Article

Prevalence of Intestinal Parasites, Protozoans and Soil-Transmitted Helminths, in Children from Communities of Northern Argentina after the Interruption of Deworming

Ernesto Candela [1,*], Rubén O. Cimino [2,3], Leonardo Sandon [4], Carla Muñoz-Antoli [1] and María Victoria Periago [3,4]

[1] Department of Pharmacy Pharmaceutical Technology and Parasitology, Universitat de València, 46100 Burjassot, Spain; carla.munoz@uv.es
[2] Instituto de Investigaciones de Enfermedades Tropicales (IIET-CONICET), Sede Regional Orán, Universidad Nacional de Salta, Orán 4530, Argentina; rubencimino@gmail.com
[3] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires C1425FQB, Argentina; vperiago@mundosano.org
[4] Mundo Sano Foundation, Buenos Aires C1061ABC, Argentina; leomadvr@gmail.com
* Correspondence: ercansen@alumni.uv.es

Abstract: Soil-transmitted helminths (STHs) are a group of parasites that are globally distributed and are the most prevalent neglected disease (NTD) in Latin America and the Caribbean (LAC); their presence is associated with health and development problems. In Argentina, their distribution is heterogenous, and there are highly endemic areas in the north of the country. The World Health Organization (WHO) recommends the mass deworming of children as a first-line strategy for the prevention and control of STHs and recently also encourage the development of more sensitive diagnostic tests. The aim of this study was to determine the prevalence of STHs in Tartagal (Salta, Argentina) after four years of deworming interruption with albendazole and ivermectin. A total of 437 fecal samples were analyzed using standard coprological techniques, a subset of which were selected to molecularly typify protozoan parasites; 257 blood samples were analyzed for the presence of specific antibodies to the STH Strongyloides stercoralis. The most prevalent protozoan species were *G. intestinalis* (19.6–49.2%) and *B. hominis* (19.1–38.5%). Molecular characterization allowed us to evidence possible zoonotic or human-to-human transmission pathways for *Giardia intestinalis* or *Blastocystis* spp., while serology for *S. stercoralis* proved to be a useful screening tool for monitoring this parasite after treatment. In general, a decrease in the prevalence of STHs was observed in the area, from 60% to 2.9–20% for hookworms and from 51% to 1–9.3% for *S. stercoralis* four years after treatment, demonstrating the effectiveness and duration of anthelmintic treatment with these two drugs.

Keywords: soil-transmitted helminths; intestinal parasites; NIE-ELISA; *Giardia intestinalis*; *Blastocystis* spp.; Tartagal; Salta; Argentina

1. Introduction

Soil-transmitted helminths (STHs) are a group of parasites that comprises *Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis*, and hookworms. They are the most prevalent neglected tropical disease (NTD) in Latin America and the Caribbean (LAC), are considered the most common infection of humankind [1,2], and are transmitted by eggs present in human feces that contaminate the soil in areas with poor sanitation systems. Several studies have highlighted the importance of these infections and the health problems associated, mainly related to children's nutrition and growth, and how the environment and sanitary conditions influence their prevalence and distribution, as well as their impact on population development and the perpetuation of poverty [1,3,4].

The endemicity of these parasites in Argentina and their different prevalences, especially in the northern regions of the country, have been observed [2,5]. Preventive chemotherapy (PC) through the mass drug administration (MDA) of albendazole or mebendazole is recommended by the World Health Organization (WHO) as a first-line strategy for the prevention and control of STHs [6]. Although the National Ministry of Health of Argentina implemented a nationwide MDA program (2005–2007) with mebendazole, it was short lived, and currently, there aren't any active deworming plans at the provincial or national level in the country.

Among STHs, *S. stercoralis* infections may be underestimated due to the low sensitivity of standard diagnostic methods [7,8] and the particularity of the parasite's life cycle, with autoinfection cycles where L3 larvae reinfect the host through the intestinal mucosa and the perianal skin, leading to persistent and lifelong infections [5,9]. Recently, the WHO categorized *S. stercoralis* as an STH, particularly regarding preventive chemotherapy, and encourages the development of more sensitive and specific rapid diagnostic tests, as well as biomarkers and procedures for disease control [10]. In this regard, previous studies have suggested that the use of serological techniques may solve the problem of having to use additional and specific parasitological methods for *S. stercoralis* for diagnosis and follow-up [5,11].

One of the areas with a high prevalence of intestinal parasites (IPs), and specifically STHs, in Argentina is in the northern province of Salta, bordering Bolivia. Both parasitological and serological studies conducted in the past have demonstrated the presence of these IPs [1,2,12]. In addition, the Ministry of Public Health of Salta launched a triennial antiparasitic plan with albendazole for children aged 2–15 years old (2013–2015) in this area to mitigate the transmission of parasites through periodic deworming [13]; ivermectin was also used for deworming in Tartagal, given the presence of *S. stercoralis* in the area [1,2]. Nonetheless, although these studies reported the presence of protozoan parasites such as *Giardia intestinalis* and *Blastocystis* spp., molecular typification has not been carried out and only one study has detected the presence of *Entamoeba histolytica* through molecular techniques (in Orán, Salta province) [14].

All of these parasitic infections associated with health, control, and distribution related to human behavior should unite multidisciplinary efforts under the concept of One Health to try to improve their diagnosis, control, and prevention. Given the previous work conducted in this area, the main objective of this study was to determine the current prevalence of STHs, as well as the prevalence of other IPs, using standard coprology and *S. stercoralis* NIE-ELISA. We also evaluated the long-term effectiveness of the STH deworming program that was implemented previously and up to 2018 [2,5,13] and determine the need for MDA specifically for STHs after deworming program interruption. Finally, molecular methods were used to determine the species, types, or subtypes of the most important species of protozoans present in the study area.

2. Results

2.1. Study Population

A total of six communities were visited (Km 3, Km 4, Km 5, Km 6, Lapacho, and Las Moras) and 580 plastic containers for fecal collection were distributed. A total of 437 fecal samples were returned (75.3% participation). For the serological study, 257 samples were obtained from four communities (*n* = 405), with a participation rate of 63.5%. Population distribution ranged from 41.5% to 59.5% females and 40.5% to 58.5% males depending on the community. Participants had a mean age of 6.5 to 7.6 years and an age range of 1–15 years. Descriptive characteristics are detailed in Table 1.

Table 1. Descriptive characteristics and prevalence of intestinal parasites in individuals aged between 1 and 15 years from different communities in Tartagal located along National Route 86, in the Department of San José de San Martín, Province of Salta, Argentina.

	Km 3	Km 4	Km 5	Km 6	Lapacho	Las Moras
Age. mean ± SD	7.2 ± 4.2	6.5 ± 3.9	7.6 ± 4.6	6.9 ± 3.9	6.9 ± 4.2	6.8 ± 3.9
Gender. *n* (%)						
Female	43 (57.3)	29 (52.7)	25 (59.5)	50 (51.5)	51 (49.5)	27 (41.5)
Male	32 (42.7)	26 (47.3)	17 (40.5)	47 (48. 5)	52 (50.5)	38 (58.5)
N	75	55	42	97	103	65
Prevalence of protozoans. *n* (%) [CI 95%]	54 (72) [60.6–81.2]	47 (85.4) [73.0–92.7]	28 (66.7) [50.6–79.6]	61 (62.9) [52.7–72.0]	73 (70.9) [61.2–78.9]	52 (80) [68.2–88.2]
Entamoeba coli	22 (29.3) [20.0–40.8]	23 (41.8) [29.2–55.5]	15 (35.7) [22.3–51.8]	31 (32) [23.3–42.0]	39 (37.9) [28.9–47.7]	25 (38.5) [27.2–51.1]
Entamoeba complex	7 (9.3) [4.4–18.6]	3 (5.5) [1.7–16.1]	2 (4.8) [1.1–18.0]	7 (7.2) [3.4–14.5]	2 (1.9) [0.5–7.6]	5 (7.7) [3.2–17.5]
Entamoeba hartmanni	12 (16) [9.2–26.4]	5 (9.1) [3.7–20.5]	3 (7.1) [2.2–20.7]	33 (34) [25.1–44.2]	13 (12.6) [7.4–20.7]	15 (23.1) [14.2–35.2]
Endolimax nana	8 (10.7) [5.3–20.2]	5 (9.1) [3.7–20.5]	5 (11.9) [4.9–26–4]	13 (13.4) [7.9–21.9]	14 (13.6) [8.1–21.8]	14 (21.5) [13.0–33.5]
Iodamoeba butschlii	-	-	-	-	2 (1.9) [0.5–7.6]	-
Chilomastix mesnili	8 (10.7) [5.3–20.2]	9 (16.4) [8.6–29.0]	-	7 (7.2) [3.4–14.5]	1 (1) [0.1–6.8]	2 (3.1) [0.7–11.9]
Giardia intestinalis	24 (32) [22.3–43.6]	23 (41.8) [29.2–55.5]	15 (35.7) [22.3–51.8]	19 (19.6) [12.8–28.9]	26 (25.2) [17.7–34.7]	32 (49.2) [37.0–61.5]
Blastocystis spp.	22 (29.3) [20.0–40.8]	15 (27.3) [16.9–40.9]	8 (19.1) [9.5–34.4]	19 (19.6) [12.8– 28.9]	28 (27.2) [19.4–36.7]	25 (38.5) [27.2–51.1]
Helminths	41 (54.7) [43.1–65.6]	28 (50.91) [37.5–64.2]	11 (26.2) [14.8–42.1]	69 (71.1) [61.2–79.4]	38 (36.9) [28.1–46.8]	29 (44.6) [32.8–57.1]
Enterobius vermicularis	2 (2.7) [0.6–10.3]	2 (3.6) [0.9–13.9]	-	5 (5.2) [2.1–12.0]	3 (2.9) [0.9–8.8]	2 (3.1) [0.7–11.9]
Hymenolepis nana	24 (32) [22.3–43.6]	18 (32.7) [21.4–46.5]	6 (14.3) [6.3–29.1]	24 (24.7) [17.1–34.5]	32 (31.1) [22.8–40.8]	18 (27.7) [18.0–40.1]
Trichuris trichiura	-	-	-	-	-	-
Ascaris lumbricoides	-	1 (1.8) [0.2–12.5]	-	14 (14.4) [8.7–23.1]	-	-
Hookworms	15 (20) [12.3–30.8]	9 (16.4) [8.6–29.0]	6 (14.3) [6.3–29.1]	50 (51.6) [41.5–61.5]	3 (2.9) [0.9–8.8]	13 (20) [11.8–31.8]
Strongyloides stercoralis	7 (9.3) [4.4–18.6]	2 (3.6) [0.9–13.9]	-	10 (10.3) [5.6–18.3]	1 (1) [0.1–6.8]	3 (4.2) [1.5–13.7]
STH	20 (26.7) [17.7–38.0]	10 (18.2) [9.9–31.1]	6 (14.3) [6.3–29.1]	57 (58.8) [48.6–68.3]	4 (3.9) [1.4–10.0]	14 (21.5) [13.0–33.5]
Total positives	63 (84) [73.6–90.8]	51 (92.7) [81.7– 97.3]	34 (80.9) [65.6–90.5]	90 (92.8) [85.5–96.6]	81 (78.6) [69.5–85.6]	54 (83.1) [71.6–90.5]

2.2. Prevalence of Intestinal Parasites

The overall prevalence of IPs in the communities ranged from 78.6% to 92.8%, and many of them were polyparasitized with more than one species (35.7% to 70.8%). The prevalence of protozoan infection ranged from 62.9% to 85.4%, while helminth infections ranged from 26.2% to 71.1%. The most prevalent protozoan species were *G. intestinalis* (19.6–49.2%) and *B. hominis* (19.1–38.5%); *Hymenolepis nana* was the most common helminth, with a prevalence of between 14.3% and 32.7%. The cumulative STH prevalence throughout the study ranged from 3.9% to 58.8%; the species found were hookworm (2.9–51.6%), *S. stercoralis* (1–10.3%), and *A. lumbricoides* (1.8–14.4%). The latter was found in only two of the six groups, while hookworm was the most prevalent. More specifically, hookworm

was most prevalent in the community of Km 6 ($p < 0.001$). Concerning the results of the serological analysis, 96.1% (247/257) of the participants were negative, and only 72 of the participants had matching parasitological and serological results. Of these, 2 were found to be positive by both coprological and serological techniques, while eight were positive only according to serology, with negative coprological results. Additionally, 23 participants were found to be infected with *S. stercoralis* through stool examination, but these lacked a blood sample, so serological evaluation using NIE−ELISA was not possible.

2.3. Molecular Characterization of Giardia instestinalis Isolates

Through the standard coprological techniques used, 139 samples were found to be positive for *Giardia* spp., of which 41 could be preserved in ethanol (29.5%) for molecular analysis, and 37 of the 41 DNA samples (90%) were successfully amplified at the β-giardin locus (described in Additional file 1: Table S1). Sequence analyses revealed the presence of assemblages A (24.3%; 9/37) and B (75.7%; 28/37).

2.4. Molecular Characterization of Blastocystis Isolates

Through the standard coprological techniques used, 117 samples were found to be positive for *Blastocystis* spp., and 40 of these were preserved in ethanol (34.2%) for molecular characterization. Finally, 31 samples with high numbers of cysts/slides were selected. After rejecting unreadable or poor-quality sequences typically associated with faint bands on the agarose gels, 19 isolates were successfully subtyped (61.3%). Sequence analysis of the SSU rDNA (barcode region) gene of the parasite revealed the presence of three subtypes (ST): ST1 (10.5%; 2/19), ST2 (26.3%; 5/19), and ST3 (63.2%; 12/19). A single allele was observed for ST1 (4), while three were found for ST2 (9,12,34) and two for ST3 (34,57).

2.5. Molecular Characterization of Entamoeba histolytica/dispar

According to the standard coprological techniques used, 26 samples were found to be positive for the "*Entamoeba* complex", and 14 (53.8%; 14/26) could be processed by PCR. Molecular characterization of the isolated samples showed that 21.4% of them (n = 3) were positive for *E. histolytica*, and *E. dispar* was not identified in any of the samples.

3. Discussion

The current WHO strategy for the control of STHs as a public health problem is focused on PC and the development of new, more-sensitive diagnostic techniques [10]. Previous studies carried out in this area reveal that the morbidity of these diseases is associated with poverty and poor access to water and sanitation [1,15,16].

The overall prevalence of intestinal parasites in the current study, performed between 2021 and 2022, ranged from 78.6% to 92.8%, in accordance with results from other studies in the area [17–19]. The prevalence of the most common fecal–oral-transmitted protozoans, such as *G. intestinalis* and *Blastocystis*, has remained high throughout different studies dating from 2003 to recent years [17,19,20]. This may be due to the lack of appropriate drugs for the treatment of protozoan parasites (i.e., metronidazole), which are not included in MDA programs, or, as the studies indicate, due to the living conditions of the population remaining the same, causing a lack of basic sanitation and access to safe water [1].

Nonetheless, despite a lack of substantial changes in the living conditions of the population, a clear decrease in the prevalence of STHs can be observed. The prevalence of hookworm was reduced from values of approximately 60.0% [5,17,18] to those currently detected (between 2.9 and 20%). A decrease in prevalence was also observed for *S. stercoralis*, from an initial prevalence of 51% to a current range between 1.0 and 10.3%. In the case of *A. lumbricoides*, practically all the infections (n = 13/14) were observed in children from Km 6; this STH was not detected in any of the other areas. Furthermore, it should also be noted that the prevalence of hookworms was significantly higher ($p < 0.001$) in this community (51.6%; 50/97) than in the rest of the areas. This may be due to two reasons: first, the location of the area. Although all the communities are peri-urban neighborhoods

along NR 86, Km 6 is more distant and isolated from the urban area of Tartagal. The other reason, and perhaps the most important, is that in previous studies carried out in the area, the chemotherapy coverage offered only reached 40% of the population, in contrast to the rest of the communities where coverage values reached up to 98.2% [2]. In this context, in order to avoid well-known antimicrobial drug resistance, the WHO recommends improving awareness and understanding of antimicrobial resistance, strengthening knowledge through surveillance and research, reducing the incidence of infection, optimizing the use of antimicrobial agents, and ensuring sustainable investment in countering antimicrobial resistance [21].

The serologic results of the NIE−ELISA assay [5], applied to evaluate the serological situation in response to previous interventions, were negative in 96.1% (n = 247/257) of the cases. This was corroborated using the Baermann technique (n = 70/72), demonstrating the effectiveness and duration of the effect of anthelmintic treatment, as has been observed in other studies [22–25]. Although other authors have pointed out that intervals of 6 months between MDA rounds is not enough to keep STH levels below the 20% threshold [26,27], in this study, the prevalence has not yet bounced back to original values.

Studies indicate that antibody levels specific for *S. stercoralis* tend to decrease after effective treatment [28–30]; therefore, serological tests are currently the only available method to assess impact of treatment for patients with (false) negative fecal test results, given the low sensitivity of standard coprological techniques. Additionally, since it takes several months to demonstrate negativization through serology, patients should be monitored 6 and 12 months after treatment. One limitation of the use of serological techniques at baseline and after treatment might be the need for a properly equipped laboratory and trained technician.

Infections with protozoan parasites can cause different gastrointestinal problems and interfere with the absorption of nutrients [31,32]. Additionally, infection with *E. histolytica* can lead to amoebiasis and is an important cause of dysentery [33,34]. Clinical diagnosis of this amoeba is usually confirmed by visualization of the parasite through light microscopy, but this has the limitation of being unable to distinguish *E. histolytica* from *E. dispar* and *E. moshkovski* cysts, which are not considered pathogenic. Herein, with the aid of molecular techniques, it was possible to identify three *E. histolytica*-infected individuals, and the remaining PCR-negative samples suggest they are microscopically identified cysts that may belong to another *Entamoeba* spp. Despite the low prevalence found in the current study, in agreement with previous studies from Northern Argentina [14,34,35], it was possible to confirm the presence of this pathogenic species though molecular techniques, as other authors have previously confirmed in fecal samples from Puerto Iguazu (Misiones) [20] and Tartagal (Salta) [18]. All cases of *E. histolytica* were detected in Km 6.

On the other hand, the pathogenicity of the protist Blastocystis is still controversial, and several studies have shown that this might be related to the specific lineage [36–38]. The genus *Blastocystis* comprises a variety of lineages, called STs, and more than 90% of human isolates are grouped into STs 1–4 [39]. The distribution of STs in our samples demonstrated that ST3 was the most frequently detected ST, followed by ST2 and ST1, as other studies have reported [20,40,41]. Several authors have reported gastrointestinal symptoms associated with ST3 [42–44], and more specifically, the possible pathogenic association of allele 34, the most common in our study (57.9%; 11/19) with urticaria [39,42].

Finally, with respect to *G. intestinalis* with zoonotic potential, genetic characterization has revealed the existence of eight groups (assemblages A to H); assemblages A and B appear to be the main *G. intestinalis* assemblages that most often infect human subjects [45]. In this study, assemblage B showed a higher prevalence (75.7%; 28/37), compared to assemblage A (24.3%; 9/37). These results coincide with the prevalence reported in a few studies from Northern Argentina and the Chaco region [20,46], where, in addition to assemblages A and B, the presence of assemblage D, usually found in animals [18], was detected, highlighting its potential zoonotic nature. Although several studies have reported correlations between assemblages and symptoms, it has not been possible to determine

which one is directly related to symptomatology [14,46,47]. Even though other studies in Argentina have detected protozoans using molecular techniques [14,20,46], our results are the first to use a molecular approach to determine the genotypes of *G. intestinalis* and *Blastocystis* present in communities from the city of Tartagal and the province of Salta. Considering the zoonotic potential of *G. intestinalis* and *Blastocystis*, domestic and wild animals such as dogs, cats, rodents, and mammals could be involved in the transmission cycle as risk agents.

It is important to point out the integral approach to balance and enhance the health of people and ecosystems with a variety of complementary techniques used for the correct identification of parasitic species or a better diagnostic sensitivity that at the same time allows for better control of the diseases.

4. Materials and Methods

4.1. Study Area and Population

This study was conducted in schools in peri-urban neighborhoods in Tartagal, a city with 64,530 inhabitants [48], located in the Department of San Jose de San Martín in the Province of Salta (22°30′54″ S and 63°47′56″ O), a tropical province of Northwestern Argentina (Figure 1), in a transition area between the "Yunga" Rainforest and the "Gran Chaco" biome. The climate is tropical with an annual average temperature of 21 °C, an annual rainfall of 1232 mm [49], and alfisol soil, composed mostly of clay, which can retain water humidity [50].
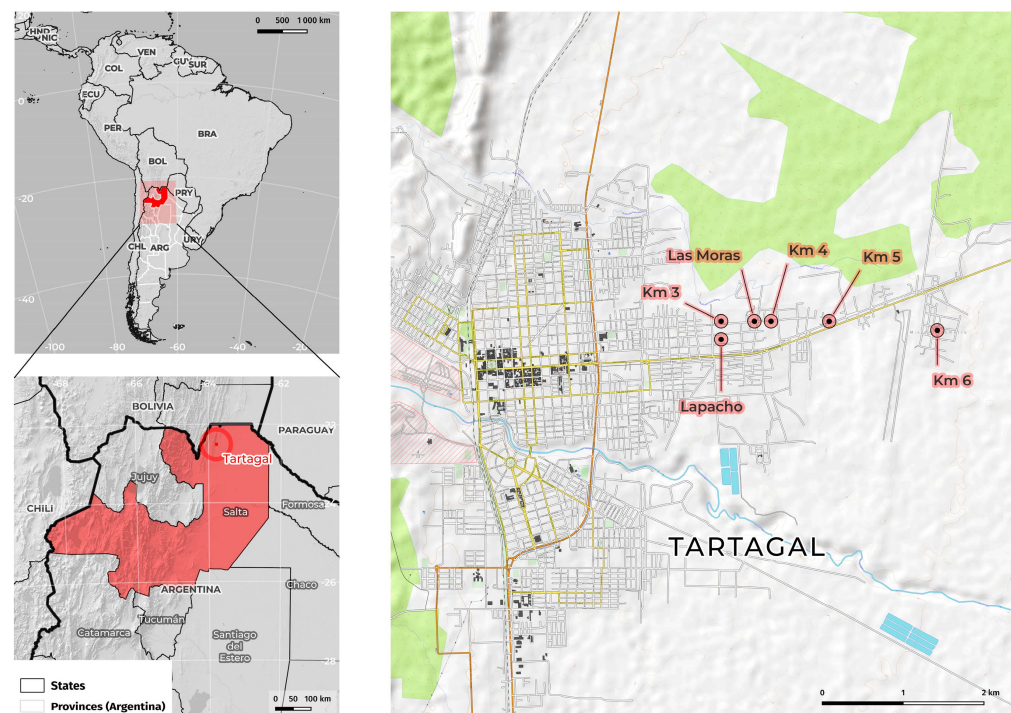
Figure 1. Map of the study area and the different communities in Tartagal.

The different indigenous communities in this area live in neighborhoods spread along National Route (NR) 86, which connects Tartagal to the rest of the province. Members of the communities Km 3, Km 4, Km 5, Km 6, Lapacho, and Las Moras, whose populations range from 91 (Km 3) to 1096 inhabitants (Km 6), were invited to participate. These communities share poor sanitation and water conditions. Most houses are made of adobe bricks, with unimproved roofs and dirt floors, and their main sources of income are government social plans and temporary jobs [2,20].

4.2. Study Design

The study was designed as a cross−sectional study, and field activities were conducted over one year (October 2021–September 2022). In each neighborhood, the study team performed house−by−house visits to enroll voluntary participants aged 1 to 15 years of age. Once signed informed consent and assent forms were obtained from parent/guardians and children, sterile containers were distributed for the collection of fecal samples. A single stool sample was collected from each individual, transported without fixative in a refrigerated icebox, and kept at 4 °C in the lab for parasitological and molecular analysis. Together with the stool samples, blood samples were drawn through venipuncture for analysis using the NIE enzyme-linked immunosorbent assay (NIE−ELISA), specifically for the detection of *S. stercoralis* antibodies [5].

4.3. Parasitological Analysis

The stool samples were processed within 24 h of collection using the modified Ritchie concentration technique [51] for the detection of both protozoan and helminth parasites. The Baermann concentration technique [52] was used for the detection of nematode larvae, and the Kato–Katz thick smear technique [53] was used to measure STH infection intensity. Aliquots of fresh fecal samples were stored partly in 10% formalin for confirmatory techniques and partly in ethanol 70% for molecular techniques. If the sample volume was insufficient to perform all the methods, the concentration technique was prioritized due to its overall higher sensitivity [54]. The present study did not consider the study of *Cryptosporidium* spp.

4.4. Serologic Test for Strongyloides Stercoralis

The individuals enrolled in the study, who had agreed and signed an informed consent form, had a 5 mL blood sample drawn through venipuncture during a second surveillance visit. All blood samples were centrifuged, and an aliquot of serum was preserved frozen at −20 °C and analyzed using the in-house enzyme−linked immunosorbent assay (NIE−ELISA) method for the diagnosis of *S. stercoralis*. NIE−ELISA detects IgG antibodies against the NIE recombinant antigen of *S. stercoralis* L3 larvae, as has been described previously [12]. A standard curve was used, and values (Unit/mL) were interpolated from that standard curve. The cut−off was defined using negative and positive control sera from stool-positive *S. stercoralis*-infected patients and healthy non−infected individuals. The cut−off was set at 120 Units/mL corresponding to a sensitivity and specificity of 75% and 95%, respectively, reported in a blinded study [12]. Patients' sera were tested in duplicate and compared to a standard positive IgG curve based on a standard curve run on each plate. The averages of duplicate results were calculated and corrected for background reactivity (no serum added). All subjects whose IgG titers against the NIE antigen were above the selected cutoff value as determined by ELISA were defined as seropositive for *S. stercoralis* and added to a database as cases [12].

4.5. DNA Extraction

Genomic DNA was extracted from 200 mg of concentrated fecal material previously washed three times with PBS using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions with brief modification. Fecal samples were mixed with stool lysis buffer and incubated for 10 min at 95 °C. DNA was eluted in 100 µL of elution buffer and stored at −20 °C for posterior use.

4.6. Molecular Identification of Giardia spp., Blastocystis spp., and Entamoeba spp.

PCR reactions were performed using an MJ Mini Thermal Cycler PTC-1148 (Bio-Rad Laboratories Inc., Hercules, CA, USA). Sterile water was used as a negative PCR control, and previously tested fecal samples containing only *G. intestinalis*, *Blastocystis* spp., *E. histolytica*, or *E. dispar* were used as positive controls. The oligonucleotides used for the molecular identification and characterization of *G. intestinalis*, *Blastocystis* spp., and

Entamoeba histolytica/dispar appear in Additional file 1: Table S1. PCR products from all of the reactions were run on a 1% agarose gel, except for the PCR products for *Blastocystis* spp., which were run on a 2% agarose gel.

4.7. Molecular Detection of Giardia intestinalis

Samples positive for *Giardia* spp. through microscopy were screened by a quantitative PCR (qPCR) method targeting a specific 62 bp region of the small subunit rRNA (SSU rRNA) gene of the parasite [55]. Amplification reactions were conducted in total volumes of 25 μL: contents included 3 μL of template DNA, 12.5 pmol of primers, and 1X TaqMan-GeneExpression Master Mix (Applied Biosystems, Foster City, CA, USA). Reactions were run using initial hold steps of 2 min at 60 °C, 10 min at 95 °C, 45 cycles of 15 s at 95 °C, and 1 min at 60 °C.

4.8. Molecular Typing of Giardia intestinalis

Giardia intestinalis isolates that tested positive by qPCR with cycle threshold values less than 37 (Ct < 37) were genotyped to an assemblage level using a nested PCR encoding a 753 bp fragment of the β-giardin (bg) gene of the parasite [56,57]. In general, the PCR mixtures (25 μL final volume) consisted of 8.5 μL of MyTaq Reaction Buffer containing 5 mM dNTPs and 15 mM MgCl$_2$, 2.5 units (U) of MyTaq DNA polymerase (Bioline GmbH, Luckenwalde, Germany), 1 μL each of a 10 μM primer pair, and 5 μL of extracted DNA for the first PCR reaction. The amplification condition for the first PCR reaction was as follows: initial denaturation at 95 °C for 7 min followed by 35 cycles (95 °C for 30 s. 65 °C for 30 s and 72 °C for 60 s), and the final extension was at 72 °C for 7 min. For the second PCR reaction, 3 μL of the product from the first PCR reaction was added, and the reaction was performed under the same conditions as used previously except for the cycling time, which was 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s.

4.9. Molecular Typing of Blastocystis spp.

Characterization of the *Blastocystis* subtypes from the microscopically positive samples was achieved by PCR by targeting the SSU rRNA gene of the parasite and amplifying a PCR product of ~600 bp. [58]. The reaction mixture (25 μL) contained 2.5 U of MyTaq DNA polymerase (Bioline GmbH, Luckenwalde, Germany), 5× MyTaq Reaction Buffer, 5 μL of template DNA, and 0.5 μM of each primer. The amplification conditions consisted of one step of 95 °C for 3 min, followed by 30 cycles of 1 min each at 94 °C, 59 °C, and 72 °C, with an additional 2 min final extension at 72 °C.

4.10. Molecular Detection of Entamoeba histolytica/dispar

Entamoeba histolytica/dispar (*Entamoeba* complex) are morphologically identical species that need to be distinguished through molecular techniques. In this study, specific primers from a PCR based on SSR rRNA [59] were used to identify either *E. histolytica* (through a specific 166 pb product) or *E. dispar* (through a specific 752 bp product). The reaction mixture contained 5 μL of DNA, 1.25 μL of each primer, 2.5 U of Taq polymerase (MyTaq DNA polymerase. Bioline GmbH, Luckenwalde, Germany), and 5× MyTaq reaction buffer containing 5 mM dNTPs. PCR amplification started with an initial denaturation at 94 °C for 3 min, followed by 30 cycles at 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 7 min.

4.11. Data Analysis

Data were analyzed using Stata 12 software (STATA Copr., College Station, TX, USA) and RStudio (PBC, Boston, MA, USA). Measures were evaluated using proportions with 95% confidence intervals (95% CI) and means with standard deviations (SD). The Chi-square test was used to compare significant associations between different variables.

4.12. Ethical Approval

The research protocol and the informed consent/assent forms were approved by the Ministry of Public Health of Salta province (Approval N° 0100321-111265/2018-0). All infected individuals were treated according to National Guidelines [60]. All participants provided written informed consent (children 14 and 15 years old) or informed assent (children 6 to 13 years old) prior to study participation, and parents provided informed consent/assent on behalf of minors (1 to 15 years).

5. Conclusions

This study highlights the efficacy and importance of adherence to treatment in reducing the prevalence of STHs, and the sustained effect over time. However, the high protozoan infection prevalence implies the need to improve access to drinking water, sanitation and jointly adapt treatments to the different parasitic species. The prevalence of *G. intestinalis* assemblage B found in the study undeniably shows that infection is mainly the ST3 *Blastocystis* subtype among human subjects, with the most common allele being 34, which could be associated with gastrointestinal symptoms. The use of serological techniques may be useful for post-treatment population monitoring as well as screening for the early detection of *S. stercoralis* infections, due to the sensitivity, rapidity, and diagnostic accuracy of NIE-ELISA. One Health integration in these communities could contribute to a better understanding of the environmental and transmission pathways of these diseases, allowing for greater action on the population and health system.

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