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Metagenomic Profiling of Human Protozoan Parasites in Wastewater and Hospital Effluents

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ABSTRACT

Objective: Advancements in metagenomic techniques have provided new tools for profiling human parasites in environmental matrices such as wastewater. This study aimed to profile protozoan parasites in wastewater from a major city, rural area, and hospital in Kayseri, Türkiye, using metagenomic techniques.

Materials and Methods: Shotgun metagenome sequencing was conducted on ten water samples collected from five sampling sites over a two-week period. The sequences were aligned to 80 human parasite genomes to evaluate the presence and relative abundance of each parasite species. A comparative bioinformatic analysis was performed on the metagenomes from each sampling point.

Results: The diversity of parasites in the city wastewater exceeded that of the rural and hospital sampling points. *Blastocystis* spp. subtypes and *Giardia intestinalis* were dominant in rural wastewater, while *Plasmodium falciparum*, *Plasmodium ovale*, *Toxoplasma gondii*, and *Acanthamoeba* species showed significant abundance in hospital effluent ($p<0.01$). Moreover, protozoan parasites not previously reported in a clinical setting were identified in the water samples.

Conclusion: This is the first study in Türkiye investigating the presence of human parasites in wastewater using metagenomics. The study highlights the risk posed by human parasites in treated wastewater to population using natural resources. Implementing a specialized wastewater treatment targeting parasites could mitigate the potential spread of these pathogens in the environment. The study revealed certain sequences associated with species not previously identified in clinical instances. This finding may result from genomic resemblances with other eukaryotic organisms that were not systematically excluded, or alternatively, the displacement of protozoa linked to the increasing influx of refugees.

Keywords: Parasites, sewage-based surveillance, wastewater, hospital effluents, metagenomics, metagenome.

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INTRODUCTION

Parasites that cause various infections in humans encompass a wide class of both single-celled (protozoa) and multicellular organisms (nematodes, cestodes, and trematodes). They generally have complex life cycles and can easily be transmitted through direct contact, vectors, or environmental routes. Infections from these parasites can occur through the ingestion of environmentally-resistant forms of the parasite (eggs, larvae, or (oo)cysts) through water, soil, or food (such as fresh produce).[1,2] Among these parasites, intestinal protozoans are a significant cause of parasite-induced diarrhea in healthy individuals, animals, and immunocompromised individuals.[3] Approximately 88% of deaths associated with diarrhea are caused by contaminated or untreated water, inadequate sanitation, and poor hygiene.[4,5]

In studies conducted to date, it has been reported that there are approximately 15,000 species of protozoan parasites worldwide that can cause various infections in vertebrate hosts.[6] Among them, *Acantamoeba* spp., *Balantidium coli*, *Blastocystis hominis*, *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia* spp, *Isospora belli*, *Naegleria* spp., *Sarcocystis* spp., and *Toxoplasma gondii* are among the waterborne protozoan parasites responsible for human infections.[7–9] Due to their small size (1–17 µm), low infection doses, resistance to chlorine, and high environmental durability in various forms, these parasites occupy an important niche among waterborne pathogens.[6] Therefore, it is crucial to fully understand the diversity of these parasites in the environment, especially in water sources.

Many parasites cannot be cultured using in vitro systems. Diagnosis is usually achieved through microscopy alone or a combination of traditional methods such as enzyme staining techniques, and molecular methods such as Polymerase Chain Reaction (PCR) and sequencing.[10] Molecular methods are typically target-specific and may include sequencing techniques such as Sanger sequencing. However, matrices with high microbial loads, such as water, soil, mud, and food, require labor-intensive concentration and purification steps for analysis.[11–13] Additionally, due to the close morphological and genomic relationships between protozoan parasite species, they may not be easily distinguished using traditional or PCR-based methods. For example, *Entamoeba histolytica* and *Entamoeba dispar* are morphologically indistinguishable,[14] and oocysts of Eimeria species may exhibit morphological variability.[15] Furthermore, the high similarity (95–97%) between the genomes of *Cryptosporidium parvum* and *Cryptosporidium hominis* complicates the differentiation of these two species when using molecular methods such as PCR and Sanger sequencing.[16] Hence, new methods are required for the detection and profiling of protozoan parasites, especially in environmental samples.

Metagenomic studies, supported by Next Generation Sequencing (NGS) technology, are widely used in microbiome studies. These can be applied without prior knowledge of microbial communities in a given sample and are not culture-dependent. They enable the detection of low-density microbial communities in complex populations and allow for faster microbial evaluation and the discovery of new species.[17] Despite the widespread use of various metagenomic techniques, metagenomic applications for eukaryotes, especially protozoan parasites, are limited compared to prokaryotes.[18] This study aims to profile human protozoan parasites by generating metagenomic data from water samples collected from a large city wastewater treatment plant before and after treatment, a town wastewater treatment plant before and after treatment, and a regional hospital wastewater.

MATERIALS AND METHODS

Sites and Sampling

This research was conducted in Kayseri, a metropolitan city in Türkiye with a population of approximately one million inhabitants. For this study, three sampling sites were chosen: the wastewater outlets of a tertiary hospital complex, consisting of five hospitals with a bed capacity of 1,300, an urban sewage treatment plant serving $1x10^6$ individuals, and a rural sewage treatment plant serving $2x10^4$ individuals. The effluent from the hospital complex (HWW) was discharged directly into urban sewage canals via two primary outlets without any prior treatment. The urban sewage treatment plant (uSTP) received effluent from community sewage, various industrial areas, hospital sites (including selected HWW), and livestock farming. The uSTP underwent a biological treatment involving four typical processes: pre-treatment, primary treatment, secondary treatment, and biological nutrient removal, then released the treated effluent into the nearby river. The rural sewage treatment plant (rSTP) was included in the study as a reference for human contribution since it receives effluent only from household waste. It underwent pre-treatment and primary treatment before discharging the treated effluent into a nearby waterway.

In August 2016, two weekly water samples were collected from five distinct sites, including: (i) the main outlets of the untreated hospital effluent (HWW), (ii) the incoming effluent of an urban sewage treatment plant (uSTP-I), (iii) the outlet of treated effluent water (uSTP-O), (iv) the incoming effluent of a rural sewage treatment plant (rSTP-I), and (v) the outlet of the rural sewage treatment plant (rSTP-O). The samples were obtained using the "grab-sampling" technique, wherein 500 ml of water was collected in a sterile microbiological container mounted onto a handle of appropriate length. The collected samples were transported on ice and processed within four hours of collection.[19]

Total Deoxyribonucleic Acid (DNA) Extraction and Shotgun Metagenome Sequencing

Total DNA was isolated from the samples using the PowerSoil DNA Isolation Kit (Qiagen) following the manufacturer's protocol. For effluent sampling points, 250 ml water samples were filtered through 0.22 µl membrane filters, and the filters were used for DNA isolation. The yield of extracted double-stranded DNA (dsDNA) was measured using the Qubit2 dsDNA Broad Range (BR) assay kit on a Qubit fluorometer (Life Technology). Shotgun DNA sequencing was performed on each of the ten samples included in the study, which comprised two samples from each of the sampling sites (urban STP - inlet, urban STP - outlet, rural STP - inlet, rural STP - outlet, and hospital wastewater). The preparation of samples for sequencing was conducted using the Nextera-XT Sample Preparation Kit following the manufacturer's recommendations. After the index PCR step, sequencing was performed using the Illumina NextSeq 500 platform in High-Output mode.

Bioinformatics Analysis

The current investigation employed metagenomic data obtained from a prior initiative (ERU-BAP TCD-2016-6041). The bioinformatic analysis involved aligning metagenomic sequences from each sample with human and protozoan parasite genomes to identify DNA sequences specific to these organisms. To facilitate this alignment, a human-parasite sequence database was constructed. This involved obtaining the genomic sequences of all 80 parasites identified in the National Center for Biotechnology Information (NCBI) GenBank database and establishing a 2.97 Gbp length panel. The quality filtering and trimming of the raw reads were conducted using the FASTX-Toolkit (version 0.0.13). To filter out DNA fragments of the human genome, the Bowtie2 short DNA sequence mapping tool[20] was utilized with the human genome reference sequence hg19 as a reference in the "--very-sensitive" option. Subsequently, the DNA readings were aligned to the established database, following filtration with the Burrows-Wheeler Aligner (BWA) DNA read aligner,[21] which enabled the identification of genomic fragments of the 80 parasites. During the alignment, sensitivity to differences arising from mutations compared to reference genomes was ensured by allowing up to six nucleotide changes and three INDELs per read. Multiple reads, due to multiplicative/homologous regions in different genomes, were not allowed in the alignments, and the corresponding coordinates were subtracted from the total genome lengths to ensure specific core-genome alignment normalization. Total DNA sequences identified for each species were normalized by the specific-genomic length of the species, and genome coverage per million nucleotides was reported.

Statistical Analysis

Data were analyzed using the Python SciPy module (version 1.10.1) and in-house Python (Version 3.7) scripts. The mean, Standard Deviation (SD), median, first, and third quartiles, and minimum and maximum values of the numerical variables were specified. Categorical variables were represented as frequency and percentage (%). The normal distribution of the variables was analyzed with the Shapiro-Wilk test. Chi-Square analysis using Fisher's exact test with contingency tables was used to compare categorical variables. A p-value less than 0.05 was accepted as statistically significant. To control for false discovery errors, Bonferroni correction was applied for multiple tests. Principal Component Analysis was conducted on relative abundance vectors and visualized for the first three principal coponents.

RESULTS

Overview of Metagenomic Data Sets

In this study, we utilized the Illumina NextSeq 500 platform to generate approximately 80 Gb of raw reads from ten samples. Following quality filtering and trimming, 6% of the raw reads were removed. The metagenomic sequences were then filtered to exclude human contamination and aligned to a panel of 80 human parasite genomes using short-read mapping. The total length of DNA sequences mapped to each parasite species was normalized by the genome length and sequencing depth to determine relative abundance. The total lengths of DNA sequences identified for each environmental sample were as follows: for urban input and output, 4.28 Gbp (median) (1.34 Gbp 1st quartile - 5.13 Gbp 3rd quartile) and 1.2 Gbp (median) (0.72 Gbp 1st quartile - 1.38 Gbp 3rd quartile); for rural input and output, 3.84 Gbp (median) (2.8 Gbp 1st quartile - 4.89 Gbp 3rd quartile) and 2.34 Gbp (median) (0.8 Gbp 1st quartile - 2.79 Gbp 3rd quartile); for hospital wastewater (HWW), 1.41 Gbp (median) (1.17 Gbp 1st quartile - 2.11 Gbp 3rd quartile). The detected length of human protozoan parasite DNA was: for urban input and output, 9.7 Mbp (median) (5.6 Mbp 1st quartile - 11.2 Mbp 3rd quartile) and 1.3 Mbp (median) (1.1 Mbp 1st quartile - 2.3 Mbp 3rd quartile); for rural input and output, 19.5 Mbp (median) (8.9 Mbp 1st quartile - 26.3 Mbp 3rd quartile) and 3.8 Mbp (median) (3 Mbp 1st quartile - 3.9 Mbp 3rd quartile); and for HWW, 13.8 Mbp (median) (7.3 Mbp 1st quartile - 15.5 Mbp 3rd quartile).

Analysis of Human Protozoan Parasites

Our study found that water samples collected from all five sampling sites were contaminated with a diverse range of protozoan parasite species. The highest parasite diversity was observed in uSTP, with 73 out of 80 species detected in uSTP-I, 75 species in uSTP-O, 65 species in both rSTP-I and rSTP-O, and 61 species in HWW. The relative abundances of the average parasite loads at the species level are presented in Figure 1, and Figure 2 displays the same information at the genus level.

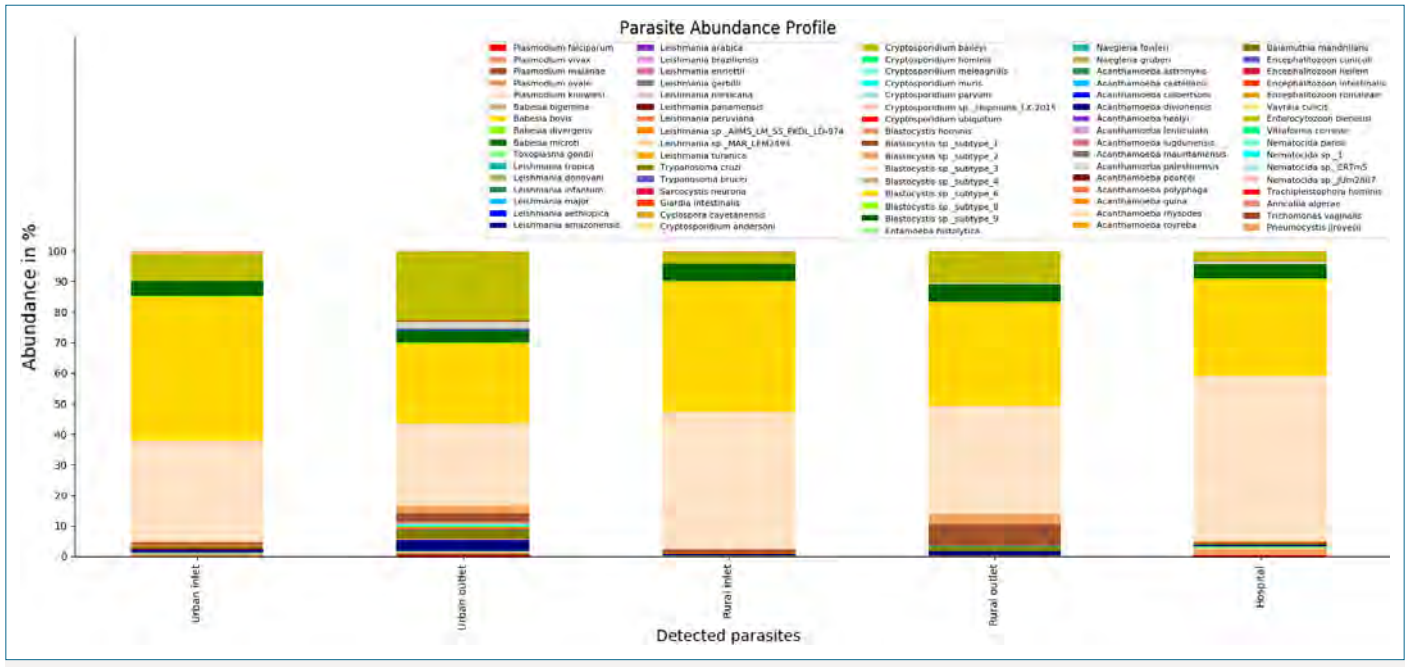

Figure 1. Relative abundances of average parasite loads at the species level across all samples.

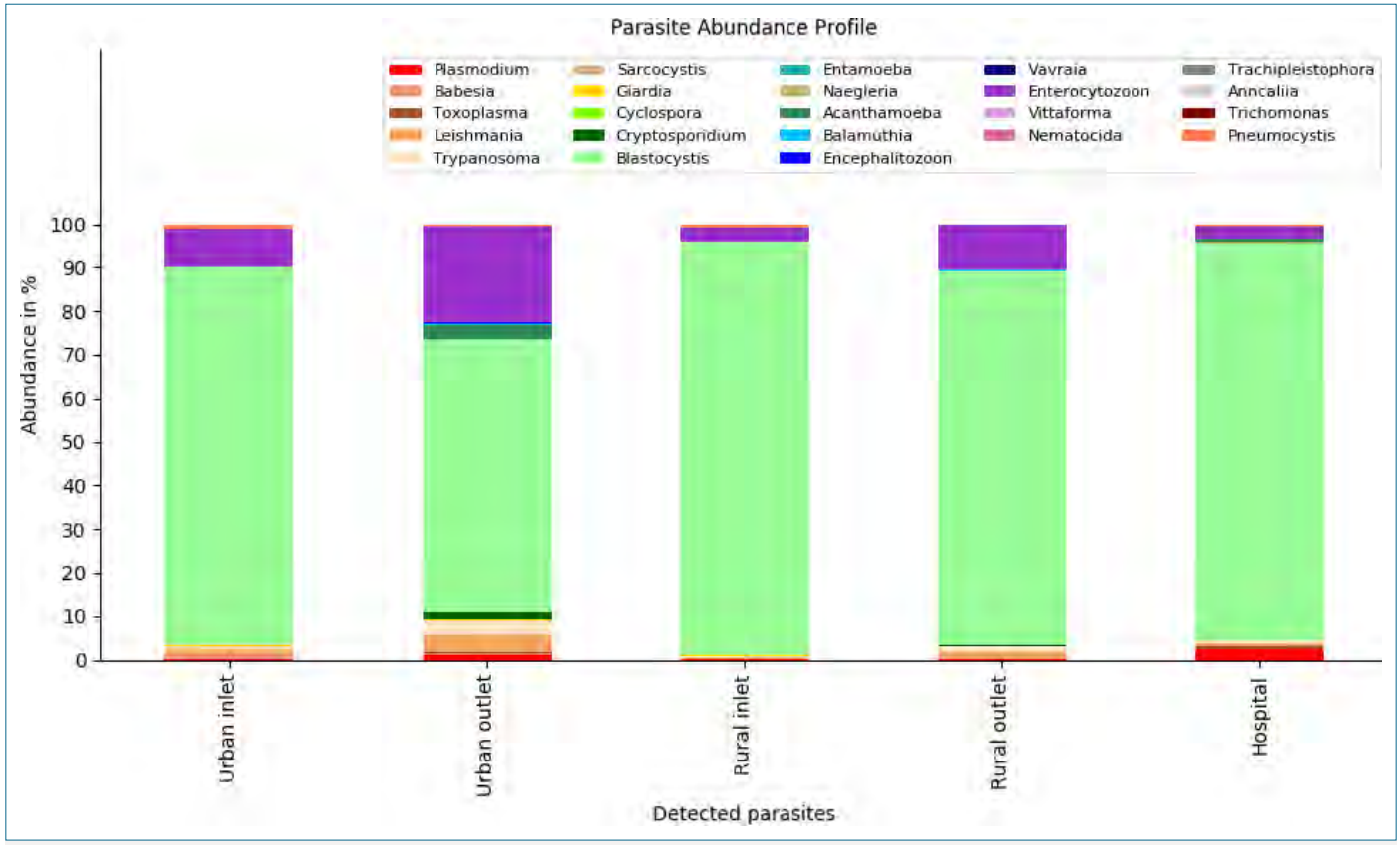

Figure 2. Relative abundances of average parasite loads at the genus level across all samples.

The most abundant species identified were *Blastocystis* sp. Subtype 6, accounting for 56.6% of the total detected abundance in urban inlets, but this shifted to *Enterocytozoon bieneusi* (27% of total detected abundance) in the urban outlets. *Blastocystis* sp. Subtype 6 was also the most abundant parasite (52.4% of total detected abundance) in rural inlets. While it remained the most abundant species in the rural outlets, its relative abundance decreased to 36.8%. In hospital wastewater, Blastocystis sp. Subtype 3 was found to be the most abundant parasite, constituting 55.7% of the total detected abundance. Appendix 1 provides the average normalized number of reads assigned to different taxa of protozoan parasites.

The relative abundances of the detected species were tested for normality. The Shapiro-Wilk test indicated non-normal distributions for the abundances. Therefore, the Mann-Whitney U-test was used to highlight the abundance differences. It was found that *Blastocystis* subtypes (STs) have the greatest relative abundance in both urban and rural STP inlets ($p<0.01$, Chi-Square test). At each sampling point, the average normalized number of reads identifying *Blastocystis* ST3 and *Blastocystis* ST6 were found to be significantly higher than those for other subtypes. *Enterocytozoon* was the second most abundant genus observed in both rural and urban STPs. Despite its classification as a fungus, *Pneumocystis jirovecii* is an opportunistic parasite that possesses distinct characteristics reminiscent of protozoa. Given its inclusion in the protozoan genome library employed in our study, we included this species in our investigation. In our findings, both *Giardia intestinalis* and *Pneumocystis jirovecii* wererelatively more abundant ($p<0.01$) in the rSTP than in other sampling points. *Babesia bovis* was detected only in uSTP samples, while *Babesia micoti* was found in both treated water samples. *Encephalitozoon* spp. were the scarcest species. *Leishmania donovani*, *Leishmania enriettii*, *Leishmania gerbilli*, and *Leishmania panamensis* were only found in the outlet of the rural STP. *Plasmodium falciparum*, *Plasmodium ovale*, *Toxoplasma gondii*, *Enterocytozoon bieneusi*, and some *Acanthamoeba* species were found to be more abundant in HWW (all with $p<0.01$).

Thirty-eigth species in uSTP and twenty-seven species in rSTP were found to be enriched after the treatment process. Additionally, four species in uSTP and eigth species in rSTP were detected only in the treated samples. Figure 3 and 4 show the log-odd ratios of normalized outlet/inlet loads for the species with the highest and lowest attenuation. According to the log-odd ratios, all *Blastocystis* spp. significantly decreased after urban treatment, but on the other hand, *Blastocystis sp. ST1* and *Blastocystis sp. ST2* increased in the rSTP. Several *Leishmania* species, along with *Babesia microti* and *Acanthamoeba palestinensis*, appear to be resistant to wastewater treatment in the rural STP. *Encephalitozoon intestinalis*, *Plasmodium knowlesi*, *Blastocystis hominis*, *Cyclospora cayetanensis*, and other *Acanthamoeba* species also proved to be among the most resistant parasites in urban STPs. The average normalized rates of reads identifying *Criptosporidium* species were higher in both treated water samples compared to untreated water. The Bray-Curtis dissimilarity measure was used to assess the beta-diversity between the parasitic compositions of the samples. A three-dimensional plot illustrating the first three principal coordinates is shown in Figure 5a and 5b. According to this, the parasitic profile of the hospital resembles that of a rural inlet instance more than the urban profiles. Although the water treatment process significantly alters the profiles, pushing treated samples into a different section of the principal components space, urban and rural parasitic profiles still do not cluster together, maintaining distinct identities even after water treatment.

DISCUSSION

The increasing prevalence of waterborne diseases caused by outbreaks has become a significant concern in recent decades. Some pathogens can survive for years, making the detection og their presence in wastewater and treated water a vital indicator for evaluating transmission risk.[22] To our knowledge, this study is the first report of a metagenomic analysis on parasite detection in untreated and treated water samples collected from metropolitan and rural areas, as well as from HWW in Türkiye. According to the metagenomic data generated in this study, the diversity of protozoan parasites varies at different sampling points. A similar investigation using shotgun metagenomic sequencing to profile the microbiome of ten wastewater treatment plants in Switzerland was conducted by Freudenthal et al.[23] Their analysis also revealed an unexpected diversity and relative abundance of active parasites, particularly in the inflow. They observed a reduction in parasite load after undergoing the treatment process. However, the present study revealed that certain detected parasites were not effectively removed by the wastewater treatment process, highlighting a potentially concerning issue.

It is well known that a significant number of emerging waterborne pathogens are zoonotic organisms. Among them, *Giardia* spp. and *Cryptosporidium* spp. are more prevalent, and outbreaks caused by these organisms are reported more frequently than those caused by other parasitic organisms.[24] The inactivation of these resistant parasites by chemical disinfectants is difficult, while the removal of *Cryptosporidium* can only be achieved by filtration using filters with a pore size of 1 micron or smaller.[25] According to the results of this study, although the relative abundance of *Giardia intestinalis* was significantly reduced after treatment, the ratio of certain *Cryptosporidium* species was significantly higher in treated water compared to untreated water. This observation suggests that the wastewater treatment systems in the investigated locations are insufficient for the removal of *Cryptosporidium oocysts*.

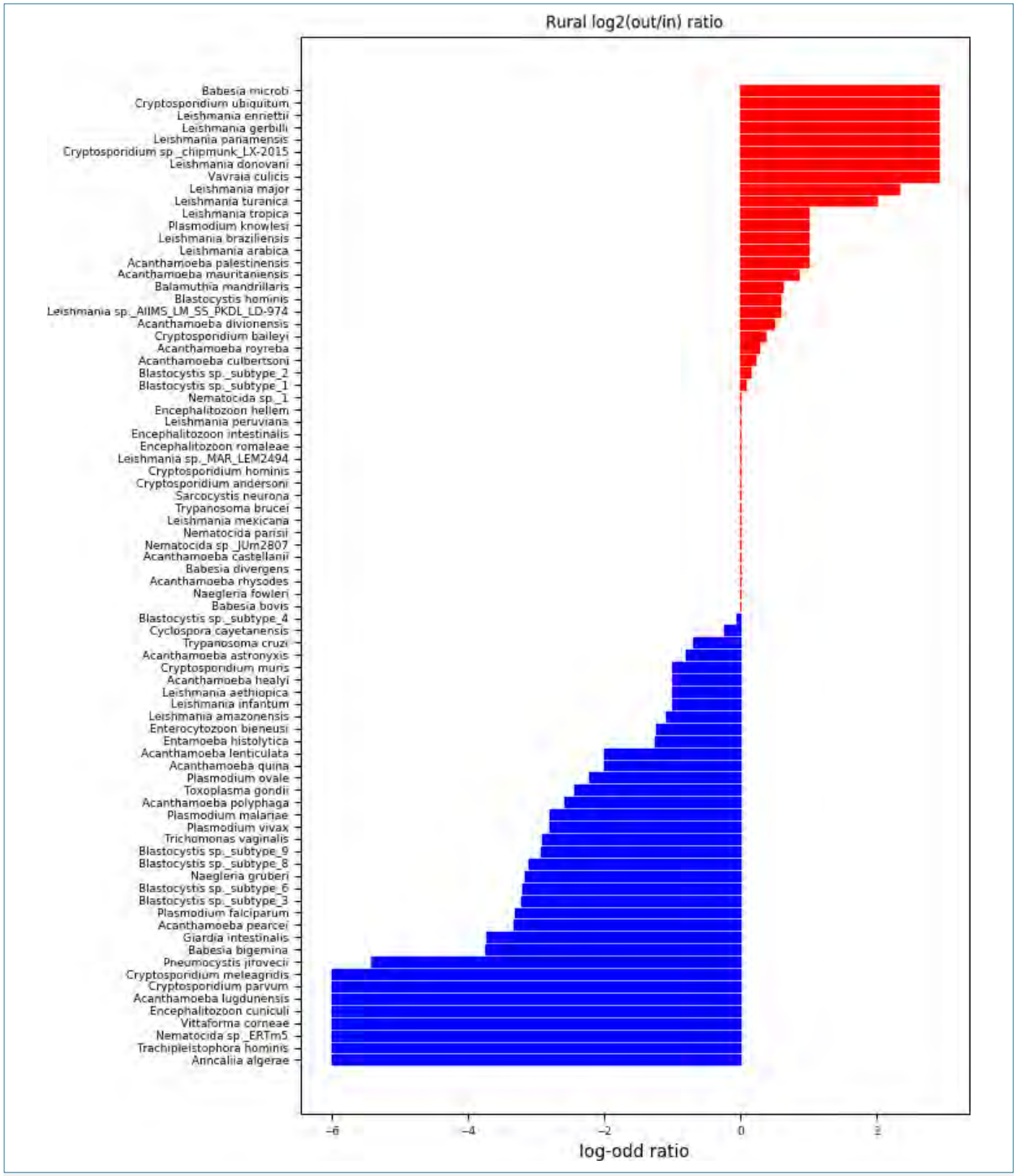

Figure 3. Log-odds ratio of normalized outlet-inlet loads for rural STP .

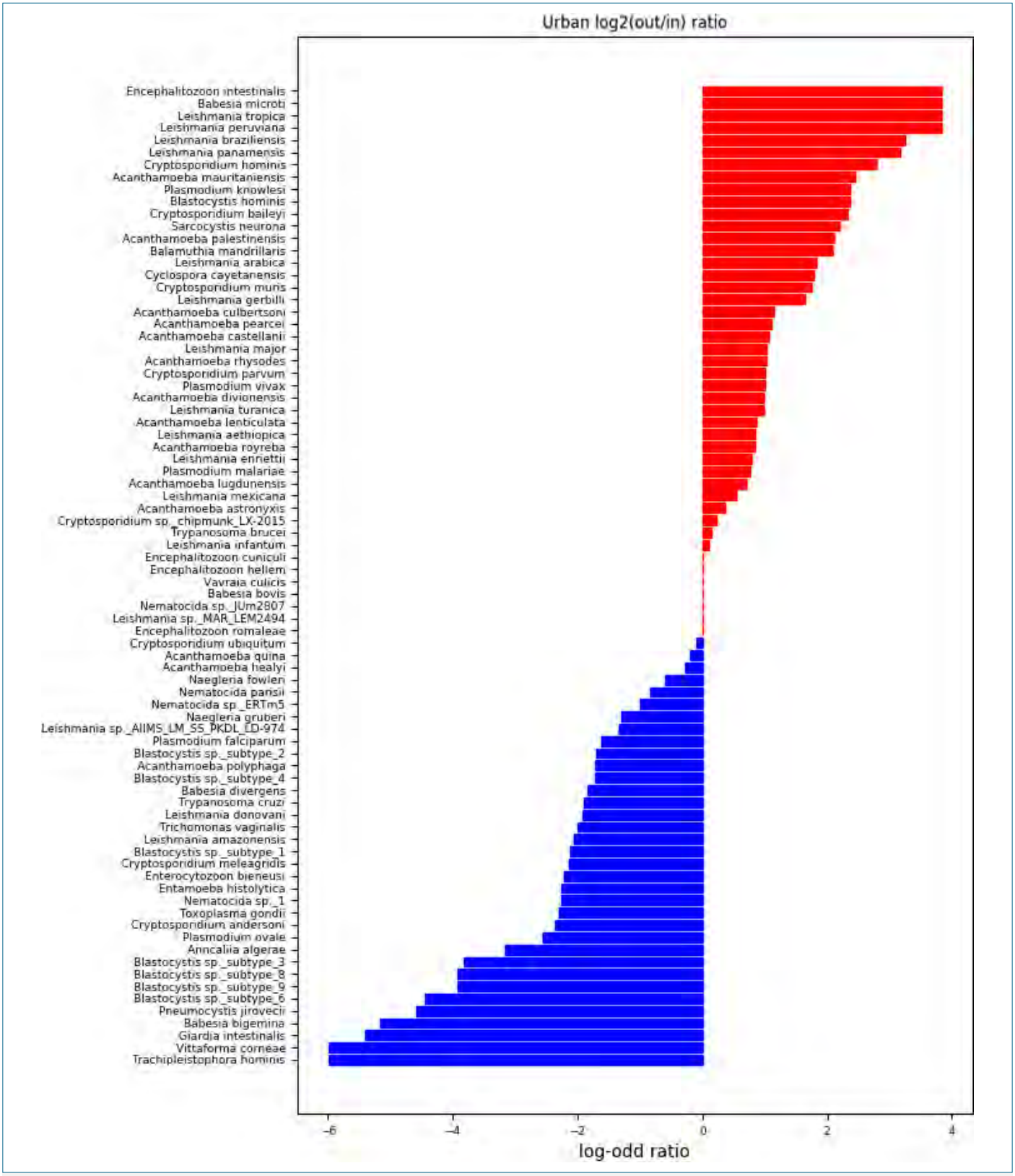

Figure 4. Log-odds ratio of normalized outlet-inlet loads for urban STP.

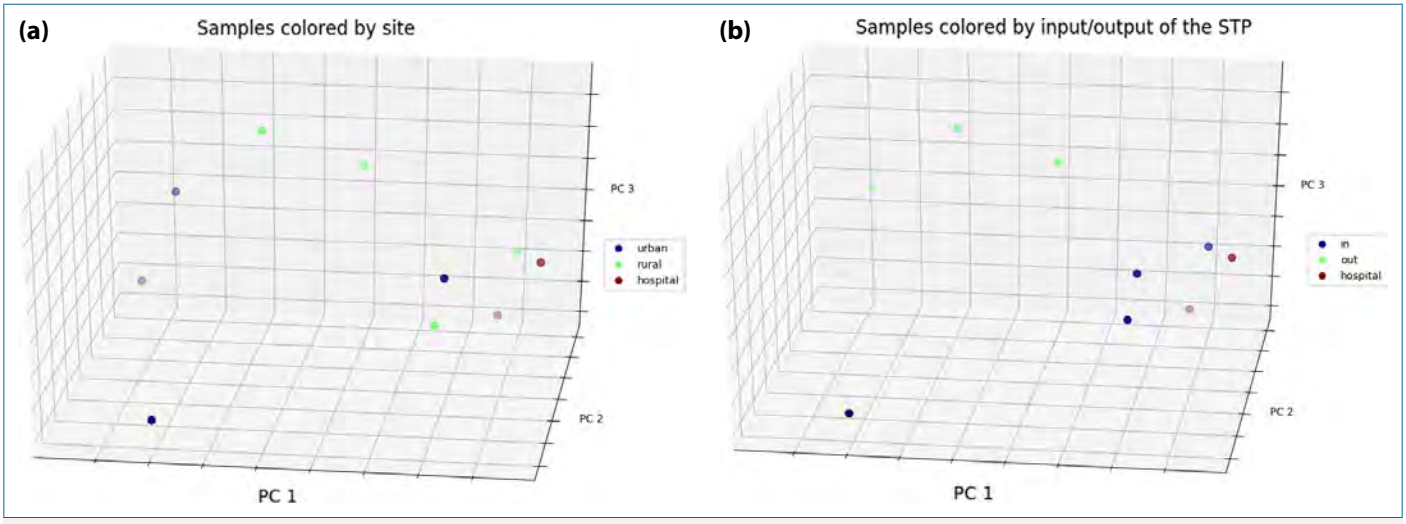

Figure 5. Principal Coordinates Analysis (PCoA) of relative abundance profiles for rural, urban, and hospital wastewater samples; **(a)** Analysis across urban, rural, and hospital wastewater samples, **(b)** Analysis across inlets, outlets, and hospital wastewater samples.

Another common zoonotic parasite, *Blastocystis* STs, can present both symptomatic and asymptomatic conditions in humans. Some researchers have conducted metagenomic analysis of fecal DNA collected from different patients to determine the prevalence of *Blastocystis* STs and to elucidate its relationship with intestinal microbiota.[26] However, no metagenomic study has been conducted so far to detect *Blastocystis* STs in environmental samples. According to our study, sequence reads identifying ST3 and ST6 were abundant in each sample for this waterborne-zoonotic and human-human transmitted parasite group. Our findings on *Blastocystis* ST3 corroborate previous studies, showing that this subtype was detected in Asia, Europe, and Africa with nearly equal frequency. Conversely, the frequency of *Blastocystis* ST6, previously found only at higher rates in Africa,[27] might be explained by Türkiye's geographical bridging these three continents. In our study, among the three sampling points, the reads identifying *Blastocystis* STs were significantly higher in the rural area. This could be due to the vast range of animal reservoirs of the parasite and the existence of numerous farms and wild animal species in the rural area.

Our study shows that the normalized sequence reads before and after the wastewater treatment process reveal that while *Blastocystis* parasites were significantly reduced, they are still detectable in treated water.

Microsporidia are spore-forming, eukaryotic, intracellular parasites that primarily parasitize in invertebrates and vertebrates and rarely infects humans. Despite the high number of species in this group, it is known that 14 species of *Microsporidia* group cause diseases in humans. *Encephalitozoon intestinalis* and *Enterocytozoon bieneusi* are reported to be the most common species infecting humans.[28] In our study, DNA fragments specific to *Enterocytozoon bieneusi* were detected more abundantly compared to other *Microsporidia* species. Given its rigid spore wall, *Microsporidia* can survive and remain infectious in the environment for extended periods. Moreover, its zoonotic, environmental, foodborne, and waterborne transmission methods allow the parasite to be widely distributed across natural ecosystems. These factors, combined with the parasite's adverse effects on public health and the economy, emphasize the necessity to remove the parasite spores from wastewater. In this study, the detection of *Microsporidia*-specific DNA in treated water indicates that the standard treatment process is inadequate for the complete eradication of *Microsporidia*.

Trypanosoma parasites, obligate intracellular organisms, cause severe diseases in humans and other vertebrates. Depending on vector arthropods, *Trypanosoma cruzi*, endemic in America, causes Chagas disease, while *Trypanosoma brucei*, endemic in the African continent, induces African sleeping sickness. Although neither of these *Trypanosoma* species are endemic in Türkiye, *T. Cruzi*-specific reads were surprisingly obtained in this study. *Leishmania* species, another type of flagellates in *Trypanosomatidae* family, are endemic in Türkiye for some of its parasitic speci.[29] However, no case caused by *L. amazonensis* has been reported to date. Interestingly, genomic DNA specific to *L. amazonensis* was detected in this study. The detection of DNA reads belong-

ing to certain protozoan parasites not previously reported in clinics (such as *Trypanosoma* spp., *Acanthamoeba* spp., *Leishmania amazonensis*) could be attributed to genomic similarities with other unfiltered eukaryotes or perhaps due to the migration of these protozoa owing to the increasing refugee population in the geographic region.

Our study demonstrates the presence of DNA reads belonging to specific human protozoan parasites in the tested water samples. This study builds upon the findings of Maritz et al.'s[18] which employed a primer-based approach to detect various parasitic protists in raw sewage. While they identified a few taxa, our study not only detected the same taxa, but also numerous additional ones such as *Dientamoeba*, *Giardia*, and so on. Many of these eukaryotic parasites are characterized by highly divergent marker gene sequences, rendering them undetectable using conventional primer-based sequencing methods. This limitation often results in the inability to detect taxa such as *Giardia*.

CONCLUSION

In conclusion our study observed that a portion of the detected parasites could not be efficiently removed after the wastewater treatment process, and some of the detected sequences belonged to clinically non-reported species. Therefore, our findings suggests that further research to determine the viability and infectivity of these organisms is necessary. This may reveal the existence of previously undetected parasites in aquatic ecosystems or confirm the genomic similarities with other unfiltered eukaryotes. The presence of human parasites in treated wastewater poses a potential risk to the public using nearby freshwater sources, as the treated water is typically discharged into these sources. To mitigate the risk of transmission of these harmful pathogens, a specialized wastewater treatment process that can effectively eliminate parasites may be necessary.

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Conflict of Interest: The authors have no conflict of interest to declare.

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Appendix 1. The parasite species detected at the sampling points and the number of normalized readings

	A uSTP-untreated	uSTP-treated	rSTP- untreated	rSTP- treated	Hospital
Plasmodium falciparum	0.042674597	0.013990038	0.025118477	0.002543643	1.307220632
Plasmodium vivax	0.003555004	0.007110008	0.003110629	0.000444376	0.000888751
Plasmodium malariae	0.01676427	0.028570504	0.013157469	0.001879638	0.021302569
Plasmodium ovale	0.142397949	0.024011095	0.064291391	0.01380077	4.705725872
Plasmodium knowlesi	0.001972372	0.010230556	0.000511528	0.001023056	0
Babesia bigemina	0.640328581	0.017920195	0.219377867	0.016475018	0.029481611
Babesia bovis	0.000978158	0.000978158	0	0	0
Babesia divergens	0.007990824	0.002223014	0.001111507	0.001111507	0.000741005
Babesia microti	0	0.011264774	0	0.001877462	0
Toxoplasma gondii	0.071643443	0.014620517	0.028692766	0.005299938	1.646757618
Leishmania tropica	0	0.002425365	0.000363805	0.00072761	0.000970146
Leishmania donovani	0.001866181	0.000493207	0	0.000369905	0.000246603
Leishmania infantum	0.000693388	0.000747247	0.000747247	0.000373624	0.000249082
Leishmania major	0.001546703	0.003165827	0.000365288	0.001826439	0.001461151
Leishmania aethiopica	0.000704157	0.001264754	0.000758852	0.000379426	0.000252951
Leishmania amazonensis	0.864628986	0.206714277	0.686704828	0.321233986	0.277548369
Leishmania arabica	0.001224056	0.004349909	0.000767631	0.001535262	0.000255877
Leishmania braziliensis	0.000498993	0.00474043	0.000374245	0.000748489	0.001746474
Leishmania enriettii	0.001504336	0.002600961	0	0.001560577	0.000520192
Leishmania gerbilli	0.001219006	0.003822318	0	0.000764464	0.000254821
Leishmania mexicana	0.001885722	0.002741043	0.001121336	0.001121336	0.000249186
Leishmania panamensis	0.000260715	0.002346439	0	0.000391073	0.000521431
Leishmania peruviana	0	0.000972541	0.000364703	0.000364703	0
Leishmania sp._AIIMS _LM_SS_PKDL_LD-974	0.00364956	0.001436539	0.001723847	0.00258577	0.000861923
Leishmania sp._MAR_ LEM2494	0.000778969	0.000778969	0.000389484	0.000389484	0.001038625
Leishmania turanica	0.002261451	0.004456024	0.000371335	0.001485341	0.000742671
Trypanosoma cruzi	0.682826347	0.183707103	0.502948235	0.309455282	0.290283911
Trypanosoma brucei	0.003944454	0.004335024	0.000541878	0.000541878	0.002528764
Sarcocystis neurona	0.001625236	0.007524794	0.001640019	0.001640019	0.000964717
Giardia intestinalis	0.181462691	0.004281068	0.353188089	0.026756673	0.072778152
Cyclospora cayetanensis	0.004294414	0.014901906	0.00350105	0.002962427	0.001615869
Cryptosporidium andersoni	0.022627095	0.004401711	0.002641027	0.002641027	0
Cryptosporidium baileyi	0.005244641	0.026376106	0.00989104	0.012717051	0
Cryptosporidium hominis	0.003395663	0.023333149	0.001346143	0.001346143	0.001794858
Cryptosporidium meleagridis	0.007832133	0.001783319	0.00133749	0	0.002674979
Cryptosporidium muris	0.006923374	0.023366386	0.007788795	0.003894398	0.001730843
Cryptosporidium parvum	0.004395054	0.008790108	0.001318516	0	0.000879011
Cryptosporidium sp._chipmunk_LX-2015	0.005048091	0.005889439	0	0.001262023	0
Cryptosporidium ubiquitum	0.008533828	0.00802761	0	0.001337935	0.000891957
Blastocystis hominis	0.006010195	0.0310394	0.002551184	0.003826775	0.000850395

Appendix 1 (cont). The parasite species detected at the sampling points and the number of normalized readings

	A uSTP-untreated	uSTP-treated	rSTP- untreated	rSTP- treated	Hospital
Blastocystis sp._subtype_1	0.594435786	0.136036537	1.480854873	1.558104192	0.154984483
Blastocystis sp._subtype_2	0.364798451	0.112201533	0.6987382	0.768706571	0.223772721
Blastocystis sp._subtype_3	15.53139467	1.104904919	72.11410199	7.793390882	27.2195284
Blastocystis sp._subtype_4	0.008134637	0.002477226	0.023223995	0.022295035	0.00185792
Blastocystis sp._subtype_6	22.10384321	1.012466401	68.66296999	7.463501681	17.21140987
Blastocystis sp._subtype_8	0.236722579	0.015689641	0.574633098	0.066680974	0.056874948
Blastocystis sp._subtype_9	2.170085764	0.14342581	9.296041419	1.228339614	1.829020566
Entamoeba histolytica	0.016450184	0.003456108	0.006912215	0.00288009	0.001536048
Naegleria fowleri	0.003906059	0.002591077	0.000863692	0.000863692	0.00115159
Naegleria gruberi	0.003821901	0.001562547	0.002636799	0.000292978	0.105276636
Acanthamoeba astronyxis	0.003786679	0.004890816	0.001006933	0.00057539	9.59E-05
Acanthamoeba castellanii	0.002351826	0.004950689	0.001142467	0.001142467	0.01218631
Acanthamoeba culbertsoni	0.004041145	0.008931052	0.001296443	0.001512517	0.000288098
Acanthamoeba divionensis	0.003140236	0.006229476	0.000707895	0.000991053	0
Acanthamoeba healyi	0.003215591	0.002655745	0.001274757	0.000637379	0.000849838
Acanthamoeba lenticulata	0.001981464	0.003635423	0.001454169	0.000363542	0.000121181
Acanthamoeba lugdunensis	0.0024644	0.004023976	0.000845035	0	0.007323636
Acanthamoeba mauritaniensis	0.0102377	0.055868395	0.002920734	0.005279788	0.043436554
Acanthamoeba palestinensis	0.032799239	0.141413841	0.01530888	0.030385806	0.334166552
Acanthamoeba pearcei	0.001926499	0.004152282	0.00103807	0.000103807	0.008373769
Acanthamoeba polyphaga	0.016500733	0.00502561	0.008754289	0.001459048	0.554924638
Acanthamoeba quina	0.003839073	0.003350157	0.001722938	0.000430735	0.013783505
Acanthamoeba rhysodes	0.002900499	0.005909397	0.000316575	0.000316575	0.006437022
Acanthamoeba royreba	0.00258533	0.004626968	0.000754397	0.000905276	0.000603518
Balamuthia mandrillaris	0.006264544	0.026726699	0.002306065	0.003547792	0.000354779
Encephalitozoon cuniculi	0	0	0.004805393	0	0.003203596
Encephalitozoon hellem	0.003553201	0.003553201	0	0	0
Encephalitozoon intestinalis	0	0.007218231	0	0	0
Encephalitozoon romaleae	0	0	0	0	0
Vavraia culicis	0.002615279	0.002615279	0	0.00196146	0
Enterocytozoon bieneusi	6.432970267	1.374009352	5.483602936	2.328355215	1.780202162
Vittaforma corneae	0.002489809	0	0.003734713	0	0
Nematocida parisii	0.003505476	0.001965208	0	0	0
Nematocida sp._1	0.008951067	0.001871133	0	0	0
Nematocida sp._ERTm5	0.01091645	0.005458225	0.002729112	0	0
Nematocida sp._JUm2807	0	0	0	0	0
Trachipleistophora hominis	0.0009415	0	0.00141225	0	0
Anncaliia algerae	0.005920169	0.000657797	0.005920169	0	0
Trichomonas vaginalis	0.001638806	0.00040817	0.001020425	0.000136057	9.07E-05
Pneumocystis jirovecii	0.632660525	0.026409359	1.128266487	0.026409359	0.178996764