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Article

Tuberculosis and Intestinal Parasites in Wayuu Indigenous Communities in La Guajira, Colombia: A One Health Approach

Adriana Arevalo-Jamaica [1], Yussely Tatiana Cobos-Leon [1], Jhindy Tatiana Pérez-Lozada [2], Beatriz Elena De arco-Rodriguez [2], Dioselina Peláez-Carvajal [2], Claudia Marcela Castro-Osorio [3], Luisa Fernanda Vasquez-Chavez [3], Mayra Alejandra Vargas-Rojas [3], Vivian Vanesa Rubio [3], Sonia Lorena Valencia-Claros [3], Carlos Esteban Franco-Muñoz [1], Amith Arelis Aldana Lyons [4], Anderson Ramírez Ayala [4] and Gloria Mercedes Puerto-Castro [3,*]

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Abstract

Acute diarrheal disease (ADD) caused by parasites and TB represent a significant public health burden worldwide and in Colombia, particularly affecting indigenous populations who are at high risk of contracting these diseases due to the social, environmental, and cultural conditions in which they live. Materials: Fifteen Wayuu indigenous communities in four areas of Manaure, in La Guajira, were subject to intervention; with prior informed consent, environmental samples and samples from individuals with clinical symptoms were collected. A total of 156 samples of human and animal feces, soil, and sediment from drinking water were analyzed for microscopic using the Kato–Katz and formalin–ether concentration techniques, 109 samples were analyzed by qPCR for the detection of helminths and 23 for metatranscriptomics targeting protozoan parasites and helminths. Additionally, 36 sputum samples from patients with respiratory symptoms were tested using Xpert/MTB Rif, and 37 milk samples were tested for *M. bovis*. Results: Among the samples tested for tuberculosis, a positivity rate of 8.3% was found, in all cases with sensitivity to rifampicin; *M. bovis* was not found in animal milk. Microscopic analysis of human samples revealed pathogenic parasites, the most common being *Blastocystis* spp. and the *Entamoeba hystolitica/Entamoeba dispar* complex each with 38.8% (n=38), *Giardia* spp. with 19.4%, *Hymenolepis nana* and *Trichuris trichiura* each with 5.1%. Commensal parasites were also identified as indicator of poor sanitary conditions. Co-infection with intestinal parasites was common in humans at 60.2%. In microscopic analysis of animals fecal samples, revealed a high incidence of *Uncinaria* spp. with 58.3%, amoebas 16.7% and *Giardia* 8.3%; this latter is also found in soil. Metatranscriptomics showed a high frequency of intestinal parasites in fecal samples (90.9%), with *Blastocystis* spp. being the most frequent (81.8%) with notable intra-species diversity, followed by *Entamoeba histolytica* (54.5%) and *Giardia duodenalis* (31.8%), and detected free-living amoebae in community water sources, highlighting potential health risks associated with exposure to untreated water in low-sanitation settings. Conclusions: The Wayuu communities studied show a significant burden of tuberculosis and intestinal parasitic infections, likely associated with poor sanitary conditions and environmental factors that facilitate their transmission. Although TB prevalence was moderate, with no evidence of rifampicin resistance or the circulation of *M. bovis* in milk, the high prevalence of intestinal parasites, including co-infections and their detection in humans, animals, and the environment suggest active transmission in the

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region. These findings highlight the need to implement comprehensive interventions in water, sanitation, and hygiene, along with surveillance and health education strategies with an intercultural approach, aimed at improving the conditions of these vulnerable populations.

Keywords: parasites; acute diarrheal disease ADD; meta transcriptomic sequencing; Wayuu indigenous; Colombia; tuberculosis; vulnerable populations

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1. Introduction

In Colombia, tuberculosis (TB) and acute diarrheal disease (ADD) remain major public health concerns, particularly in socially and geographically vulnerable populations, including indigenous communities [1,2]. Among these populations, the Wayuu people constitute one of the largest indigenous groups in the country and are primarily located in the department of La Guajira, in the northernmost region of Colombia, which is characterized by an arid climate throughout the year. The Wayuu people are of Amerindian descent and are traditionally organized into clans known as e'irukuus. They maintain their own language, Wayuunaiki, and preserve a distinct cultural identity within the region [3,4].

The Wayuu communities face persistent challenges related to limited access to safe water, health services, food security, and citizen participation [5]. In La Guajira, mortality rate from acute diarrheal disease in children under five is 28.95 cases per 100,000 inhabitants, more than 5.8 times higher than the national rate [6]; as for TB, the incidence rate is 61.20 cases per 100,000 inhabitants, contrasting with the national average of 38 cases per 100,000 inhabitants; on average, approximately 510 TB cases are reported annually in La Guajira [7]. These indicators highlight the persistent burden of infectious diseases in this region and underscore the need for integrated surveillance and control strategies.

Health models propose an integrated and unifying approach that seeks to balance and optimize the health of people, animals, and ecosystems in a sustainable manner, recognizing that these three dimensions are closely linked and interdependent; that is, changes in any one of them can influence the occurrence of diseases in others [8]. Such an approach is particularly relevant in rural and indigenous settings, where close interactions between people, animals, and shared environmental resources can increase the risk of the emergence and transmission of existing or new diseases among humans and animals.

To address the challenge of TB and EDA in vulnerable populations and analyze it within the One Health approach, a study was conducted in fifteen Wayuu communities in the municipality of Manaure in La Guajira to identify circulating pathogens using conventional and molecular methods, including metagenomics, to understand the relationships between the dimensions and propose interventions tailored to indigenous communities. This approach can be useful in supporting local epidemiological surveillance, outbreak control, and infectious disease care strategies in vulnerable and geographically remote populations.

2. Materials and Methods

2.1. Participants, Samples and Study Area

Between November 2023 and March 2025, a sampling was conducted under the program "Formulation of a Strategy for the Prevention, Control, and Management of Communicable Diseases with a One Health Approach in Manaure, La Guajira". The study included inividuals from the Wayuu population belonging to the indigenous communities of Ichien, Polousira (Santa Rosa zone), Parritchon, Kauracira, Paliawo (Sabana zone), Pasito de la Raya, Kousepa, Pondores, Sabana Larga (Mayapo zone), Lapuntachon, Mekoloquimana, Yuselamana, Ratip e Ishamana (Pajaro zone) (Figure 1). After obtaining informed consent and authorization from the indigenous authority, environmental samples and samples from animals and people with symptoms of tuberculosis or acute diarrhea were collected, as follows: 36 sputum samples were collected for microbiological and

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molecular analysis for TB. Moreover 156 samples for microscopic analysis of parasites, included 98 human fecal samples, 12 animal fecal samples (goat, pig, cow, sheep, and peacock), 42 soil samples, and 4 drinking water sediment samples. In addition, 109 samples were analyzed by quantitative PCR (qPCR) for the detection of geohelminths. These comprised 51 human fecal samples, 12 animal fecal samples, 42 soil samples, and 4 water samples collected from a jagüey, a rainwater pond commonly used for human consumption. Finally, 23 human stool samples preserved in RNAlater were processed for metatranscriptomic analysis. Furthermore, 49 samples of milk from cattle, sheep and goats were also collected for *Mycobacterium bovis* analysis. All samples were transported under cold chain conditions to the Laboratories of National Institute of Health in Bogotá, Colombia for processing.

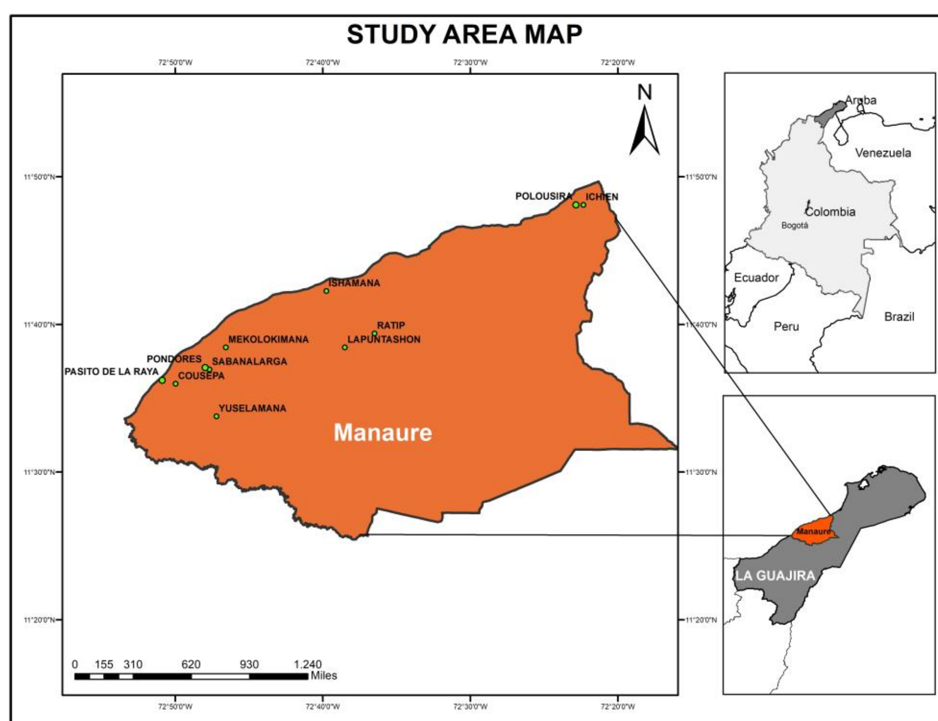

Figure 1. Location map of the study area. The map shows Colombia with the department of La Guajira shaded in dark gray. A zoomed view of the municipality of Manaure, shown in terracotta, highlights the Indigenous communities. For national context, the location of Bogotá is also indicated. The map was created using ArcGIS v10.6. .

2.2. Detection of Tuberculosis in Human Samples: Xpert MTB/RIF Assay for TB

Sputum processing was performed according to the manufacturer's instructions [9]. In summary, in a biological safety cabinet, the sample reagent (a mixture of NaOH and isopropanol) was added to each sputum sample in a 1:2 ratio in a Falcon tube. The mixture was shaken vigorously until a clear solution was obtained and incubated at room temperature for 15 minutes. Next, 2 ml of clear solution was added to the labeled cartridge using a sterile Pasteur pipette, and the cartridge was incubated in the system GeneXpert Dx. The results were obtained after 1.5 hours. Samples that tested positive were tuberculosis confirmed by Xpert MTB/RIF.

2.3. Cultured on BACTEC MGIT 960™ for TB

An aliquot of the decontaminated sputum sample was cultured on BACTEC MGIT 960™ systems (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and solid LJ media. Identification of *M. tuberculosis* complex was performed from the axenic colonies by the method

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STANDARD Q TB MPT64 Ag Test (SD Biosensor, Suwon, South Korea). It is a rapid chromatographic immunoassay for the qualitative detection of MPT 64 antigen.

2.4. Detection of Tuberculosis in Animal Samples: Processing Milk for Bovine TB. Cultured on BACTEC MGIT 960™ for TB

Aseptically, 50 mL of milk was collected and centrifuged at 4,000 × g for 30 min at 4°C. 2ml of the sediment was decontaminated with 3 ml of lauryl sulfate of sodium, incubating at ambient temperature for 30 minutes. Subsequently, 3ml of neutralizer was added and left to act for 2 minutes. Later, this was centrifuged for 30 minutes at 4,000 x g and 4°C. The supernatant was discarded and the sediment was re-suspended with distilled sterile water. The sediment obtained was cultured by duplicate in culture middles: Lowenstein-Jensen and Stonebrink-Giraldo's modified (STG). The cultures were incubated at 37ºC for growth readings, weekly up to completing 12 weeks when the negative cultures were discarded [10].

2.5. DNA Extraction for PCR Mycobacterium bovis

An aliquot of sediment obtained by processing of each one of milk, were submitted to extraction of DNA according to the recommendations of van Soolingen *et al* [11], method based in CTAB, chloroform-isoamyl alcohol, and precipitating with isopropanol. Every DNA sample obtained was re-suspended in a buffer TE 0.1X. The quantification of DNA was performed using a Qubit™ 4 Fluorometer with the Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA), and DNA quality was assessed using a NanoDrop™ Lite Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.6. PCR Mycobacterium bovis

The reaction mixture was prepared using FastStart Essential DNA Green Master (Roche Diagnostics, Indianapolis, IN, USA), which contains a ready-to-use double-concentrated PCR master mix. Primers were added, specific to the 16S, RD9 and IS6110 genetic regions [12,13]. Amplification was performed in a real-time PCR thermocycler. Positive controls were incorporated using *M. bovis* and *M. tuberculosis* H37Rrv reference DNA, negative control and nuclease-free water. Amplification was monitored by observing the increase in Sybr green fluorescence, and melting-curve analysis.

2.7. Detection of Parasites: Microscopy Analysis of Stool Samples (Human, Animal) and Soil Samples

The coproparasitological analysis of the samples was performed using the Kato-Katz and modified formalin–ether (SAF–ether) concentration techniques at the Parasitology Research Laboratory of the National Institute of Health. For Kato-Katz, fecal and soil samples were filtered to remove impurities and transferred to 15 mL conical tubes, filling the volume to 10 mL with sodium acetate-acetic acid-formalin (SAF) solution. After centrifuging at 2,264 × g for 10 minutes, the supernatant was discarded and excess moisture was removed by inverting the tubes. The sample was then deposited into the hole of a Kato-Katz slide on a microscope slide, covered with a cellophane sheet coated with glycerol, and smeared. Finally, the helminth eggs were counted under a microscope, and the result is expressed as the number of eggs per gram of feces [14].

In the case of the method modified formalin–ether (SAF–ether), the suspension is poured into a 15 ml conical tube, then 8 ml of SAF and 2 ml of ether are added. It was then centrifuged at 2,264 × g for 10 minutes and four layers were observed. The top three layers were discarded by inverting the tube. Finally, the coprological sample is prepared with Lugol's solution and saline solution and observed under a microscope.

2.8. DNA Extraction for Intestinal Parasites from Stool Samples

DNA was extracted using the NucleoSpin® DNA Stool Kit (Macherey-Nagel, Düren, Germany). Prior to extraction, samples underwent a pretreatment that included three washes with phosphate-

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buffered saline (PBS) 1x, with centrifugation at 1,369 × g for 5 minutes during each wash. DNA was finally eluted in a total volume of 40 µL, and DNA quality and concentration were assessed using a NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.9. DNA Extraction for Intestinal Parasites from Soil Sample

DNA was extracted from 250 mg of sediment and soil samples. Prior to extraction, the samples underwent three wash cycles with PBS 1x, each followed by centrifugation at 1,369 x g for 5 min. Subsequently, DNA was extracted using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research, Irvine, CA, USA), following the manufacturer's instructions. Finally, DNA quality was assessed using a NanoDrop™ Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.10. Multiplex Real-Time PCR for Detection of Geohelmints

A multiplex qPCR was performed for the identification of intestinal parasites *Ascaris lumbricoides*, *T. trichiura*, *Ancylostoma duodenale* and *Necator americanus*. Primers and probes previously described in the literature were used for Azzopardi KI et al. 2021 [15]. For the multiplex detection of geohelminths, the following protocol was used: Cools [16] adapted by the Parasitology Research Laboratory of the INS (Table 1). The reaction mix was prepared using the Luna® Universal Probe qPCR Master Mix (2X) (New England Biolabs, Ipswich, MA, USA), with primers and probes at a final concentration of 10 µM, completed with nuclease-free water and 2 µL of DNA. The reactions were set up in triplicate, including three no-template controls (NTC) and one negative control. The thermal cycling conditions were as follows: an initial cycle at 95 °C for 5 minutes, followed by 39 cycles of denaturation at 95 °C for 10 seconds and alignment/extension at 60 °C for 30 seconds. Detection was performed using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Data analysis was performed using the CFX Maestro 3.1 software, which automatically determined the cycle thresholds (Ct).

Table 1. Protocol for the Detection of Geohelminths by Multiplex qPCR.

Cycles	Step	Temperature	Time
1	Initial denaturation	95º C	5 minutes
39	Denaturation	95º C	10 seconds
	Annealing and Elongation	60º C	30 seconds

To evaluate the DNA extraction efficiency and detect the presence of inhibitors in the PCR, the pcDNA3.4-TOPO construct of 6,011 bp (Invitrogen) with the Cytomegalovirus (CMV) promoter downstream was used as an internal control in the geohelminth qPCR [17]. For its detection, the Luna® Universal Probe qPCR Master Mix (2X) (NEB, Ipswich, MA, USA) was used, along with specific primers at a concentration of 10 µM: forward primer 5′-CATCTACGTATTAGTCATCGATATTACCA-3′, reverse primer 5′-GAAATCCCCGTGAGTCAAACC-3′, and the TaqMan probe (Cy5-5: 5′-TCAATGGGCGTGGATAG-3′) along with nuclease-free water. A calibration curve was standardized using serial 1:10 dilutions processed in quintuplicate to determine the optimal dilution with a cycle threshold (Ct) close to 25. The reaction efficiency was evaluated using the correlation coefficient (R≈1), The slope was close to -3.32, with the intercept and efficiency percentage ranging between 90–110% (E). Additionally, a no-template control (NTC) was included to ensure the absence of contamination.

2.10. Processing, Concentration and RNA Extraction of Water Samples for Metatranscriptomic Analysis

A total of 20 water samples were collected from 14 rural indigenous communities, representing water sources commonly used for human consumption and domestic purposes. These included jagüey (rainwater reservoirs), shallow artisanal wells, and deep wells operated by windmills. Samples were initially subjeted to a microfiltration process, followed by tangential ultrafiltration and

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a final concentration step using AMICON® ultra centrifugal filter (10 kDa molecular weight cutoff) (Merck, Darmstadt, Germany). Subsequently, RNA was performed extrated from the concentrated samples.

2.11. Total RNA Extraction of Stool, and Soil Samples for Metatranscriptomic Analysis

RNA extraction was extracted from 250 mg of fecal material or soil sediment free of RNAlater® (Thermo Fisher Scientific, Waltham, MA, USA), obtained after centrifugation for 5 min at 15,000 × g. The samples were subjected to mechanical lysis by agitation for 15 min in ZR BashingBead™ tubes containing 0.1 and 0.5 mm zirconia beads to ensure efficient disruption and homogenization of the fecal material. Total RNA was subsequently extracted using the ZymoBIOMICS™ DNA/RNA Miniprep Kit (Zymo Research, Irvine, CA, USA), following the manufacturer's instructions. RNA concentration was determined by fluorometry using Qubit™ (Thermo Fisher Scientific, Waltham, MA, USA), and purity was assessed by spectrophotometry using NanoDrop™ (Thermo Fisher Scientific, Waltham, MA, USA). RNA integrity was additionaly evaluated by agarose gel electrophoresis.

2.12. Metatranscriptomic Sequencing

Metatranscriptomic sequencing was performed to capture the actively transcribed fraction of parasite diversity. Libraries were prepared using the automated MGISP-100 system (MGI Tech, Shenzhen, China). The purified RNA was fragmented and converted to complementary DNA (cDNA) via reverse transcription with random primers, followed by second-strand synthesis. The resulting double-stranded cDNA underwent end-repair, A-tailing, adapter ligation and PCR enrichment according to the manufacturer's RNA-seq Library Prep Kit Protocol (MGI Tech, Shenzhen, China). PCR products were subsequently purified using magnetic beads. Library concentration was quantified using the Qubit™ dsDNA High Sensitivity (HS) Assay Kit (Life Technologies, Carlsbad, CA, USA), and quality was assessed by evaluating the expected fragment size with D1000 DNA ScreenTape assays on a TapeStation 4150 system (Agilent Technologies, Santa Clara, CA, USA).

All individual libraries were pooled in equimolar amounts and subsequently circularized for DNA nanoball (DNB) synthesis. DNBs were generated from the single-stranded DNA circles obtained during library preparation and used as templates for sequencing. Library concentration was maintained at ≥2 fmol/µL, with each DNB reaction requiring 40 fmol of library in a 100 µL reaction volume. A minimum DNB concentration of 12 ng/µL was required for sequencing. Finally, sequencing was performed on a DNBSEQ-G50 platform (MGI Tech, Shenzhen, China) using a single-end 100 bp (SE100) FCL flow cell with 120 sequencing cycles.

2.13. Bioinformatics Analysis

Raw sequencing reads were quality-filtered and processed using the CZ-ID pipeline v8.3 [18]. CZ-ID is an open-source cloud platform used to identify microbial agents in NGS datasets. This pipeline integrates multiple bioinformatic tools into an automated workflow that enables researchers to analyze sequencing data without requiring local computational infrastructure. Specifically, in this study the pipeline performed the following steps: Fastp for removal of short and low-quality reads, CZID-dedup to collapse PCR/optical duplicates without affecting transcript diversity, Bowtie for the initial subtraction of host human reads, and Hisat2 for the removal of remaining host reads. The remaining reads were aligned against the NCBI nucleotide (NT) and non-redundant protein (NR) databases (index date: 6 February 2024) using Minimap2 and DIAMOND, respectively, and preliminary taxonomic assignments were generated. Reads passing filters were assembled into contigs with SPAdes, followed by refinement through BLAST-based (v2.16.0) reassignments against candidate NT and NR references, improving taxonomic accuracy. The final outputs included

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annotated contigs, refined taxon counts, coverage statistics, and non-host FASTQ files for downstream analyses.

To determine the presence of parasite taxa in each sample, five filters were applied to generate a heatmap to compare the relative presence of viral taxa across samples: 1. Category: Eukaryota; 2. NT rPM ≥ 10 (number of reads aligning to a taxon in the NCBI NT database, per million reads sequenced); 3. NT alignment length ≥ 50 base pairs; 4. NR rPM ≥ 1 (number of reads aligning to a taxon in the NCBI NR protein database); and 5. Z-score filter > 1, based on a standard background model, to retain only taxa more abundant in samples than in negative controls. NT rPM was used to estimate parasite abundance in each sample.

2.14. Ethical Considerations

The study was conducted following the principles set out in the Declaration of Helsinki and local regulations in Colombia. All participants voluntarily signed the informed consent form, previously approved by Comité de Ética y Metodologías de Investigación from INS, Colombia (CEMIN 18-2023), in addition to having the prior authorization of the indigenous leader.

3. Results

A total of 130 members of the Wayuu community were surveyed. Their ages ranged from 1 to 93 years. The most common age group was 27 to 59 (35.4%), and women made up the majority of the sample (60.8%). Among those over 18 years of age (n=67), the most common occupation was artisan (49.3%), followed by domestic work (10.4%) and various trades (7.5%), while 32.8% reported having no specific occupation. 62.6% of adults reported having no schooling, 22.4% had partially completed primary school, and 7.5% had completed it; 6.0% had partially completed secondary school, and only 1.5% had completed it. Regarding sources of water for human consumption, the settlements primarily use rainwater collection wells ("Jaguey") and windmills (40.0%), 24.6% use only windmills, 14.6% use only jaguey, 10% use both jaguey and dug wells, 7.75% use only dug wells, 2.3% use a desalination plant, and only one community purchases drinking water from the nearest urban center. A total of 94.6% of the community stated that they do not boil water before drinking it.

3.1. Tuberculosis Detection

A total of 36 sputum samples were processed using the Xpert MTB/RIF assay, with a positivity rate of 8.3% (n=3) for the MTB complex and no detection of mutation in the rpoB gene, indicating the absence of rifampicin resistance in the samples analyzed. The positive samples were also for TB MPT64 Ag Test and only one sample was positive using the BACTEC MGIT methodology cultured. Of the positive cases, one occurred in a 44-year-old woman who was the head of a household living with four minor children in the zone Santa Rosa, and two cases in young men (aged 35 and 19); in the Pájaro community; the first was an index case and the second was a contact identified during a second visit to the community.

3.2. Tuberculosis Detection

All milk samples analyzed were negative for *M. bovis*.

3.3. Detection of Parasites

Microscopic analysis using the Kato–Katz and formalin–ether concentration tech-niques enabled the identification of various intestinal parasites in samples of human and animal feces and in soil from different communities. The representative photo-graphs illustrate the morphological characteristics of the eggs of *Chilomastix mesnili*, *Entamoeba coli*, *Giardia* sp., *Hymenolepis diminuta*, *Entamoeba hartmanii*, the *E. histolytica*/*E. dispar complex*, *H. nana*, *Iodamoeba butschlii*, *Endolimax nana* and *T. trichiura* in samples of human feces, larvae in soil, and *Uncinarias* spp. in animal feces. The observation was initially conducted using a 10x objective, followed by the addition 40x.

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Morphometric measurements were subsequently performed using ImageJ software (V 1.54 g) National Institutes of Health, Bethesda, MD, EE. UU.) to this end, the images were calibrated according to the microscope objective scale, and morphometric measurements of length were taken; the values obtained were compared with those reported in the literature for species identification (Figure 2).

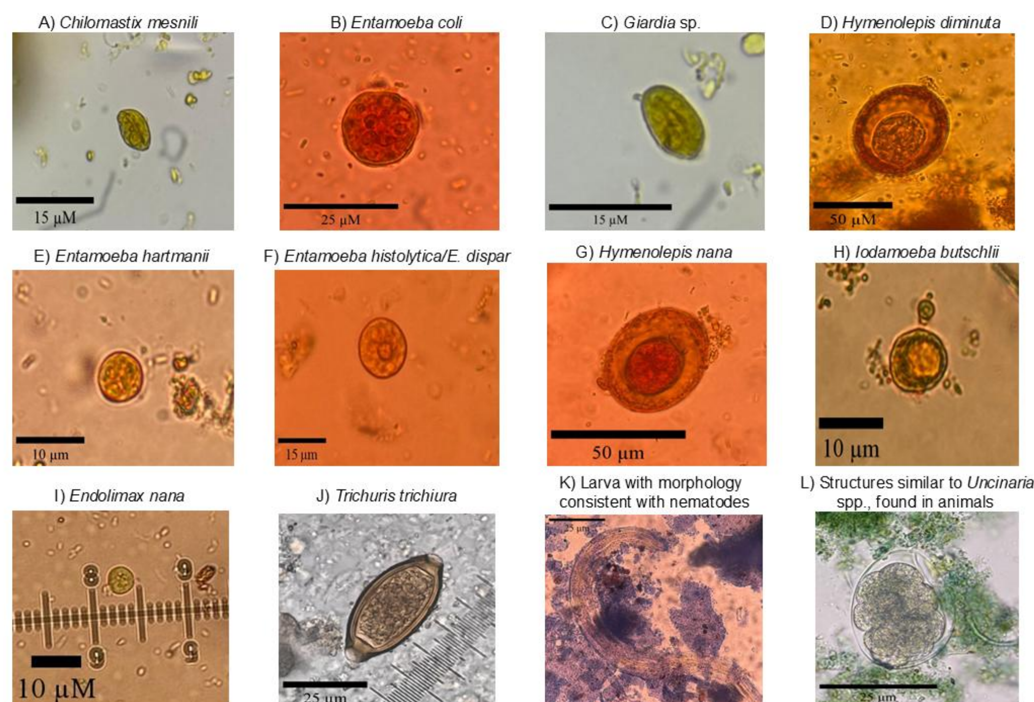

Figure 2. Photographs A), B), C), D), E), F), G), H), I), and J) show cysts of commensal and pathogenic parasites found in human feces; K) shows a parasite found in soil; and L) shows a parasite found in animal feces. Edited using ImageJ software.

Analysis of human samples reveals pathogenic parasites; the most common were Blastocystis sp. 38.8% (n=38), and the complex *Entamoeba hystolitica* / *Entamoeba dispar* with 38.8% (n=38). The presence and prevalence of other pathogenic parasites are shown in Figure 3A. In addition, cysts of non-pathogenic organisms that are part of the large intestine microbiota, such as *Entamoeba coli* confirmed in a 46,9% (n=46), *Endolimax nana* in 13.3% (n=13), *Entamoeba hartmanni* in 11.2% (n=2), *Chilomastix mesnili* in 5.1% (n=5) and *Iodamoeba bütschlii* in 4.1% (n=4) (Figure 3B); its presence serves as an indicator of exposure to fecal matter and poor sanitary conditions.

Microscopic analysis of fecal samples from animals revealed a high incidence of *Uncinaria* spp. with 58.3% (n=7) in the areas of Mayapo (Sabana Larga, Pasito de la Raya, Kousepa) and Pájaro (Mekoloquimana), the presence of amoebas was also detected. 16.7% (n=2) in two communities in Mayapo (Sabana Larga and Kousepa) and *Giardia* 8.3% (n=1) in the community of Kousepa. In addition, soil analysis revealed the presence of *Giardia* in 2.2% (n=1) of the Mekoloquimana community (Figure 3A). There is a high prevalence of intestinal parasites, including protozoa and helminths associated with fecal-oral transmission, in the municipalities of Manaure, La Guajira.

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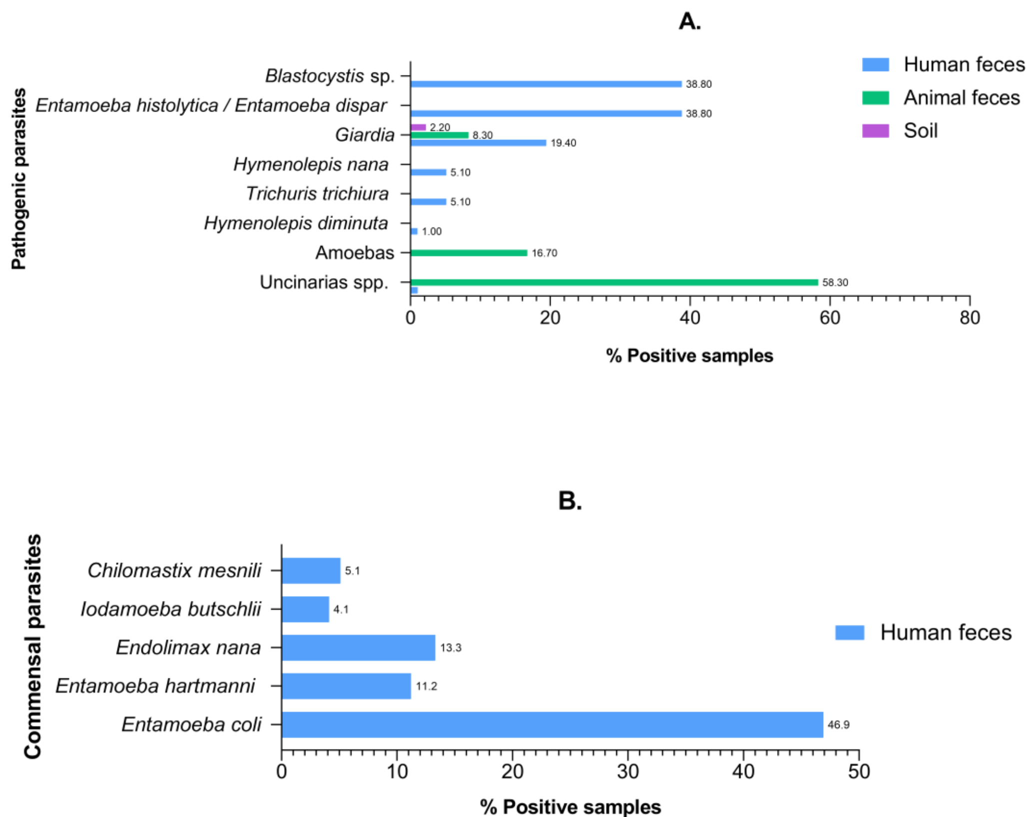

Figure 3. Microorganisms detected by formalin-ether and Kato-Katz concentration methods in samples of human feces, animal feces, soil, and sediments from La Guajira, Colombia. Both A) pathogenic parasites and *Blastocystis* spp., *E. histolytica*, *E. dispar*, *Giardia duodenalis*, *H. nana*, *T. trichiura*, and B) commensal parasites such as *C. mesnili*, *I. butschilil*, *E. nana*, *E. hartmani*, *E. coli*.

The analysis of co-infection among intestinal parasites in humans was visualized using an UpSet plot generated with the UpSetR package in R software (R Foundation for Statistical Computing, Viena, Austria) (Figure 4). In this graph, the top bars represent the frequency of each parasite combination, while the side bars indicate the total number of positive samples for each identified parasite species. The dot matrix shows the combinations of parasite species, with black dots indicating the sets involved in each intersection. This analysis identified the most common combinations of parasites found in human fecal samples; multiple co-infections were observed between intestinal protozoa and soil-transmitted helminths. Among the most common parasitic associations was co-infection between *E. coli* and the *E. histolytica/E. dispar complex* 9.18%, detected in a total of nine samples. In addition, other combinations of parasites were identified less frequently, including *E. coli* the most common parasite in these communities. In addition, co-infections involving up to six different parasite species were observed in a single sample, reflecting the diversity of parasitic infections present in the analyzed samples. The overall frequency of coinfection by parasites was 60.2% (Figure 4).

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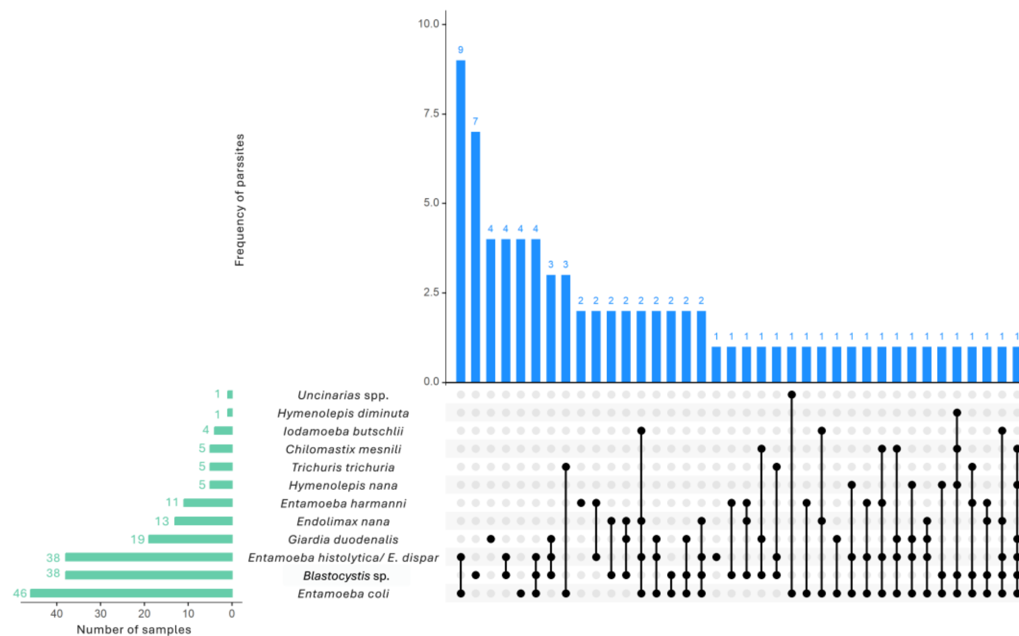

Figure 4. UpSet plot showing the co-infection combinations among intestinal parasites indentified in human fecal samples.

Figure 5 also shows the distribution of the number of parasites per individual, revealing a high frequency of multiple infections among the study participants from the Wayuu communities; most individuals had between 1 and 3 parasites, with 2 parasites per individual being the most common.

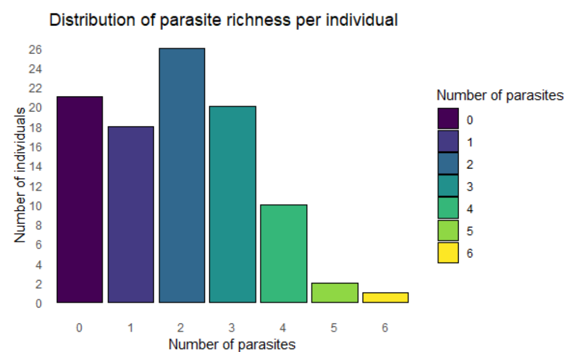

Figure 5. Distribution of the number of parasites per individual.

3.4. Detection of Geohelminths for qPCR Multiplex

As for molecular detection using multiplex qPCR, this method made it possible to identify DNA from the geohelminth *T. trichiura* in the 5.50% of the samples processed (n=109): 2.75% in soil, 1.83% in human and 0.92% in animal. The CMV internal control amplified 100% of the samples, indicating adequate efficiency in both the DNA extraction process and the test performance.

There was agreement between the microscopic and qPCR tests for the identification of *T. trichiura* and the absence of *Ascaris lumbricoides*. Regarding the geographic distribution of the identified geohelminth, it was limited to the communities of Kauracira (Sabana area), and Polousira (Santa Rosa area), confirming environmental contamination by human feces and the local circulation of the parasite.

3.5. Metatranscriptomic Detection of Protozoan Parasites in Fecal and Enviromental Samples

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A subset of fecal samples preserved in RNAlater (n = 23) was selected for metatranscriptomic analysis, of which 22 met quality and were included in downstream analyses. Sequencing generated an average of 38,696,363 reads per sample prior to quality filtering and host read removal, resulting in an average of 2,340,879 reads per sample retained for taxonomic classification. Relative abundance was estimated using normalized read counts (nt RPM), reflecting the contribution of each taxom within individual samples.

At least one intestinal parasite was detected in 90.9% (n=20) of the analyzed samples, reflecting a high frequency of sequence-based detection in the study population. *Blastocystis* spp. was the most frequent parasite, identified in 81.8% (n =18) of samples, followed by *E. histolytica* (54.5%; n = 12) and *Giardia duodenalis* (31.8%; n = 7), while *E. dispar* in 3 samples (13.6%) and *Dientamoeba fragilis* were detected less frequently (4.5%; n = 1). Furthermore, subtype resolution of *Blastocystis* spp. was achieved in 16 of the 17 samples, revealing notable intra-species diversity. *Blastocystis* sp. subtype 2 was the most frequently detected (33.3%; n = 6), followed by subtype 4 in 16.7% (n = 3) and subtype 3 in 11.1% (n = 2), and was identified across both pediatric and adult age groups.

In children aged 0–5 years, detection patterns varied between samples. In Kauracira (Saban zone) (A14) and Ratip (Pajaro zone) (L20), *G. duodenalis* represented 100% protozoan reads. In contrast, other samples in this age group (A54, F12) showed co-detection of *Blastocystis* spp., *E. histolytica*, and *G. duodenalis*, each contributing a smaller proportion of reads. In A54, *Blastocystis* subtypes 2 and 3 were both detected, although at different nt RPM values. In Ichien (D28), multiple taxa were detected with high nt RPM values, including *Blastocystis* subtype 2 (19991.2), subtype 4 (19.4), *D. fragilis* (10591.2), and *E. histolytica* (5627.8), without a single taxon representing most reads.

Among individuals aged 6–11 years, co-detection of *Blastocystis* spp. and *E. histolytica* was observed in both Kauracira (A3, A6) and Ichien (Santa Rosa zone) (D11). In D11, *E. histolytica* accounted for 73.9% of protozoan reads (1768.0 nt RPM), followed by *E. dispar* and *Blastocystis* spp. In D8 (Ichien), *Blastocystis* subtypes 3 (1650.4 nt RPM) and 4 (11.5 nt RPM) were detected, representing most of the protozoan signal in that sample. In contrast, A6 showed higher nt RPM values for *Blastocystis* spp. (1458.3) than for *E. histolytica*.

The adolescent sample (A36, Kauracira) showed detection of multiple protozoa, including *Blastocystis* sp. subtype 2 (2069.9 nt RPM), *E. histolytica*, *E. dispar*, and *G. duodenalis*, with subtype 2 presenting the highest nt RPM within that sample. In individuals >60 years (A25 and A29), detection was limited to *Blastocystis* sp. subtype 4 (A29), with no reads assigned to *E. histolytica* or *G. duodenalis*.

Among the samples included in the metatranscriptomic analysis, a subset of 16 samples had corresponding coproparasitological results, allowing partial comparison between microscopy-based detection and sequencing-based profiles.

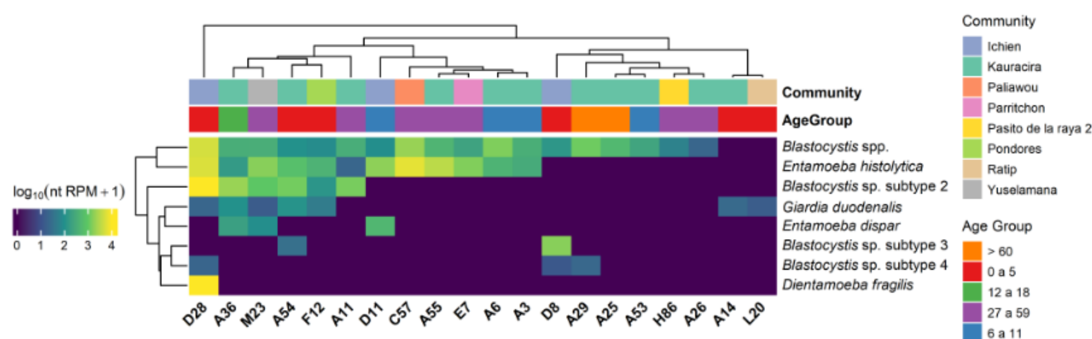

Figure 6. Heatmap of intestinal protozoa detected in fecal samples. Relative abundance of intestinal protozoan taxa across individual fecal samples. Rows represent protozoan species and subtypes, and columns represent individual samples. Cell color intensity corresponds to the log10-transformed normalized read counts (log10nt_RPM+1]) obtained from metatranscriptomic sequencing. Hierarchical clustering was applied to both samples and taxa to visualize similarity patterns in protozoan composition among samples. Top annotations

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indicate sample metadata, including community of origin and age group, represented by distinct color-coded bars. .

Regarding environmental matrices, 20 water samples were processed using metatranscriptomics, in which reads associated with *Acanthamoeba sp*. were identified in 2 samples (10%) and *Naegleria sp.* in 1 sample (5%), all originating from the windmills. Additionally, soil samples were collected from three specific areas (common area, animal corral, and human fecal matter disposal site) across 14 indigenous communities. Subsequently, a pooled sample combining the three areas was generated for each one, resulting in a total of 14 pools. These were analyzed using metatranscriptomics; however, no reads associated with parasites were detected.

4. Discussion

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted. In Colombia, the number of tuberculosis (TB) cases reported in indigenous communities has been on the rise in recent years. By 2024, 1,026 cases had been reported among the indigenous population, representing 4.8% of the national total, while 391 cases were reported among the Wayuu people, accounting for approximately 0.10% of their estimated total population [19,20]. These data reflect a disproportionate burden of disease in these communities, consistent with recent studies showing higher rates of TB among indigenous populations compared to the general population [21,22].

Although this study involved only 15 villages and was limited to individuals with respiratory symptoms who agreed to participate, the positivity rate found is significant and suggests that the actual number of cases in the Wayuu community may be underestimated. This finding could be influenced by previously documented sociocultural factors, such as the stigma associated with the disease, discrimination, and limited knowledge about TB, all of which limit the pursuit of care and timely diagnosis [23,24].

Although this study does not estimate incidence or prevalence in the population, the identification of positive cases including an index case and a positive contact within the same community suggests the presence of active household or community transmission. This pattern has been widely documented among indigenous populations, where factors such as overcrowding, intergenerational cohabitation, and barriers to accessing health care contribute to the spread of the disease [24]. Particularly relevant is the identification of a case involving an adult woman living with four minors, which highlights the risk of transmission in household settings and the potential impact on children. The literature has indicated that in indigenous communities, the prevalence of tuberculosis among children may be considerably higher than in the general population, reflecting uncontrolled chains of transmission, delayed diagnosis, and deficiencies in contact tracing [25,26].

The results show a high prevalence of intestinal protozoa in indigenous communities, with *Blastocystis* spp. (38.8%) being particularly prevalent to *E. histolytica/E. dispar* complex (38.8%). These findings are consistent with studies conducted among vulnerable populations in Latin America, where intestinal parasites remain a public health problem closely linked to poor water, sanitation, and hygiene conditions. In municipalities across Antioquia, the presence of these two parasites has been reported in school-age children; the National Survey on *Blastocystis* spp. and *E. histolytica/E. dispar* complex. Parasitism also found high prevalence rates, primarily in the Sierra Nevada de Santa Marta, where indigenous communities are also located [27,28].

In particular, the high prevalence of *Blastocystis* spp.is consistent with reports from vulnerable communities in Brazil, where prevalence rates exceeding 50% have been documented, making this protozoan one of the most common in areas with socioeconomic limitations [29]. Although its pathogenic role remains controversial, it has been described both as a potential commensal of the gut microbiome and as being associated with alterations in the microbiota and specific clinical conditions, suggesting that its interpretation must take into account the epidemiological and clinical context.

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Furthermore, the coexistence of multiple parasitic species observed in this study suggests a scenario of polyparasitism, which is common in environments with high environmental exposure. This situation may have significant health implications, particularly for vulnerable populations such as children, where it has been linked to malnutrition, developmental delays, and increased susceptibility to other infections [30].

Giardia was also detected in all three study matrices (human and animal feces, soil) (Figure 4A), indicating the potential for transmission across different reservoirs. The detection of this parasite in animal samples suggests the possibility of zoonotic transmission, particularly from cattle, as they can excrete up to 10^6 cysts per gram of feces and contaminate the soil and water in Wayuu communities [31]. The communities of La Guajira rely heavily on livestock farming (cattle, goats, sheep, and pigs) as their primary source of economic livelihood and food; the presence of this parasite in animals can have implications that affect the region's economic stability. It is also important to note that, since this protozoan is detected in the environment, water sources may be contaminated, as the cysts can remain viable for long periods in moist areas and have been linked to waterborne outbreaks; this flagellated protozoan is the causative agent of *Giardia*sis and can be asymptomatic or, in other cases, cause severe gastrointestinal symptoms, including chronic diarrhea, abdominal pain, and malabsorption [32]. From an epidemiological perspective, this infection can affect children's quality of life, particularly among vulnerable populations, by impairing their development and causing nutritional deficiencies. In Colombia, it is not classified as a disease of interest in the Public Health Surveillance System (SIVIGILA), which is a cause for concern given that it is a common pathogen in both rural and urban communities; this situation limits the monitoring of its impact on public health in Colombia [33].

Furthermore, *H. nana* was also identified in human stool samples from children aged 6 to 11 in the communities of Ichien and Polousira at a prevalence of 5.10%. Reports have shown that it is one of the most common tapeworms in humans, particularly among children, It is commonly found in areas with warm climates and poor sanitation, which underscores the importance of environmental and socioeconomic factors in the persistence of these infections [28,34].

Parasites of the genus *Uncinaria* were identified by microscopy in animal fecal samples (Figure 2L); cystic forms of amoebae and cysts of *Giardia duodenalis* were also observed. In contrast, only *Giardia* was detected in soil samples, indicating environmental contamination by fecal matter (Figure 3A). The detection of the helminth *H. nana*, along with the presence of *T. trichiura* in human, animal, and environmental samples, suggests that exposure to contaminated sources is constant. This is consistent with limited access to safe drinking water, basic sanitation, and waste management, where the transmission of enteroparasites tends to remain endemic [35,36].

As evidenced in the municipalities of Manaure, La Guajira, there is a high prevalence of intestinal parasites associated with environmental and sanitary conditions; these results highlight the need to strengthen more targeted control strategies. The high prevalence of pathogenic protozoa, such as *Blastocystis* spp., the *E. histolytica/E. dispar* complex, and *Giardia duodenalis*, suggests the importance of developing therapeutic strategies that allow for the treatment of protozoal and helminth infections, including cestodes such as *H. nana*. The WHO recommends periodically implementing antiparasitic programs involving the administration of broad-spectrum anthelmintics, especially to school-age children [37]. In this context, it is recommended that periodic deworming programs be implemented that target these microorganisms, not only helminths, but also smaller tapeworms and other parasites that are frequently underdiagnosed.

In the samples analyzed by microscopy and metatranscriptomics, it was observed that metatranscriptomics enabled the identification of several parasites that were not detected by microscopy. This finding is expected, as microscopy requires the presence of viable parasitic forms, whereas sequencing allows the detection of nucleic acids from both living and dead organisms [38]. Additionally, metatranscriptomics made it possible to differentiate between *E. histolytica* and *E. dispar*, which represents a significant advantage, since *E. histolytica* is a pathogenic species associated with amoebiasis, while *E. dispar* is considered non-pathogenic; this distinction is not possible by

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conventional microscopy due to the morphological similarity between both species [39]. Overall, the co-detection of multiple protozoan taxa within and across residence communities, particularly in younger individuals, highlights the complexity of the intestinal eukaryotic signal captured by metatranscriptomic sequencing. These findings provide insight into the circulation of intestinal protozoan parasites in the evaluated population and underscore the utility of metatranscriptomics for the surveillance of gastrointestinal pathogens.

In contrast, microscopy of some samples allowed the identification of *E. coli* (n=9), *Giardia duodenalis* (n=3), *T. trichiura* (n=3), *E. histolytica/dispar* complex (n=1), and *H. nana* (n=1); however, these microorganisms were not detected by metatranscriptomics in the same samples. This discrepancy could be explained by limitations associated with nucleic acid extraction in molecular assays, considering that some protozoa may be present as cysts and helminths as eggs, which hinders the recovery of their genetic material and may require more robust lysis methods [40]. Despite the limitations of both techniques, it is essential to approach these techniques in a complementary manner: microscopy and qPCR remain key tools for diagnostic confirmation, while metatranscriptomics provides higher taxonomic resolution, enables the differentiation of pathogenic species, and facilitates the detection of parasites that may go unnoticed using conventional methods. This integrated approach is critical for advancing toward high precision public health strategies, particularly in regions such as La Guajira, where the distribution and transmission of parasites vary across communities.

Environmental samples showed no parasites in soil. In contrast, metatranscriptomic analysis of water sources used by the indigenous community for consumption and domestic activities identified free-living amoebae (*Acanthamoeba* sp. and *Naegleria* sp.) in two samples. These microorganisms are considered opportunistic pathogens associated with encephalitis and keratitis, particularly in settings with limited sanitation, hygiene, and access to diagnostics [41]. This finding is consistent with the conditions of the studied indigenous communities, most of which lack aqueduct systems and rely on untreated natural water sources. In this context, the results highlight a potential public health risk associated with continuous exposure to untreated water and underscore the need to strengthen surveillance of these microorganisms, as well as to implement intervention strategies aimed at improving water quality in these communities [42].

5. Conclusions

This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex. The study demonstrates a high prevalence of parasites across human, animal, and environmental matrices in Wayuu indigenous communities from Manaure, confirming the presence of an active transmission system sustained by the interaction among these three components. The simultaneous detection of protozoa and helminths across different reservoirs suggests the coexistence of both anthroponotic and zoonotic transmission cycles within a context of widespread fecal contamination, reflecting a complex and persistent transmission dynamic.

The findings indicate that the burden of disease is strongly influenced by social, cultural, and economic determinants, which play a critical role in disease management and outcomes. These include limited access to safe drinking water, inadequate sanitation conditions, geographic barriers to healthcare access, and sociocultural practices that shape risk perception and health-seeking behaviors. In particular, stigma surrounding tuberculosis and the normalization of symptoms such as childhood diarrhea-combined with limited awareness of warning signs-contribute to the persistence of these conditions within Wayuu communities.

From a One Health perspective, the results highlight that the parasitic burden in these communities cannot be addressed in isolation but is instead driven by structural determinants involving environmental factors, sociocultural practices, and human–animal interactions. Therefore, control strategies must move beyond individual-level treatment and focus on comprehensive, intersectoral interventions. In this context, active community-based screening, the implementation of timely diagnostic tools, and the coordinated involvement of human and animal health professionals-

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alongside strong community participation—are essential to strengthen epidemiological surveillance, improve public health responses, and foster trust in healthcare systems.

Additionally, in the context of other infectious diseases such as Tuberculosis, these findings reinforce existing evidence positioning indigenous populations as highly vulnerable groups. This underscores the urgent need for integrated, culturally appropriate, and sustainable interventions aimed at reducing health inequities and interrupting transmission cycles.

Overall, this study emphasizes the importance of adopting intersectoral approaches grounded in the One Health framework, which go beyond individual-level interventions to structurally address the determinants of disease, as a key strategy to reduce the burden of infectious diseases in Wayuu communities of La Guajira.

6. Limitations

Among the study's limitations are the sample size and the cross-sectional nature of the analysis, which prevent the establishment of robust estimates of prevalence or risk. Nevertheless, the findings provide relevant evidence regarding the presence of tuberculosis and pathogenic parasites in Wayuu communities and underscore the need to strengthen strategies for active case finding, community surveillance, timely diagnosis, and the provision of adequate sanitary conditions.

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Institutional Review Board Statement: The study was conducted following the Declaration of Helsinki. All subjects enrolled in this study voluntarily signed an informed consent form previously reviewed and approved by CEMIN (18-2023, 14/07/2023), and prior authorization was obtained from the indigenous leader.

Informed Consent Statement: Written Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data supporting the findings of this study are publicly available in the Chan Zuckerberg ID (CZ ID) platform. The metatranscriptomic sequencing datasets generated and analyzed during the current study can be accessed at the following link: https://czid.org/my_data?currentDisplay=table¤tTab=samples&mapSidebarTab=summary&projectId=20261&showFilters=true&showStats=true&workflow=short-read-mngs. Additional information may be available from the corresponding author upon request.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

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TB	Tuberculosis
ADD	Acute diarrheal disease
qPCR	quantitative polymerase chain reaction
STG	Stonebrink-Giraldo's modified culture middle
SAF	ether: formalin–ether concentration techniques
PBS	phosphate-buffered saline
NTC	no-template controls
CMV	Cytomegalovirus
cDNA	complementary DNA
HS	High Sensitivity
DNB	DNA nanoball
FIS	Fondo de Investigación en Salud

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