.arb-silva.de/documentation/release-1381/). By aligning the reads against these reference databases, we were able to filter out sequences originating from the cattle host and bacterial contaminants, leaving us with a dataset focused on viral genetic material for further analysis. (Li, 2018). For file format conversions during the analysis, we utilized Samtools (https://github.com/samtools/samtools) and Bam2fastq tools (https://github.com/jts/bam2fastq). These tools facilitated the conversion and manipulation of file formats as needed (Danecek et al., 2021).

To assess bacterial contamination in the enriched viromes, we employed the computational tool ViromeQC (https://github.com/SegataLab/viromeqc). This tool allowed for the comparison and quantification of bacterial contamination using pre-established databases containing rRNA 16S sequences. These analytical steps enabled us to process the sequencing data, remove non-viral reads, and evaluate bacterial contamination, ensuring the focus on the virome of interest (Zolfo et al., 2019).

2.4.2. Taxonomic assignment and metagenomic data partial assembly

We employed two approaches to analyze the reads that did not map to bacterial and host reference genomes. To perform taxonomic assignment, we created a Bovine Virus database using high-quality, non-duplicated, and unambiguous complete genome sequences of both DNA and RNA obtained from GenBank via NCBI Virus, specifically focusing on those sequences with hosts belonging to the *Bovidae* family. We employed the metagenomic sequence classifier Centrifuge to assign taxonomic information to the reads. To ensure accuracy, computations were executed in parallel across 8 CPU cores. We only considered a single primary assignment for each read and enforced a minimum partial match length of 50, following the command structure "centrifuge -p 8 –min-hitlen 50 -k 1 -x" (Kim et al., 2016). The resulting outputs were converted to Kraken-Report format using the Centrifuge-kreport function. The outcomes derived from metagenomic classification at both the species and family levels were subjected to in-depth analysis and visualization through the utilization of the Pavian package, this tool facilitated a comprehensive examination and graphical representation of the taxonomic assignments (Breitwieser and Salzberg, 2020). The second approach focused on partial assemblies. Using the Genome Detective Virus Tool, we constructed partial assemblies from the reads. The partial assemblies with the best quality parameters, including nucleotide identity percentage (>80%) and alignment match value (>75%), were selected (Vilsker et al., 2019). To validate the taxonomic assignment, a BLASTn search was performed against the NCBI BLAST database with strict parameters (identity percentage: >80%, query cover: >75%, *E*-value: ≤0.0). For this search, the method optimized for highly similar sequences (megablast) was selected, targeting the organism Viruses (taxid:10239).

2.4.3. Analysis of viral community abundance and diversity

To assess the abundance of viral families and species, we converted the read counts into relative values, offering an evaluation of their presence at individual sites. Abundance bar plots were generated utilizing the ggplot2 package in RStudio (http://www.rstudio.com/). Further analysis of diversity based on metagenomic data was conducted using the Rhea Alpha Diversity pipeline within RStudio v3.4.0. This enabled the computation of richness, Shannon index, and Simpson index at the viral species level, mirroring a study examining viromes from wild bird feces (Wille et al., 2018). In scrutinizing diversity indices across our study sites (Ubaté, Simijaca, and Cucunuba), non-parametric Kruskal-Wallis tests and Dunn posthoc tests were conducted. Additionally, Levene's test confirmed variance homogeneity ($p < 0.05$). For the assessment of beta diversity, examining viral diversity inter-municipality, we employed a Bray-Curtis dissimilarity matrix and performed ordination via principal coordinate analysis (PCoA). This analytical approach has found application in diverse studies exploring intestinal viromes in humans and farm animals, such as cattle (Cook et al., 2021). The evaluation of beta diversity utilized the vegan and phyloseq packages within RStudio v3.4.3 (Jari et al., 2022; McMurdie and Holmes, 2013). Statistical measures included similarity analyses and PERMANOVA tests, with resulting visual representations generated using ggplot2 (Wickham, 2016). These analyses provided insights into variations in viral diversity across the distinct municipalities examined in our study.

2.4.4. Phylogenetic analysis

Phylogenetic analyses were conducted to investigate the relationships between the viral sequences obtained from the partial assemblies and those present in the NCBI GenBank database. We specifically focused on sequences derived from *Bos taurus* fecal samples. For conducting the analyses, nucleotide sequences were aligned utilizing the iterative refinement method available in MAFFT version 7.450. The "—auto" option was used to internally determine the most suitable alignment method and settings, considering factors such as sequence length, evolutionary divergence, and data size (Katoh and Standley, 2013).

The alignment was meticulously inspected using Unipro UGENE 3 software. Throughout this process, specific emphasis was placed on validating the precise alignment of pertinent regions essential for species-specific phylogenetic analysis. This involved identifying potential gaps or misalignments while ensuring the alignment's overall quality (Okonechnikov et al., 2012). To determine the most suitable evolutionary model for the sequences, IQTree was employed, considering criteria such as the Bayesian Information Criterion (BIC), Akaike Information Criterion (AIC), and corrected Akaike Information Criterion (AICc). The preference for BIC was due to its penalty against more complex models, favoring those offering a better fit with fewer parameters. Subsequently, maximum likelihood trees were reconstructed using IQTree. To evaluate the robustness of the tree topology, a bootstrap analysis involving 1000 iterations was conducted, following the methodology outlined by (Nguyen et al., 2015). The resulting trees underwent further refinement using the iTOL software (Letunic and Bork, 2021) to enhance their visual representation. This additional step aimed to improve the graphical depiction of the trees, facilitating a clearer interpretation and presentation of the evolutionary relationships among sequences.

2.4.5. Genomic partial assembly annotation

Genomic annotation was conducted to identify crucial features within the sequence. This process involved the utilization of two

Table 1
Positive samples for viral species detected by metagenomic sequencing in municipalities of the Ubaté Province.

Family	Virus	Baltimore classification	Biology	Ref.	Number of positive samples per municipality			
					Ubaté	Simijaca	Cucunuba	Total
Picornaviridae	Bopivirus A	IV (ssRNA+)	Unknown pathogenic role	(Nagai et al., 2015)	3/14	4/14	4/14	11/42
Smacoviridae	Smacoviridae sp.	II (ssDNA+)	Common in fecal matter of various vertebrate species (Cattle).	(Krupovic & Varsani, 2021)	6/14	5/14	2/14	13/42
Picornaviridae	Kobuvirus Bovino	IV (ssRNA+)	Potential pathogenic role.	(Reuter et al., 2011)	7/14	8/14	7/14	22/42
Picornaviridae	Enterovirus E	IV (ssRNA+)	Potential pathogenic role.	(Zhang et al., 2021)	7/14	9/14	9/14	25/42
Astroviridae	Bovine Astrovirus	IV (ssRNA+)	Potential pathogenic role.	(Zhu et al., 2022)	3/14	4/14	1/14	8/42
Smacoviridae	Bovismacovirus bovas1	II (ssDNA+)	Common in fecal matter of various vertebrate species (Cattle).	(Kim et al., 2012)	4/14	4/14	1/14	9/42

complementary annotation tools: Prokka and Vgas (Seemann, 2014; Zhang et al., 2019). Prokka integrates ab initio and similarity-based methods to predict coding regions and subsequently assigns putative functions to these regions. It is known for its robustness in identifying conserved domains and annotating protein functions. On the other hand, the Vgas system employs a diverse range of computational approaches to comprehensively annotate genomic sequences, focusing on specific genomic structures, regulatory elements, or functional motifs that may not be prominently identified by other tools. This combination of Prokka and Vgas allowed for a more comprehensive annotation, highlighting various genomic elements, including coding regions, non-coding elements, and potential regulatory sequences, offering a multifaceted understanding of the sequence characteristics. To visualize and present our characterization findings, we employed the web-based tool Proksee, which utilizes the BLAST formatter tool to illustrate genetic identity on a gene-by-gene basis against previously identified genomes from the online BLASTn search. It is important to note that we focused exclusively on ORFs exceeding 100 codons in length. Proksee facilitated graphical representation of partial assembly annotations, providing a comprehensive overview of predicted features and their respective positions within the sequence (Grant and Stothard, 2008). Through the application of these annotation tools and visualization techniques, we successfully identified and annotated pertinent genomic features, including coding regions. This aided in predicting proteins or functional products inferred from the coding sequences identified within our most refined partial assembly.

3. Results

3.1. Metagenomic analysis of bovine fecal virome

We extracted nucleic acids (DNA/RNA) from 42 fecal samples obtained from adult bovines in three municipalities in the Ubaté Province of Cundinamarca, Colombia (Fig. 1). The samples underwent viral enrichment using SMRT-9 methodology and were sequenced using Oxford Nanopore technology. Prior to decontamination, the metagenomic viral data was analyzed using NanoStat software, revealing an average of 248,285 raw reads per sample with an average length of 516 base pairs and an average quality score of 9.97 (Supplementary Table 1). After removing host and bacterial sequences and performing the taxonomic assignment, the virome data was visualized using Pavian. The results showed an average of 168,156 raw reads per sample. Approximately 99.59% (~167,466) of the reads were classified as unclassified sequences, while the remaining 0.41% (~689) corresponded to bovine viral reads (Supplementary Table 2).

3.2. Characterization of viral communities

The virome in bovine feces from the Ubaté Province predominantly consists of RNA viruses known to impact the physiology of animals (Table 1). A consistent pattern is observed in the relative abundance of viral species, with Bovine Kobuvirus and Enterovirus E being the most frequent collectively accounting for over 50% of the virome in all municipalities (Fig. 2a). At the family level, a similar trend is observed, with the *Picornaviridae* family being the most abundant, which is expected given its inclusion of the two major viral species along with Bopivirus A. This is followed by the *Smacoviridae* family, which includes single-stranded DNA viruses commonly found in animal feces, such as Bovismacovirus bovas1 and Smacoviridae sp. In the taxonomic classification of reads using Centrifuge, we were only able to reach the family level for Herpesviridae and Toboviridae due to not meeting the assignment filters for species-level identification. This is noteworthy because within these families, species commonly associated with enteric diseases in symptomatic bovines, such as Bovine Alfaherpesvirus 1 and Torovirus, are included (Fig. 2b). Notably, all six viral species identified in this study were found in at least one sample from each municipality, with Enterovirus E being the most frequently detected viral species in over half of the fecal samples (Table 1).

3.3. Analysis of viral community diversity

To assess the diversity within each municipality (alpha diversity), three metrics were employed. Firstly, the richness, which quantifies the number of different observed taxa at the family level, revealed a notable difference between municipalities due to the lower number of families in Cucunuba compared to the other municipalities in the Ubaté Province (Kruskal-Wallis *p-value*: 0.01948). When considering both richness and the evenness of taxon abundances, low values of the Shannon index were found, indicating low diversity across all municipalities at both the family and species levels. This finding aligns with the relative abundance of the virome described in the previous section, where certain taxa dominate. Comparison of these indices between municipalities did not yield significant differences, indicating low and similar diversity within the Ubaté Province (Kruskal-Wallis p-value for families: 0.1155; Kruskal-Wallis p-value for species: 0.2583). This result is further supported by Simpson's indices, which exhibit high values at the species level, suggesting a high probability of two randomly selected entities belonging to the same species, indicating dominance patterns and thus low diversity (Kruskal-Wallis p-value for families: 0.3281; Kruskal-Wallis *p-value* for species: 0.2309) (Fig. 3a and b).

On the other hand, in terms of differentiation between sampling sites

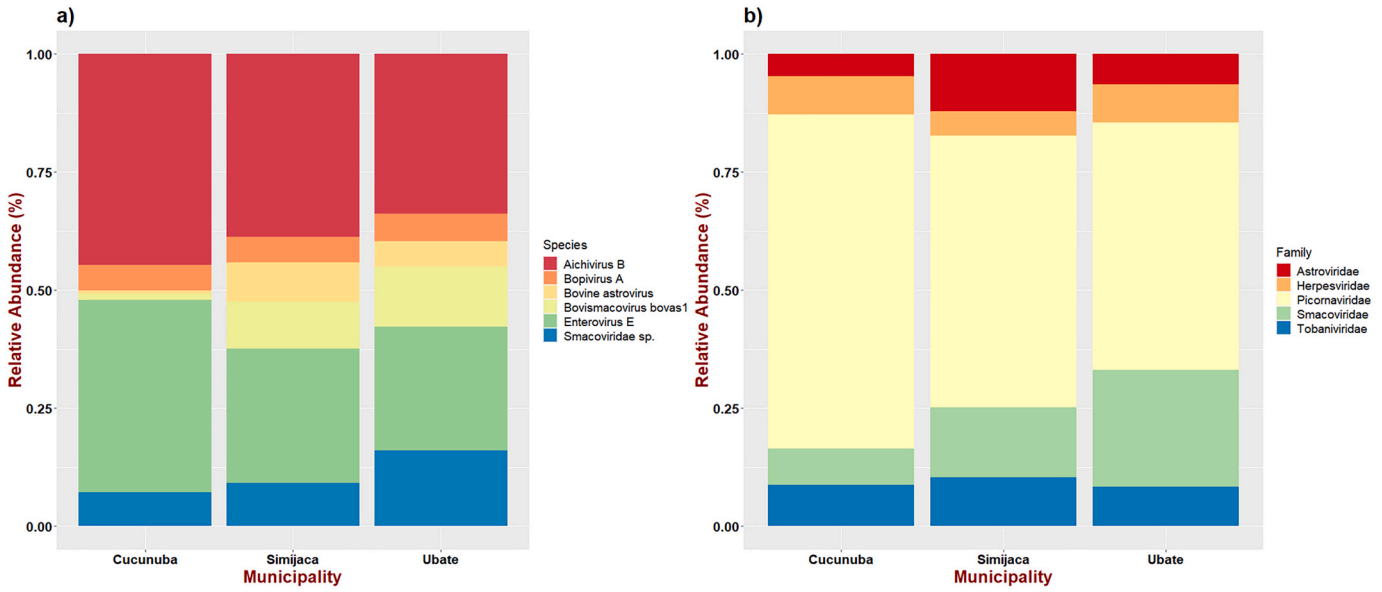

Fig. 2. Composition of viral species and families in bovine fecal samples from the Ubaté Province.
The bar graphs show the relative abundance of (a) viral families and (b) viral species detected by metagenomic sequencing at the municipal level. The figure was created in Rstudio using the ggplot2 package.

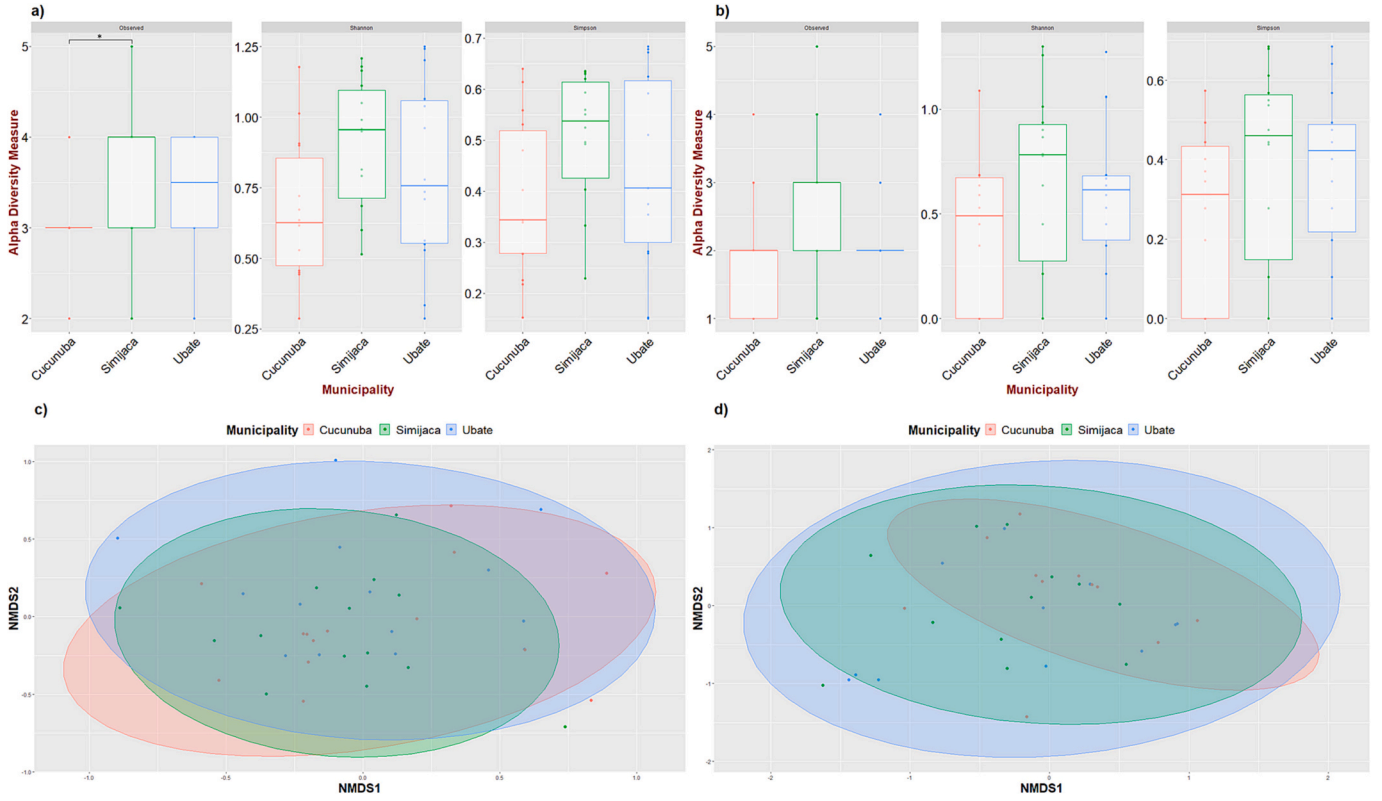

Fig. 3. Analysis of viral community diversity in bovine fecal samples.
Comparison of alpha diversity measures among municipalities in the Ubaté Province. Group differences were determined using a Kruskal-Wallis test at the viral family level (a) (Number of families: 0.01948; Shannon index: 0.1155; Simpson index: 0.3281) and viral species level (b) (Number of species: 0.1032; Shannon index: 0.2583; Simpson index: 0.2309). Pairwise differences between each pair of municipalities were determined using a posthoc Dunn test (*: $p < 0.05$). Beta viral diversity in fecal samples from cattle in municipalities of the Ubaté Province. Non-metric multidimensional scaling based on Bray-Curtis dissimilarity matrix. Similarity analysis and PERMANOVA at the family level (c) (R statistic: −0.02958; Significance: 0.814 | F: 0.73968; Pr > F: 0.7273) and at the species level (d) (R statistic: −0.03436; Significance: 0.851 | F: 0.53463; Pr > F: 0.8182).

(beta diversity), no significant changes were observed in the composition of the virome, with a considerable overlap observed among the municipalities. This pattern is evident at both the viral family level (Similarity analysis: R statistic: −0.02958; Significance: 0.814 | PERMANOVA: F: 0.73968; Pr > F: 0.7273) and the viral species level (Similarity analysis: R statistic: −0.03436; Significance: 0.851 | PERMANOVA: F: 0.53463; Pr > F: 0.8182) (Fig. 3c and d).

3.4. Phylogenetic reconstruction from viral partial assemblies

Partial assemblies are valuable for genomic research as they provide an approximate representation of the genome, revealing aspects of its structure, organization, and genetic content. They offer insights into phylogenetic relationships, genetic variations, and can aid in understanding virulence factors. Additionally, partial assemblies facilitate the identification of genes involved in various biological processes and pathways. Using the Genome Detective Tool, we performed reference-based partial assemblies, resulting in the generation of specific sequences for Enterovirus E, Bovine Astrovirus, and Bovine Kobuvirus. The Enterovirus E sequence obtained from a sample collected in the municipality of Simijaca (length: 1290 bp; coverage: 18%; Nucleotide Identity: 80%; #Contigs: 1; *E*-Value: 0). From a sample of the same municipality we obtained a sequence of BoAstV (length: 6334 bp; coverage: 99%; Nucleotide Identity: 81%; #Contigs: 1; E-Value: 0). Furthermore, a Bovine Kobuvirus sequence obtained from a sample collected in the municipality of Ubaté (length: 8461 bp; coverage: 95%; Nucleotide Identity: 89%; #Contigs: 1; E-Value: 0).

Phylogenetic analyses were performed using partial sequences from specific coding regions corresponding to each virus species. Additionally, sequences from other representative species within the same genus, including viruses associated with humans or other farm animals, were incorporated into these phylogenetic trees. For Enterovirus E, the VP1 region, which plays a role in receptor binding on the cell surface, was analyzed (Ji et al., 2021). Clade separation was observed among Enterovirus species commonly found in cattle (EVE and EVF). The EVE sequence identified in this study is closely related to previously characterized sequences from various countries, including Brazil (OL660538.1) (Best-fit Model: TVM + F + 1 + G4) (Fig. 4a). For Bovine Astrovirus, the ORF2 region, which encodes the capsid proteins, was selected. Amino acid identity >75% is used for Astrovirus classification as the same species (De Benedictis et al., 2011). The BoAstV sequence from the Simijaca municipality clustered together with sequences found in bovine feces from Japan and Hong Kong (LC047796.1; HQ916313.1) (Best-fit Model: SYM + I + G4) (Fig. 4b). The Bovine Kobuvirus genome consists of three structural regions (VP0, VP3, and VP1) and seven non-structural regions (2 A-2C and 3 A-3D). The region of the 3D gene, which encodes a viral RNA-dependent RNA polymerase (RdRp), is highly conserved and commonly used for phylogenetic analyses (Reuter et al., 2011). Sequence identities at the nucleotide level for Bovine Kobuvirus can vary between 74% and 81%. The Ubaté partial assembly sequence grouped into different clades compared to sequences from Europe and Asia, showing close relation to two recent sequences obtained from calves in Brazil (KC921391.1; KJ402443.1) (Best-fit Model: TN + F + G4) (Fig. 4c).

3.5. Annotation of Bovine Astrovirus partial assembly detected in a sample from Colombia

Using Vgas and Prokka for annotation, four coding sequences were identified in the BoAstV partial assembly obtained from a sample in Colombia. The partial assembly had a size of 6334 bp and contained one ORF1a, two partial ORF1b, and one ORF2. These coding sequences corresponded to specific regions of the viral genome. The ORF1a encoded a non-structural protein called nsp1a, while ORF1b encoded its counterpart, the non-structural protein nsp1ab. Both proteins are involved in various viral functions. The ORF2 was identified as encoding a precursor of the capsid protein, which plays a crucial role in the formation of the viral capsid structure (Fig. 5; Supplementary Table 3). This could be an indication of viral particle presence in the sample; however, it does not necessarily mean they are complete and viable. Additional complementary tests are required to confirm the presence of viable viral particles.

4. Discussion

The use of next-generation sequencing technologies has greatly advanced our understanding of infectious diseases by allowing us to characterize viral agents present in samples. Metagenomic approaches have been instrumental in identifying important viruses and even discovering previously unknown ones (Blomström, 2011; Govender et al., 2021). In our study, we employed an Oxford Nanopore Technologies metagenomic approach to analyze the virome of fecal samples from cattle in municipalities that are key centers of animal production in Colombia.

The most common representatives of vertebrate viruses were found in fecal samples, and no new species or striking proportions were reported. At the family level, we found that *Picornaviridae* and *Astroviridae* viruses were commonly detected in the samples. These two families are among the most frequently found in farm animals, including cattle, regardless of the animal's health status (Kwok et al., 2020b). We also identified viruses belonging to the *Smacoviridae* family, previously known as CRESS-DNA viruses. These viruses have been detected in fecal samples from a variety of farm animals, including sheep, pigs, camels, and cattle, suggesting their widespread presence in nature. While the exact reasons have not been fully determined, high rates of mutation and recombination are considered potential key factors (Zhao et al., 2019). These have been observed in CRESS virus of birds and pigs, for example. It is noteworthy that none of these CRESS-DNA viruses have been cultivated or directly isolated from the samples (Steel et al., 2016). These viruses were solely identified using metagenomic approaches, emphasizing the significance of these methods in veterinary research and indicating a potential for vast diversity. Devoting efforts to cultivate and study representatives of the major families is essential to unravel various aspects, including their potential pathogenicity, through in-depth biological characterizations (Zhao et al., 2019). Among the detected ruminant viruses, we identified six different viruses, including Bovismacovirus bovas1, which is commonly found in cattle feces and with an unknown pathogenic potential. We identified viruses potentially acting as enteric pathogens in cattle, including Bopivirus A and Bovine Kobuvirus, the latter of which has been described in virome studies involving other bovines (Chen et al., 2015). Similar findings were reported in a study conducted in the Sichuan Province, China, which investigated diarrheic calf samples and found a comparable number of viruses, with a high proportion of bovine enteroviruses (Guo et al., 2018).

We present the frequencies of the identified viruses using viral metagenomics (Table 1). Our findings show that BKV had a frequency of 52%, which is consistent with reports from other countries such as Canada (56.2%), Brazil (18.8%), South Korea (34.6%), and Egypt (66.7%) using molecular methods (Jeoung et al., 2011; Mohamed et al., 2017; Ribeiro et al., 2014; Savard et al., 2022). Bovine Astrovirus was detected in 19% of the samples, like reports from other Latin American countries like Brazil (14.34%) and Uruguay (25.6%) (Candido et al., 2019; Castells et al., 2020). Enterovirus E had the highest frequency in our study at 59%, while Spain reported a frecuency close to 78% and lower values were reported in China (10.7%) and the USA (15%) (Jiménez-Clavero et al., 2005; Luo et al., 2023). It is important to note that the high rates observed in the Ubaté Province may be influenced by the relatively small sample size in our study ($n = 42$). These descriptive findings present potential scenarios for the management and comprehension of viral infections in the country. Firstly, these findings suggest that enhancing our understanding of the role of these viruses in bovine

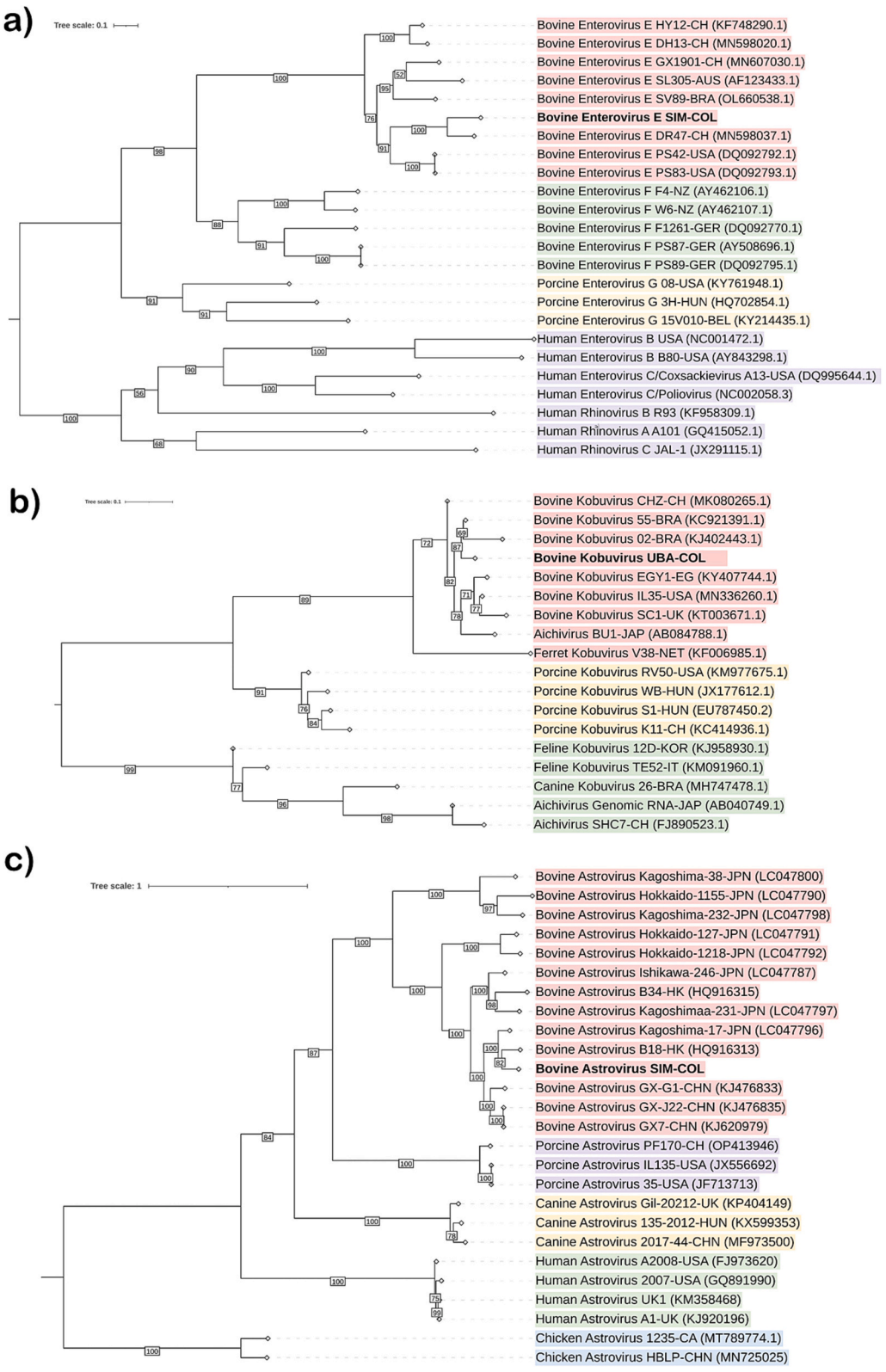

Fig. 4. Phylogenetic analysis of viruses detected in bovine feces in the Ubaté Province.
The phylogenetic trees were reconstructed using maximum likelihood analysis with topology evaluation based on 1000 bootstrap replicates. The accession numbers of the sequences obtained from the NCBI database are shown in parentheses. The numbers on the nodes represent the percentage of bootstrap support. Viruses found in this study are highlighted in bold. a) Bovine Enterovirus E. Analysis based on partial nucleotide sequences corresponding to the VP1 gene. b) Bovine Kobuvirus. Analysis based on partial nucleotide sequences corresponding to the RdRp gene. c) Bovine Astrovirus. Analysis based on partial nucleotide sequences corresponding to the ORF2 gene.

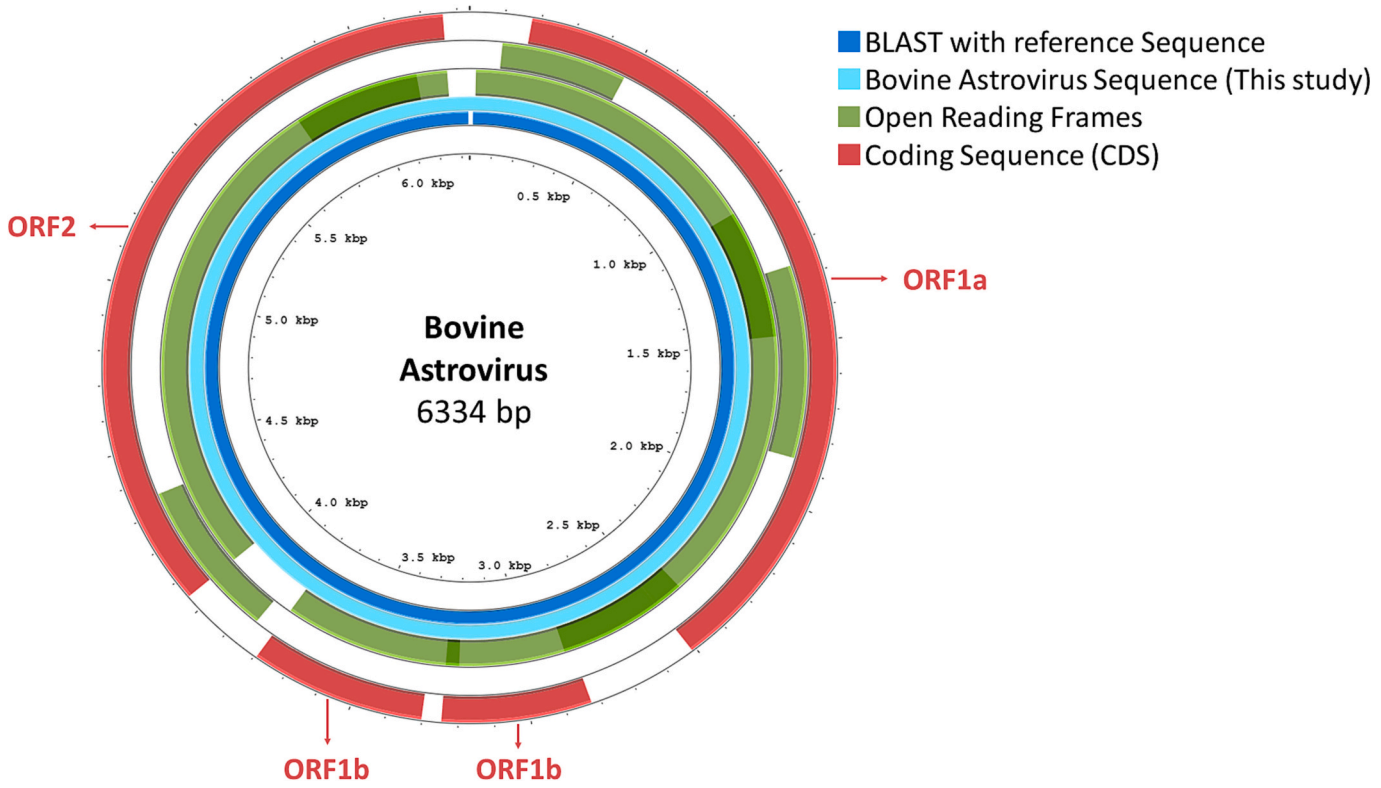

Fig. 5. Genomic characterization of Bovine Astrovirus.
Coding sequences were determined through annotation in VGAS and Prokka. Visualization and editing were performed using Proksee. Only ORFs with a size >100 codons were considered. Accession number of the reference sequence: NC_023631.1.

livestock physiology is crucial. This understanding can then pave the way for identifying key factors influencing their transmission and impact on animal health. Consequently, it can guide the development and implementation of targeted strategies to mitigate the spread of viral diseases among the Colombian cattle population. Such strategies may involve implementing stringent biosecurity protocols, conducting regular surveillance, and establishing customized vaccination programs to address specific viral threats. Additionally, these findings underscore the importance of both epidemiological and genomic surveillance. Such surveillance efforts help determine the endemicity of these viral agents and provide a better understanding of their presence in the bovine population. Furthermore, genomic surveillance can aid in identifying evolutionary processes and routes of viral dispersion. This knowledge can eventually lead to identifying phenotypic associations, enabling early interventions.

We observed a consistent pattern of low viral diversity across different sampling sites (Fig. 3). In virome studies, plant viruses and bacteriophages represent a substantial proportion of viral communities. This has been evidenced in studies, such as the one characterizing the virome diversity across multiple ruminants' rumen, where over 99% of the classified viruses belonged to the Caudovirales order—a pattern also observed in the human gut virome (Yan et al., 2022). Bearing this in mind, we attribute the limited diversity of viruses in part to the database used. Although we acknowledge the significant role bacteriophages play in host energy acquisition and modulating bacterial communities, our focus was solely on host-specific eukaryotic viruses. Hence, we utilized a filtered database of complete genomes containing sequences solely from the *Bovidae* family. Similarly, this can be attributed to the limited number of confirmed viral agents and the predominance of two main viruses, EVE and BKV. Enteroviruses have a stable presence in the gastrointestinal tracts of their host animals and are excreted in large quantities through feces. They can also persist in the environment for extended periods, increasing the risk of infection for healthy animals (Income et al., 2018). Bovine Enteroviruses have been detected in water sources near livestock farms, where the genetic sequences closely resemble those found in bovine feces, indicating the potential for environmental contamination (Income et al., 2018; Jiménez-Clavero et al., 2005). While cattle are considered the primary reservoir for EVE, other farm animals have also been found to be infected. Additionally, there is evidence of viral replication in the cells of sheep, goats, and horses, along with their high frequency, suggesting potential transmission and persistence in the population and highlighting their possible zoonotic role (Fieldhouse et al., 2018; Gür et al., 2019).

The Oxford Nanopore sequencing technology has proven to be a powerful tool in virus research and viral epidemiological surveillance (Cook et al., 2021). In our study, we successfully generated a partial partial assembly corresponding to an Enterovirus E in the municipality of Simijaca (Fig. 4a). Enteroviruses belong to the *Picornaviridae* family and are categorized into 12 species, including 3 species of Rhinoviruses (RV-A, B, C) and 9 species of Enteroviruses (EV-A, B, C, D, E, F, G, H, J). Among these, Enterovirus E (EVE) is commonly found in animals. This virus has a non-enveloped, icosahedral structure and a positive-sense, single-stranded RNA genome, and it has been previously isolated from cattle in different regions of the world (Zhang et al., 2021).

Enterovirus E has been implicated in various clinical manifestations in cattle, including enteric, reproductive, and respiratory diseases. However, it has also been detected in the feces of apparently healthy animals, and its pathogenicity and virulence are still not fully understood (Blas-Machado et al., 2011). EVE has been detected in bovine fecal samples from several countries, including Mexico, the USA, Japan, and China (Chen et al., 2015; Guo et al., 2018; Mitra et al., 2016). However, there is currently no literature documenting the detection of Bovine Enterovirus E in Colombia. Hence, this study marks the first identification of this virus in the country using this molecular approach. However, since we lacked metadata associated with veterinary reports on the health status of the cattle, further investigations are needed to explore its

presence in other regions of the country and establish any potential relationship with the animals' health. Although cases of transmission to humans are limited, there is biological plausibility for cross-species transmission, highlighting the importance of surveillance at the human-animal interface (Fieldhouse et al., 2018). The presence of a potential new etiological agent, which could be involved in the presentation of enteric or respiratory symptoms, further complicates the diagnostic challenges for veterinarians. The overlapping symptoms with other diseases make it difficult to identify and pinpoint the exact cause of the illness in livestock. Moreover, the productivity and reproduction of the cattle could be adversely affected, leading to significant economic losses for the producers. For instance, the manifestation of symptoms could hinder the trade and exchange of animals, disrupting the commercial activities in the livestock industry.

Bovine Kobuviruses, like Enteroviruses, are commonly found in bovine feces and can exhibit a high viral load. In a previous study by Savard et al., they reported Ct values <20 in 30 positive samples, indicating a significant presence of the virus (Savard et al., 2022). Moreover, the management and conditions of livestock within the framework of Colombia's economic activity may have played a role in the observed homogeneity of the virome composition. The confinement of animals in relatively small spaces, higher population density, and similar care practices associated with cattle domestication have created an environment conducive to the emergence and spread of viruses in new populations. Additionally, the commercialization of cattle in areas with shared geographic conditions has facilitated the transmission of these viruses, amplifying their impact on bovines (He et al., 2021; Hodnik et al., 2022). This scenario not only emphasizes the importance of cattle for human communities but also underscores the complex challenge posed by infectious dynamics, which involve multiple levels of interaction and intervention.

In Ubaté, we identified Bovine Kobuvirus from the Picornaviridae family (Fig. 4b). This virus induces gastrointestinal infections in cattle and has been observed in diseased cattle globally. For instance, its complete genome sequence was generated via NGS in Egypt (Mohamed et al., 2017). Recent reports from the Americas, particularly Brazil, have also noted BKV occurrences, displaying genotypic differences across regions (Candido et al., 2017). Our study marks the first detection of Bovine Kobuvirus in Colombia, implying a possible transmission route between Colombia and Brazil through the exchange of infected animals. Further investigation is necessary to comprehend the transmission direction and stages, emphasizing the necessity for enhanced surveillance and monitoring in potentially affected regions. The role of these viral infections in Colombian cattle warrants thorough examination due to limited available information on the cattle's health status in our study. Although no anthropozoonotic events involving BKV have been reported, the possibility of host jumps and interspecies transmission remains due to close contact and the fecal-oral route. Notably, Kobuviruses' evolution has shown recombination events and host barrier crossings, necessitating continuous vigilance (Khamrin et al., 2014; Lu et al., 2018).

In Simijaca, another viral partial assembly was constructed, this time revealing a BoAstV, a species belonging to the Mamastrovirus genus and the *Astroviridae* family (Fig. 4c). This virus has been detected in many countries worldwide, where it is known to affect cattle, causing intestinal, digestive, and even neurological diseases. Frequency studies have been conducted in Europe (Switzerland), the Americas (Brazil and Uruguay), and Asia (China) country where mNGS was employed and the frequency was similar to that found in this study (25% and 19%, respectively). However, no cases of infection by this virus in Colombian cattle have been previously reported (Alfred et al., 2015; Bouzalas et al., 2014; Castells et al., 2020; Chen et al., 2015). Therefore, this study provides the first sequence of the virus in Colombia and one of the few sequences available in the southern hemisphere.

The Bovine Astrovirus shows a broad tropism for different types of tissues, making it unclear which cells are responsible for its proliferation and transmission. However, a study from Uruguay on cattle with encephalitis caused by BoAstV reported a high frequency of enteric infection, suggesting a fecal-oral route of transmission (Doncel Díaz et al., 2022). Additionally, the virus has the potential to synergize with other viruses, particularly in calves and immunocompromised animals, leading to more severe gastrointestinal disorders such as diarrhea. Therefore, the identification and characterization of this virus are crucial, confirming its presence in the continent. During the annotation process of the viral partial assembly, it was observed that the coding region ORF1b did not show significant similarity with the reference Bovine Astrovirus, despite encoding an RNA-dependent RNA polymerase that is typically conserved. However, a high percentage of nucleotide identity (>80%) was observed in the ORF2 region, which is commonly used for phylogenetic analysis of the Mamastrovirus genus (Supplementary Table 3). This clustering with sequences from China, derived from fecal samples from cattle, provides insights into the potential effects of the virus. Phylogenetic comparisons generally indicate that astroviruses derived from nervous tissues are related to isolates previously associated with encephalitis, while those derived from feces are associated with enteric or asymptomatic presentations (Zhu et al., 2022).

Evolutionary analysis elucidates genetic diversity drivers, highlighting viral evolution, dissemination, and potential inter-species transmission. This knowledge is pivotal for anticipating and mitigating zoonotic risks, enhancing public health strategies, and bolstering disease surveillance (Harvey and Holmes, 2022). Evidence suggests the possibility of anthroponotic infections in humans with animal enteroviruses. In Turkey, a seropositivity rate of 33% for Enterovirus E was observed in healthy individuals, indicating potential infection through contact with contaminated bovine feces (Gür et al., 2019). Genomic changes in enteroviruses can lead to genetic diversity and facilitate the crossing of host species barriers. Recombinant strains of bovine enterovirus and porcine enterovirus have been detected in sheep fecal samples, highlighting the potential for interspecies transmission (Boros et al., 2012). Phylogenetic analysis using complete genomes has revealed the clustering of some Bovine Astroviruses with porcine and ovine Astroviruses, suggesting the ability to infect other farm animals. Additionally, there is a significant risk of new astrovirus recombinants emerging and potentially infecting humans (Wohlgemuth et al., 2019). Recombination events with Porcine Astroviruses, for instance, are of concern in farm animals and their interactions with other species like cattle (Ulloa and Gutiérrez, 2010). Viruses that can overcome adaptation restrictions often cause more severe diseases in the new host, as seen in alpacas with Bovine Enterovirus F, where illness progresses rapidly and severely (McClenahan et al., 2013). Given the rising prevalence of zoonotic pathogens, it is crucial to carefully examine the potential role domestic animals may play as sources of diseases. The high rate of human-cattle contact, coupled with inherent commercial dynamics, creates a framework where professionals in the veterinary, environmental, and human health sectors must collaborate to enhance our understanding of zoonotic ecology and prevention, underscoring the importance of a One Health approach. When constructing phylogenetic trees, it is crucial to acknowledge that different model selections can result in diverse trees due to variations in data adjustments. Information criteria (BIC, AIC, AICc) may exhibit biases such as overfitting or underfitting, being sensitive to parameters like sample size and statistical assumptions (Susko and Roger, 2020). Moreover, selecting a specific region for phylogenetic analysis requires consideration of various factors including the rate of variation, evolutionary conservation, and suitable length (Ji et al., 2022).

In our descriptive study, we characterized the virome in one transmission route of veterinary agents, providing insights into the gastrointestinal health of cattle. However, there are methodological limitations to consider in future studies. We obtained reads of lengths that, although longer than those obtained with other platforms, were somewhat short for ONT standards. This discrepancy can be attributed

to several factors, such as the inherent nature of viral genomes, which are inherently smaller and structurally more variable. Experimental and environmental conditions may also play a role, as lower viral concentrations or association with other cellular components can increase the likelihood of fragmentation during extraction. To minimize this impact, we suggest working with recently collected samples. However, we were able to reconstruct partial assemblies that met the necessary parameters for length, coverage, and quality, allowing for well-supported phylogenetic relationships to be established. We lacked information on the health status of the sampled cattle, and viral compositions can vary between symptomatic and asymptomatic animals (Ng et al., 2015). Comprehensive evaluations should involve multiple sample types to capture viral diversity and consider the tropism and transmission routes of the agents involved. Fecal sampling only represents distal portions of the gastrointestinal tract, potentially underestimating upper regions (Shkoporov et al., 2022). The underrepresentation of South American studies affects our understanding of viral evolution and classification. Further advancements in bioinformatics and metagenomic next-generation sequencing approaches will enhance our ability to detect novel organisms and provide valuable insights into the ecology and evolution of ruminant-associated viruses (Nooij et al., 2018).

5. Conclusions and perspectives

The application of metagenomic sequencing techniques provided valuable insights into the virome present in bovine fecal samples and emphasized the importance of virological surveillance in veterinary research. Our study revealed a consistent and uniform composition of viral communities in Holstein breed or crossbreeds of Normado-Holstein across different municipalities at the same region, suggesting similar bio-sanitary, care, and climatic conditions, as well as a high commercial flow in the region. The predominance of commonly. The predominance of commonly found fecal viruses and the low abundance of confirmed pathogens may contribute to this homogeneity. We identified several viruses of veterinary importance, including BoAstV, EVE, and BKV, which were reported for the first time in Colombia. Additionally, the presence of a CRESS Virus in animal feces was confirmed. Our findings shed light on the frequency of these potentially pathogenic viruses and emphasize the need for further research to establish possible correlations with disease occurrence by considering associated metadata, such as symptom presentation. Based on the abundance and frequency of these viruses, we recommend that diagnostic testing, whether it is routine or non-routine, should include these viruses as targets. By doing so, it ensures the early detection and timely intervention of markers of interest in the population. Additionally, incorporating these targets will prove valuable in specific situations such as suspected pathogen cases, outbreak investigations, or research studies where a comprehensive approach to disease detection is required. This will contribute to a better understanding of their impact on animal health and the economic activity associated with livestock production.

Declaration of generative AI and AI-assisted technologies in the writing process

Statement: During the preparation of this work the author(s) used ChatGTP in order to improve readability and language. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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Authorship

JEM, LP, MC, DJG, APM, MM participated in the standardization and experimental design process. JEM, SC, MG, NL, ALR analyzed the data and performed bioinformatics analyses. JEM wrote the manuscript. JDR led the project and wrote the manuscript. All authors have read and approved the final version of the manuscript.

CRediT authorship contribution statement

Julián Esteban Medina: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – original draft. **Sergio Castañeda:** Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Writing – review & editing. **Luisa Páez-Triana:** Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Writing – review & editing. **Milena Camargo:** Investigation, Methodology, Resources, Writing – review & editing. **Diego J. Garcia-Corredor:** Investigation, Methodology, Resources, Writing – review & editing. **Marcela Gómez:** Formal analysis, Investigation, Methodology, Resources, Writing – review & editing. **Nicolas Luna:** Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Angie L. Ramírez:** Formal analysis, Investigation, Methodology, Resources, Writing – review & editing. **Martín Pulido-Medellín:** Investigation, Methodology, Resources, Writing – review & editing. **Marina Muñoz:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – review & editing. **Juan David Ramírez:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2023.105543.

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