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

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Abstract

Acute diarrheal disease (ADD) caused by parasites and Tuberculosis (TB) remain major public health concerns in vulnerable indigenous communities with limited access to sanitation, safe water, and healthcare, and where humans, animals and the environment interact closely. Using a One Health framework, this study investigated TB and Intestinal parasites in human, animal and environmental samples from 15 Wayuu indigenous communities in Manaure, La Guajira. A total of 190 samples, including human sputum and feces, animal milk and feces, soil and drinking water, were analyzed according to sample type, preservation suitability, and availability using parasitological concentration techniques, qPCR for helminth detection, metatranscriptomic sequencing, Xpert ® MTB/RIF assay, and mycobacterial culture. *Mycobacterium tuberculosis* was detected in 8.3% of human sputum samples, with no evidence of rifampicin resistance, whereas *Mycobacterium bovis* was not detected in animal milk. Human fecal samples analyzed by microscopy showed *Blastocystis* sp. and the *Entamoeba histolytica/Entamoeba dispar* complex (38.8% each), followed by *Giardia* (19.4%), *Hymenolepis nana* and *Trichuris trichiura* (5.1% each) and *Hymenolepis diminuta* (1%). Commensal parasites were also identified, with *Entamoeba coli* (46.9%) being the most frequent species, indicating inadequate sanitary conditions and poor hygiene practices. Co-infections were common in humans (60.2%). In animal fecal samples, strongylids (66.7%), amoebas (16.7%) and *Giardia* (8.3%) were observed. *Giardia* sp. was detected in 2.38% of soil samples by microscopy, supporting environmental circulation, whereas no parasites were detected in water sediments. Multiplex qPCR detected *Trichuris trichiura* DNA in human feces and *Trichuris* spp. DNA in soil and sheep fecal samples. Metatranscriptomic analysis of 22 human fecal samples revealed a high diversity and frequency of parasitic protozoa (90.9%), with *Blastocystis* spp. being the most frequent (81.8%). Additionally, reads of free-living amoebae, including *Acanthamoeba* spp. (10%) and *Naegleria* spp. (5%) were detected in community drinking water sources. These findings suggest active transmission of TB and parasitic-associated ADD in Wayuu communities and highlight the need for

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integrated surveillance and culturally appropriate interventions focused on sanitation, hygiene, veterinary services and community health education to improve the living and health conditions of these vulnerable populations.

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

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].

In 2025, the municipality of Manaure (La Guajira) had a population of 97.010 Wayuu indigenous people, with a balanced gender distribution (approximately 51% women and 49% men). This community resides in 1210 indigenous reserves mainly in rural and dispersed areas, and 81.72% face unmet basic needs, which creates great inequalities in access to drinking water, health services, education and food security [5,6]. The Wayuu communities of La Guajira are characterized by rural and semi-nomadic lifestyles, with limited access to potable water and sanitation services. Goat, sheep, and cattle rearing are common practices, and animals are frequently kept close to households. These environmental and sanitary conditions may facilitate the transmission of intestinal parasites and other infectious diseases through fecal–oral routes [7]. Furthermore, overcrowded living conditions in vulnerable and Indigenous communities have been recognized as important social determinants favoring tuberculosis transmission. The social vulnerability of Wayuu communities is characterized by precarious housing conditions, overcrowding, and limited access to healthcare [5,6].

The Wayuu communities face persistent challenges related to limited access to safe water, health services, food security, and citizen participation [8]. In La Guajira, where the population is approximately 918.062 inhabitants [9], the 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 [10]; 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 [11]. 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 [12]. 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 [13].

To address the challenge of TB and EDA in vulnerable populations from a One Health perspective, a study was conducted in fifteen Wayuu communities in the municipality

of Manaure, La Guajira, to identify circulating pathogens in humans, animals and the environment using conventional methods (microscopy and PCR) and metatranscriptomic sequencing, a sequencing approach based on the analysis of total RNA present in a sample, has emerged as a powerful tool for the unbiased detection and identification of known and potentially emerging infectious agents that may not be detected through conventional methodologies. This integrative strategy expanded microbial detection and potential transmission dynamics across the different dimensions of One Health. The study aimed to improve the understanding of pathogen circulation and potential transmission dynamics in indigenous communities with high social and environmental vulnerability, contributing to epidemiological surveillance strategies in remote settings.

2. Materials and Methods

2.1. Participants, Samples and Study Area

Between November 2023 and August 2025, 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 individuals from the Wayuu population belonging to the indigenous reserves; 850 indigenous people of Ichien, Polousira (Santa Rosa area), Parritchon, Kauracira, Paliawo (Sabana area), Pasito de la Raya, Kousepa, Pondores, Sabana Larga (Mayapo area), Lapuntachon, Mekoloquimana, Yuselamana, Ratip and Ishamana (Pajaro area) attended the invitation (Figure 1). After obtaining informed consent or assent and authorization from the indigenous authorities, environmental samples and samples from animals and people presenting symptoms of tuberculosis or acute diarrhea disease (in total 130) were collected, as follows: A total of n = 190 samples were obtained and, depending on sample availability and preservation suitability, different analyses were performed for parasite detection. For microscopic analysis using the Kato–Katz and formalin–ether concentration techniques, (n = 156) samples were processed, including (n = 98) human fecal samples, (n = 12) animal fecal samples from goats (n = 5), pigs (n = 3), cattle (n = 1), sheep (n = 2), and one peacock; (n = 42) soil samples collected from common areas, animal corrals, and human fecal disposal sites; and (n = 4) sediment samples from drinking jagüeys (rainwater reservoirs). In addition, (n = 109) samples were analyzed by quantitative PCR (qPCR) for the detection of geohelminths, including (n = 51) human feces, (n = 12) animal fecal samples, (n = 42) soil samples, and (n = 4) sediment samples. Furthermore, (n = 57) samples were analyzed by metatranscriptomics, including (n = 23) human stool samples preserved in RNAlater, (n = 20) drinking water samples, these included (n = 7) samples from jagüeys (rainwater reservoirs), (n = 8) from shallow ground-water wells and (n = 5) from deep wells operated by windmills, and (n = 14) soil samples. Finally, (n = 36) human sputum samples were collected for microbiological and molecular analyses for *M. tuberculosis*, and (n = 37) milk samples from (n = 27) goats, (n = 7) sheep and (n = 3) cows were analyzed for the detection of *Mycobacterium bovis*. All samples were transported under cold chain conditions to the laboratories of the National Institute of Health in Bogotá, Colombia, for processing. Due to the exploratory and community-based nature of the study, only descriptive analyses were performed. Frequencies and percentages were used to summarize the parasitological and microbiological findings.

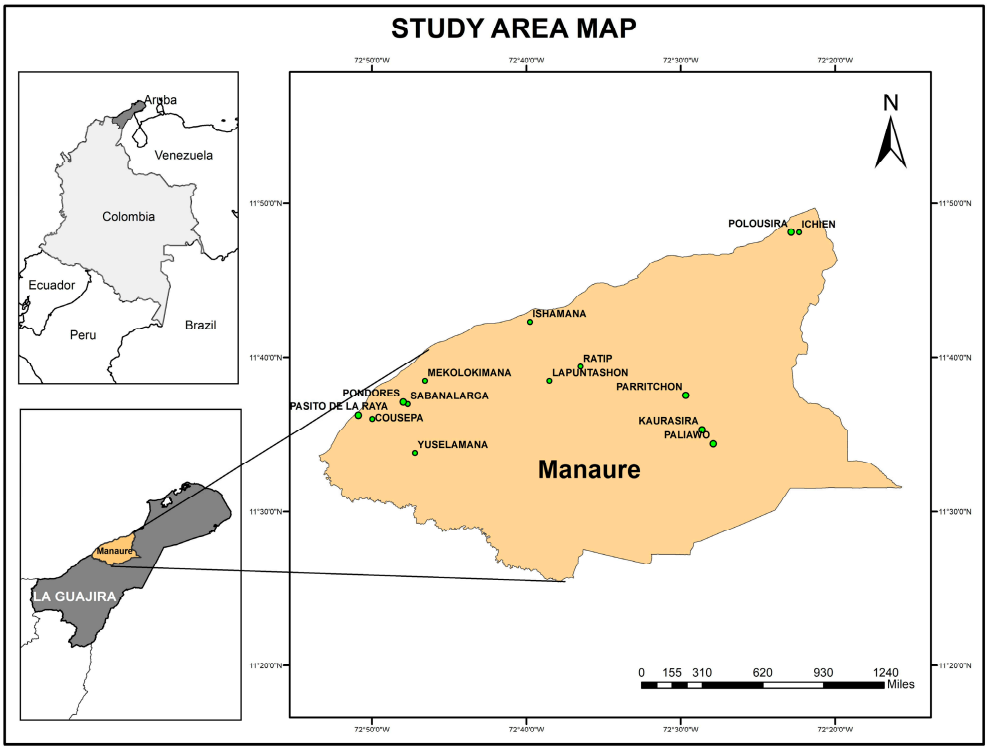

Figure 1. Location map of the study area. The map shows Colombia, with the department of La Guajira shaded in dark gray. A zoomed-in view of the municipality of Manaure, shown in yellow, highlights the Indigenous communities. Black lines indicate the boundaries of the municipality of Manaure. For national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

2.2. Detection of Mycobacterium tuberculosis in Human Samples: Xpert® MTB/RIF Assay

Sputum samples were processed and analyzed using the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) for the detection of *Mycobacterium tuberculosis* and rifampicin resistance. This automated molecular test, based on real-time PCR, was performed on the GeneXpert® (Cepheid, Sunnyvale, CA, USA) system following the manufacturer's instructions [14]. Samples were treated with reagent (NaOH/isopropanol, 1:2), incubated for 15 min, and 2 mL was loaded into the cartridge. The automated system performed lysis, DNA amplification, and detection, with results available in 1.5 h.

2.3. Sputum Sample Culture on BACTEC MGIT 960™ for Isolation of Mycobacterium tuberculosis

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* was performed from the axenic colonies by the method STANDARD Q TB MPT64 Ag Test (SD Biosensor, Suwon, Republic of Korea). It is a rapid chromatographic immunoassay for the qualitative detection of MPT 64 antigen.

2.4. Culture on Löwenstein–Jensen (LJ) and Stonebrink (STG) Media for the Isolation of M. bovis from Milk Samples

The (n = 37) milk samples (50 mL) were aseptically collected and centrifuged at 4000× g for 30 min at 4 °C. The sediment (2 mL) was decontaminated with 3 mL of sodium lauryl sulfate and incubated at room temperature for 30 min, followed by neutralization (3 mL, 2 min). The final sediment was resuspended in sterile distilled water and cultured in duplicate on LJ and STG-Giraldo's modified media and incubated at 37 °C, with weekly readings for up to 12 weeks [15].

2.5. DNA Extraction from Milk Samples for Molecular Detection of Mycobacterium bovis

An aliquot of sediment derived from processed milk samples was subjected to DNA extraction for mycobacteria according to the recommendations of van Soolingen et al. [16]. Every DNA sample obtained was re-suspended in a buffer, TE 0.1X.

2.6. Molecular Detection of Mycobacterium bovis by Real-Time PCR

An in-house real-time PCR assay was standardized using the primers described by Pinsky et al. (2008) and Capcha et al. (2005) [17,18]. The reaction mixture was prepared with FastStart Essential DNA Green Master (Roche Diagnostics, Basel, Switzerland) at a final concentration of 1X. Primers targeting 16S, RD9, and IS6110 were used at final concentrations of 0.4 µM, 0.8 µM, and 0.15 µM, respectively. Real-time PCR amplification was performed, including appropriate controls: *M. bovis*, *M. tuberculosis* H37Rv, a negative control, and nuclease-free water. Amplification was monitored through SYBR Green fluorescence detection followed by 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 2264× *g* for 10 min, 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 from the Kato–Katz Stool Examination Kit (Vestergaard Frandsen, Disease Control Textiles, Copenhagen, Denmark) on a microscope slide (0.0417 g of feces according to the template), 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 [19].

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 2264× *g* for 10 min, 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.

Samples were initially examined at 100× magnification and subsequently confirmed at 400× magnification. Morphometric measurements were performed using ImageJ software (v1.54g; National Institutes of Health, Bethesda, MD, USA) after calibration with the microscope scale, and the obtained values were compared with reference measurements reported in the literature for species identification [20,21].

2.8. DNA Extraction 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-buffered saline (PBS) 1X, with centrifugation at 1369× *g* for 5 min 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 from Soil Samples

Prior to extraction, 40 g of the soil samples underwent three wash cycles with PBS 1X, each followed by centrifugation at 1369× *g* for 5 min. DNA was extracted from 250 mg of

sediment 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 Geohelminths

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 by Azzopardi KI et al. 2021 [22]. For the multiplex detection of geohelminths, the following protocol was used: Cools [23], 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 min, followed by 39 cycles of denaturation at 95 °C for 10 s and alignment/extension at 60 °C for 30 s. 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	95 °C	5 min
39	Denaturation	95 °C	10 s
	Annealing and Elongation	60 °C	30 s

To evaluate the DNA extraction efficiency and detect the presence of inhibitors in the PCR, the pcDNA3.4-TOPO construct of 6011 bp (Invitrogen, Carlsbad, CA, USA) with the Cytomegalovirus (CMV) promoter downstream was used as an internal control in the geohelminth qPCR [24]. 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 ($R \approx 1$). The slope was close to −3.32, with the intercept and efficiency percentage ranging between 90 and 110% (E). Additionally, a no-template control (NTC) was included to ensure the absence of contamination.

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

A total of $n = 20$ water samples were collected from 14 rural indigenous communities, representing water sources commonly used for human consumption and domestic purposes. These included ($n = 7$) samples from jagüeys (rainwater reservoirs), ($n = 8$) from shallow groundwater wells and ($n = 5$) from deep wells operated by windmills. Samples were initially subjected to a microfiltration process, followed by tangential ultrafiltration and a final concentration step using an AMICON® ultra centrifugal filter (10 kDa molecular weight cutoff) (Merck, Darmstadt, Germany). Subsequently, RNA was extracted from the concentrated samples.

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

RNA 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 additionally evaluated by agarose gel electrophoresis.

2.13. 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 $\geq$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.

2.14. Bioinformatics Analysis

Raw sequencing reads were quality-filtered and processed using the CZ ID pipeline v8.3 [25]. CZ ID is an open-source cloud platform used to identify microbial agents in NGS datasets. This pipeline integrates multiple bioinformatics 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 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 $\geq$ 10 (number of reads aligning to a taxon in the NCBI NT database, per million reads sequenced); 3. NT alignment length $\geq$ 50 base pairs; 4. NR rPM $\geq$ 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 values were used to estimate parasite abundance across samples. Heatmaps and hierarchical clustering analyses were generated in R (v4.4.2) using the ComplexHeatmap package (v2.22.0) after log10 transformation of normalized read counts [log10(NT rPM + 1)].

2.15. 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 the 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%). Other variables of the people who provided samples for analysis are shown in Table 2. Regarding sources of water for human consumption, respondents indicated that they use rainwater collection wells (jaguey) and windmills in 40.0% of cases, windmills alone in 24.6% of cases, and jaguey alone in 14.6% of cases; 10% used both jaguey and dug wells, 7.75% used only dug wells, and 2.3% used a desalination plant, with only one community purchasing drinking water from the nearest urban center. A total of 94.6% of those surveyed stated that they do not boil water before drinking it.

Table 2. Description of the people who provided samples for analysis.

	Indigenous Reserve								**Total**	
	Pajaro		**Mayapo**		**Sabana**		**Santa Rosa**			
	n	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**
Sex										
Female	24	30	6	7.5	22	27.5	28	35	80	100
Male	15	30	4	8	14	28	17	34	50	100
Age in years										
0–5	9	42.9	0	0	7	33.3	5	23.8	21	100
6–11	4	12.1	2	6.1	5	15.2	22	66.7	33	100
12–18	3	33.3	1	11.1	2	22.2	3	33.3	9	100
19–26	6	66.7	2	22.2	0	0	1	11.1	9	100
27–59	14	30.4	4	8.7	16	34.8	12	26.1	46	100
≥60	3	25	1	8.3	6	50	2	16.7	12	100
Occupation over 18 years old										
Artisan	15	45.5	0	0	10	30.3	8	24.2	33	100
Domestic work	5	71.4	0	0	1	14.3	1	14.3	7	100
Various trades	2	40	0	0	1	20	2	40	5	100
No occupation	1	4.5	7	31.8	10	45.5	4	18.2	22	100

Table 2. *Cont.*

	Indigenous Reserve								Total	
	Pajaro		Mayapo		Sabana		Santa Rosa			
	n	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**
					Schooling					
No schooling	14	33.3	4	9.5	15	35.7	9	21.4	42	100
Partially completed primary	3	20	1	6.7	7	46.7	4	26.7	15	100
Completed primary	3	50	1	17	0	0	2	33	6	100
Partially completed secondary	3	75	1	25	0	0	0	0	4	100
Completed secondary school	0	0	1	100	0	0	0	0	1	100

3.1. Detection of M. tuberculosis in Sputum Samples

A total of $n = 36$ sputum samples were processed using the Xpert® MTB/RIF assay, with a positivity rate for *Mycobacterium tuberculosis* of $n = 3$ (8.3%). No mutations associated with rifampicin resistance were detected in the *rpo*B gene. All Xpert-positive samples were also positive by the TB MPT64 antigen test, where only one sample yielded a positive culture using the BACTEC MGIT methodology.

Among the confirmed cases, one corresponded to a 44-year-old woman from the Santa Rosa area who was the head of a household composed of four minor children. The other two cases were identified in male participants aged 35 and 19 years from the Pájaro area. The 35-year-old participant was identified as the index case during the initial screening, while the 19-year-old participant was identified as a household/community contact during a follow-up visit conducted in the same community.

3.2. Detection of M. bovis in Milk Samples

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

3.3. Microscopic Detection of Parasites in Feces

Microscopic analysis using the Kato–Katz and formalin–ether concentration techniques enabled the identification of various intestinal parasites in samples of human and animal feces, as well as soil samples from different communities. Representative photographs illustrate the morphological characteristics of cysts of *Chilomastix mesnili*, *Entamoeba coli*, *Giardia*, *Entamoeba hartmanii*, the *E. histolytica*/*E. dispar* complex, *Iodamoeba butschlii* and *Endolimax nana*; eggs of *Hymenolepis diminuta*, *Hymenolepis nana*, and *T. trichiura* in human fecal samples; strongylid eggs in animal feces from goats, pigs, and sheep, and a nematode larva detected in soil, morphologically compatible with strongylids (Figure 2).

The analysis of human fecal samples revealed the presence of several pathogenic parasites, with *Blastocystis* sp. and the *Entamoeba histolytica/Entamoeba dispar* complex being the most prevalent, both detected in 38.8% ($n = 38$) of the samples. In addition, cysts of non-pathogenic organisms were confirmed; these microorganisms are part of the large intestine microbiota and included *Entamoeba coli* with a prevalence of 46.9% ($n = 46$), *Endolimax nana* at 13.3% ($n = 13$), *Entamoeba hartmanni* at 11.2% ($n = 11$), *Chilomastix mesnili* at 5.1% ($n = 5$), and *Iodamoeba bütschlii* at 4.1% ($n = 4$) (Figure 3). The presence of these organisms serves as an indicator of exposure to fecal contamination and poor sanitary conditions.

Microscopic analysis of animal fecal samples revealed a high frequency of strongylid eggs, with 66.7% ($n = 8$) detected in the areas of Mayapo (Sabana Larga, Pasito de la Raya, and Kousepa) and Pájaro (Mekoloquimana). Strongylid eggs were identified in goat ($n = 5$), pig ($n = 1$), and sheep ($n = 2$) samples. In addition, amoebae were detected in 16.7% ($n = 2$) of the samples, corresponding to one pig sample and one goat sample from two communities

in Mayapo (Sabana Larga and Kousepa). Likewise, *Giardia* spp. were identified in 8.3% (n = 1) of the samples, corresponding to a peacock sample from the Kousepa community.

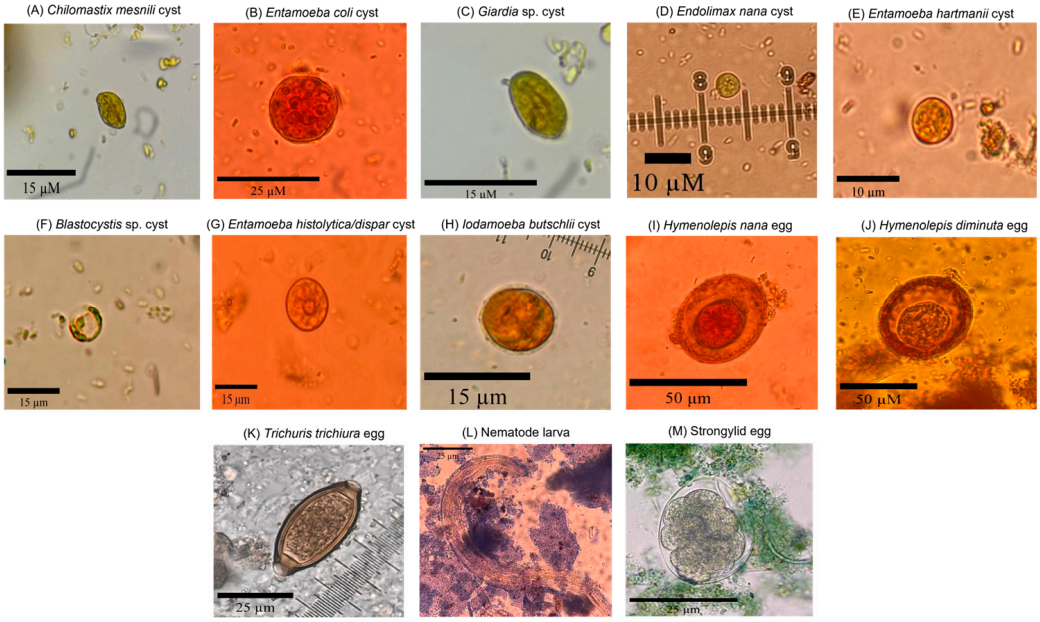

Figure 2. Photographs (**A**–**H**) show cysts of commensal and pathogenic parasites identified in human feces, whereas (**I**–**K**) show eggs of pathogenic parasites identified in human feces. A nematode larva detected in soil is shown in (**L**), and (**M**) shows a strongylid egg identified in goat feces. Images were edited using ImageJ software.

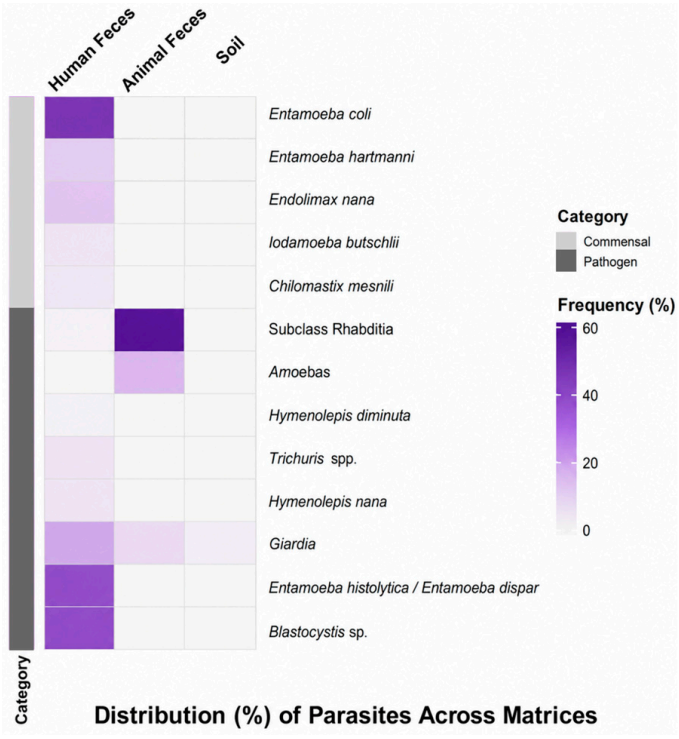

Figure 3. Pathogenic parasites: *Blastocystis* sp., *E. histolytica*, *E. dispar*, *Giardia* spp., *H. nana*, *Trichuris* spp.; and commensal parasites: *C. mesnili*, *I. butschilil*, *E. nana*, *E. hartmani*, *E. coli*. detected in human stool, animal feces, soil, and sediment samples from La Guajira, Colombia, using formalin–ether and Kato–Katz concentration methods.

Furthermore, soil analysis revealed the presence of *Giardia* spp. in 2.38% (n = 1) of the samples from the Mekoloquimana community (Figure 3). Overall, these findings

demonstrate a high frequency of intestinal parasites, including protozoa and helminths associated with fecal–oral transmission, in the municipalities of Manaure, La Guajira. Water sediment analysis did not reveal the presence of parasites.

The presence and frequency of parasites identified by microscopy in human fecal, animal fecal, and soil samples are shown in Figure 3.

The analysis of co-infection among intestinal parasites in humans was visualized using an UpSet plot generated with the UpSetR package (v.1.4.0) in R software (R Foundation for Statistical Computing, Vienna, 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. Overall, the frequency of parasitic coinfection was 60.2%, corresponding to 59 out of 98 human fecal samples that presented coinfection by two or more parasite species. 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, the most common parasite identified in these communities was *E. coli*, followed by *Blastocystis* sp. 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. (Figure 4).

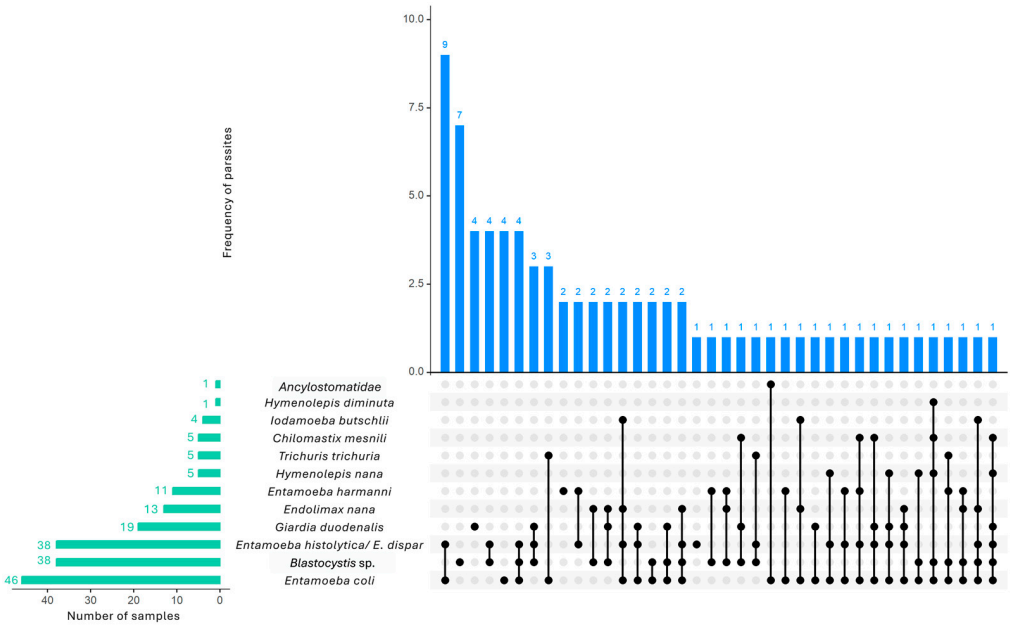

Figure 4. UpSet plot showing the co-infection combinations among intestinal parasites identified in human fecal samples.

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.

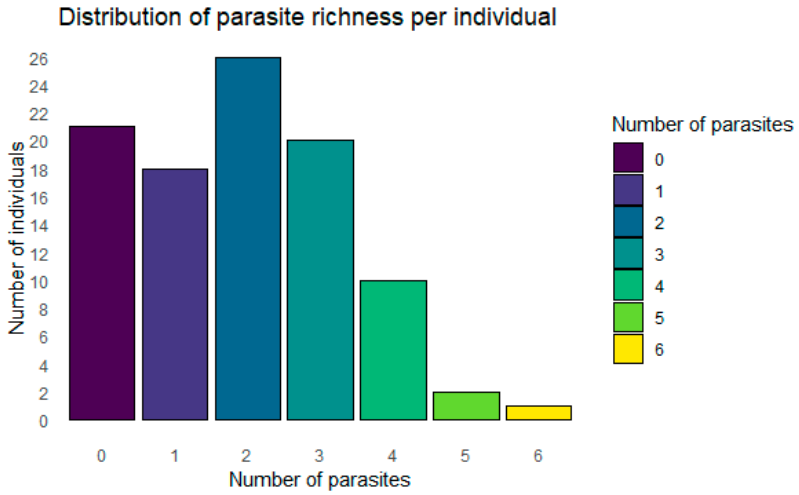

Figure 5. Distribution of the number of parasites per individual.

3.4. Detection of Geohelminths for qPCR Multiplex

As for molecular detection using multiplex qPCR, *Trichuris trichiura* DNA was identified in human fecal samples, while *Trichuris* spp. DNA was detected in soil samples and in a sheep fecal sample. Specifically, *T. trichiura* DNA was found in human fecal samples at 3.92% (n = 2), *Trichuris* spp. in soil samples at 7.14% (n = 3), and in a sheep fecal sample at 8.33%. The CMV internal control amplified 100% of the samples, indicating adequate efficiency in both the DNA extraction process and the test performance.

Concordance was observed between microscopy and qPCR assays for the identification of *T. trichiura* and the absence of *Ascaris lumbricoides*. qPCR-positive samples for *T. trichiura* in human feces were detected exclusively in the communities of Kauracira (Sabana area) and Polousira (Santa Rosa area), indicating a localized distribution of the parasite in the study areas.

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

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 38,266,631 reads per sample prior to quality filtering and host read removal, resulting in an average of 2,366,523 reads per sample retained for taxonomic classification. Relative abundance was estimated using normalized read counts (NT rPM), reflecting the contribution of each taxon 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* was detected less frequently, in 4.5% (n = 1). Notably, reads assigned to *Blastocystis* subtypes were identified in 8 of the 18 positive samples, revealing genetic diversity among the detected *Blastocystis* populations. *Blastocystis* sp. subtype 2 (ST2) was the most frequently detected, at 33.3% (n = 6), followed by subtype 4 (ST4) in 16.7% (n = 3) and subtype 3 (ST3) in 11.1% (n = 2), and was identified across both pediatric and adult age groups (Figure 6).

Although *Giardia* spp. were initially identified by microscopy based on parasite morphology, as previously described, metatranscriptomic analysis of the seven positive samples enabled species-level characterization to *Giardia duodenalis*. The highest read abundance was observed in samples A36 and A54, where contigs extracted from CZ ID of 423 nt and 275 nt showed high nucleotide identity (99.6 and 100%, respectively) to *G.*

duodenalis ribosomal RNA reference sequences (L29192.1 and X52949.1, respectively). In the remaining positive samples, the taxonomic assignment was supported exclusively by read-level detection without contigs recovery.

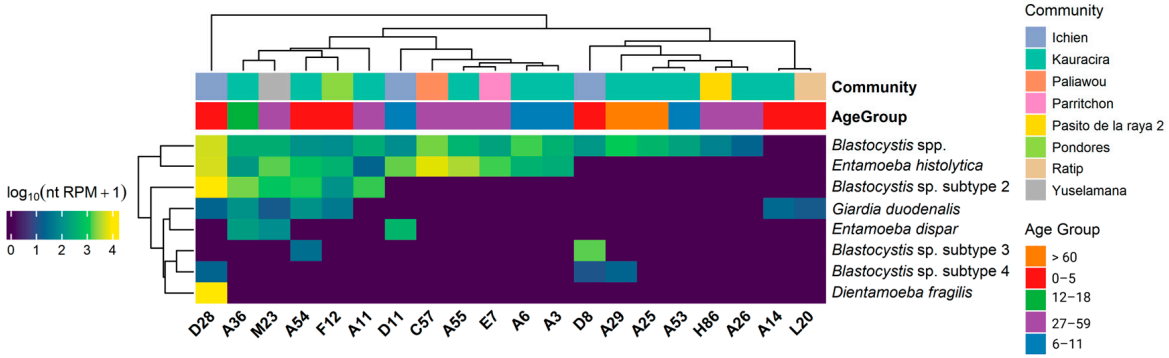

Figure 6. Heatmap showing the relative abundance of intestinal protozoan taxa detected in human fecal samples. Rows represent protozoan taxa and columns represent individual samples. Cell color intensity corresponds to log10-transformed normalized read counts ($\log_{10}$ NT rPM + 1). Hierarchical clustering was applied to samples and taxa, and top annotations indicate community of origin and age group.

In the 0–5-year age group, detection patterns varied between samples. In samples A14 and L20 from Kauracira (Saban area) and Ratip (Pajaro area), respectively, *G. duodenalis* represented 100% of the protozoan reads. In contrast, other samples in this age group (A54 and F12) showed co-detection of *Blastocystis* spp., *E. histolytica*, and *G. duodenalis*, with differing relative abundances among the detected taxa. In sample A54, *Blastocystis* subtypes 2 and 3 were both detected, although ST2 had a substantially higher NT rPM value than ST3. Notably, D8 and D28 samples from the Ichien community showed distinct *Blastocystis* profiles. In D8, ST3 was detected at substantially higher nt rPM values than ST4. In contrast, the D28 library showed co-detection of multiple taxa, including *D. fragilis* and *E. histolytica*, while ST2 showed markedly higher nt rPM values than ST4. Overall, heterogeneous protozoan profiles were observed in the 0–5-year age group, ranging from apparent monoparasitism to multiple protozoan co-detection.

Among individuals aged 6–11 years, co-detection of *Blastocystis* spp. and *E. histolytica* was observed in both Kauracira (A3, A6) and Ichien (D11). In contrast, sample A53 showed exclusive detection of *Blastocystis* spp. The D11 library from the Ichien community exhibited a distinct profile, dominated by *E. histolytica* (73.9%; 1768.0 nt rPM), with lower abundances of *E. dispar* and *Blastocystis* spp. Notably, microscopy of D11 had previously identified cysts compatible with the *E. histolytica/dispar* complex, while metatranscriptomic analysis revealed the coexistence of both species within the same sample.

The only adolescent sample (12–18 years; A36, Kauracira) showed co-detection of multiple protozoa, including *Blastocystis* sp. Subtype 2, *E. histolytica*, *E. dispar*, and *G. duodenalis*, with ST2 presenting the highest nt rPM value within the sample. Among adults aged 27–59 years, heterogeneous protozoan detection patterns were observed across samples from multiple communities. *Blastocystis* spp. and *E. histolytica* were the most frequently detected taxa, commonly identified in co-detection profiles. In samples A55 (Kauracira), C57 (Paliawo), E7 (Parritchon), and M23 (Yuselamana), *E. histolytica* showed the highest nt rPM values within each sample, whereas in A11 (Kauracira), *Blastocystis* ST2 predominated over *E. histolytica*. Sample M23 additionally showed co-detection of *E. dispar* and *G. duodenalis*. In contrast, samples A26 (Kauracira) and H86 (Pasito de la Raya) showed exclusive detection of *Blastocystis* spp. Finally, in two individuals older

than 60 years, protozoan detection was limited to *Blastocystis* spp. in both samples from Kauracira (A25 and A29), with subtype 4 identified only in A29.

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 from Kauracira and Sabanalarga communities.

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

In Colombia, the number of tuberculosis (TB) cases reported in indigenous communities has been on the rise in recent years. By 2024, 1026 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 0.05% of their estimated total population (711.965 Wayuu people in the country) [11,26]. 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 [27,28].

Although this study involved only 15 indigenous reserves in Manaure 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 stigma and discrimination associated with tuberculosis, which may discourage Wayuu individuals from reporting respiratory symptoms, particularly in community-based screening settings where multiple community members are present during sample collection. In addition, limited knowledge about TB may prevent individuals from recognizing disease symptoms and seeking timely medical attention, thereby contributing to delayed diagnosis and potential underestimation of cases within the community [29,30].

Although the present study does not allow estimation of tuberculosis incidence or prevalence at the population level, the identification of an index case and a subsequently confirmed positive contact from the same community may indicate ongoing local transmission dynamics. The detection of a secondary case during follow-up contact investigation underscores the potential role of household and close-community exposure in sustaining transmission in these rural settings.

This pattern has been widely documented among indigenous populations, where factors such as overcrowding, precarious living conditions, and barriers to accessing health care in indigenous communities contribute to the spread of the disease [30]. Particularly relevant was the identification of an adult female case living with four minor children, highlighting the potential risk of household exposure and transmission among close contacts. Previous studies in indigenous communities have shown that tuberculosis in children may reflect ongoing and uncontrolled transmission chains, delayed diagnosis, and deficiencies in contact tracing [31,32].

The results show a high prevalence of intestinal protozoa in indigenous communities, with *Blastocystis* sp. (38.8%) being particularly prevalent compared 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* sp. 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 [33,34].

In particular, the high prevalence of *Blastocystis* sp. 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 [35]. 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.

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, in whom it has been linked to malnutrition, developmental delays, and increased susceptibility to other infections [36].

Giardia spp. was also detected by microscopy in all three study matrices (human and animal feces, and soil) (Figure 4), indicating the potential circulation of the parasite across different reservoirs. The detection of *Giardia* spp. in animal samples suggests the possibility of zoonotic transmission in the presence of zoonotic assemblages, particularly considering that cattle can excrete large numbers of cysts that may contribute to environmental contamination of soil and water sources in Wayuu communities [37]. 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 *Giardiosis* and can be asymptomatic or, in other cases, cause severe gastrointestinal symptoms, including chronic diarrhea, abdominal pain, and malabsorption [38]. 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 [39].

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 [34,40].

Strongylids were identified by microscopy in animal fecal samples (Figure 2L); cystic forms of amoebae and cysts of *Giardia* spp. were also observed. In contrast, only *Giardia* was detected in soil samples, indicating environmental contamination by fecal matter (Figure 3). The detection of the helminth *H. nana*, together with the presence of *T. trichiura* in human samples and *Trichuris* spp. eggs in animal and environmental samples suggest constant exposure to contaminated sources. The detection of *Trichuris* eggs in animal samples may reflect environmental dissemination or pseudoparasitism. These findings are consistent with

limited access to safe drinking water, basic sanitation, and waste management, conditions under which enteroparasite transmission tends to remain endemic [41,42].

The findings revealed a high circulation of intestinal parasites across human, animal, and environmental matrices, supporting the relevance of a One Health approach to understanding transmission dynamics in rural communities of La Guajira. Pathogenic protozoa and helminths, including *Blastocystis* sp., *Giardia* sp., the *Entamoeba histolytica*/*E. dispar* complex, and *Trichuris* spp., were detected in humans, animals, and soil, while molecular analyses confirmed *T. trichiura* DNA in human samples and *Trichuris* spp. DNA in soil and sheep feces. The occurrence of shared parasitic genera among these matrices suggests active fecal–oral transmission pathways associated with environmental contamination, deficient sanitation, and close human–animal interaction, consistent with previous One Health studies conducted in Latin America [12].

Likewise, the identification of *Giardia duodenalis* through metagenomics strengthens the evidence of zoonotic transmission potential, as this protozoan is commonly shared among humans, livestock, and environmental sources. Previous One Health investigations have emphasized that environmental matrices, particularly soil and water, can act as reservoirs for intestinal protozoa and helminths, facilitating persistence and reinfection in endemic settings [43].

As evidenced in the municipality 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* sp., the *E. histolytica/E. dispar* complex, and *Giardia* spp., 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 [44]. In this context, the implementation of periodic deworming and integrated parasite control programs is recommended to reduce the burden of helminths and other intestinal parasites that are frequently underdiagnosed in these communities.

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 [45]. 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 conventional microscopy due to the morphological similarity between the two species [46]. 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* ($n = 3$), *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 [47]. 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 [48]. 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 [48].

5. Conclusions

The study demonstrated the circulation of intestinal parasites across human, animal, and environmental matrices in Wayuu indigenous communities from Manaure, supporting the presence of active fecal–oral transmission cycles associated with environmental contamination, deficient sanitation, and close human–animal interaction. The detection of shared parasitic genera, including *Giardia* spp. and *Trichuris* spp., across different matrices reinforces the relevance of a One Health approach to understanding transmission dynamics in these rural settings.

Overall, these findings highlight the need for integrated and culturally appropriate control strategies involving human health, animal management, environmental sanitation, safe water access, and molecular surveillance. Strengthening intersectoral interventions and community-based public health actions will be essential to reducing the burden of enteric parasitic diseases in vulnerable Wayuu communities of La Guajira.

6. Limitations

Among the study's limitations are the relatively small sample size, the cross-sectional design, and the recruitment of participants based on the presence of symptoms, which may limit the generalizability of the findings and prevent robust estimates of prevalence or risk. Another limitation was the reduced number of animal fecal samples collected, as access to livestock, an important means of subsistence in Wayuu communities, is culturally restricted and dependent on owner authorization. In addition, because fecal samples were preserved in SAF solution to maintain parasite morphology, coproculture and larval development techniques could not be performed, limiting species-level identification of some nematodes. Finally, metatranscriptomic analyses were performed only on a subset of samples due to sample availability and quality criteria. 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 July 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 on 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&currentTab=samples&mapSidebarTab=summary&projectId=20261&showFilters=true&showStats=true&workflow=short-read-mngs (accessed on 1 May 2025). 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:

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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