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Original Article

Intestinal parasitic infections and associated epidemiological drivers in two rural communities of the Bolivian Chaco

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Abstract

Introduction: In 2013 a coproparasitological survey was carried out in two rural communities of the Bolivian Chaco to determine the prevalence of intestinal parasitic infections (IPIs) and to investigate on possible infection drivers through a questionnaire interview.
Methodology: Faecal samples were examined by microscopy. Samples positive for *Entamoeba histolytica* complex and *Blastocystis* were molecularly examined to identify the species/subtypes involved.
Results: The overall infection rate was 86%, identical in both communities and mostly due to protozoa. Soil-transmitted helminths were detected in <3% of children and adults.
Discussion: The protozoa detected, including *Blastocystis* subtypes, indicate faecal contamination of the environment by both humans (as confirmed by the presence of *Hymenolepis nana*) and animals. Nested-PCR identified *E. histolytica*, thus signalling the possible occurrence of invasive amoebosis. Lack of safe water, environmental contamination, poor sanitation and hygiene, shared by both communities, are the main drivers of IPIs. In addition, unlike gender and socioeconomic factors, childhood (only for some species), crowding and cohabitation with animals proved to be further significant protozoon infection risk factors.
Conclusions: These results highlight the need for the promotion of access to clean water, improved sanitation and better hygiene, thus reducing the frequency of preventive chemotherapy for STHs while continuing to monitor the population for possible recrudescence.

Key words: humans; intestinal parasites; epidemiological drivers; soil-transmitted helminths; molecular diagnostics; Bolivia.

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Introduction

Intestinal parasitic infections (IPIs) are distributed throughout the world, and more than half of the world's population is at risk of infection [1] with a high prevalence in poor and socio-economically deprived communities in the tropics and subtropics. Therefore, IPIs are public health problems, also because they can increase the susceptibility to infection with other pathogens. These infections are due to Cestoda (genera *Taenia* and *Hymenolepis*), to soil-transmitted Nematoda (*Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis*, and hookworms), and to Protozoa (such as *Entamoeba histolytica, Giardia intestinalis,* and *Cryptosporidium*). They have been described as 'the cancers of developing nations' [2] and are associated with high morbidity particularly among young children, women of childbearing age, and immunocompromised subjects.

Depending on the parasitic species and relative burden, infections can be asymptomatic or acute and severe, as they can induce severe/fatal diarrhoea, intestinal occlusion, or severe anaemia. Moreover, *E. histolytica* can cross the intestinal barrier and disseminate to the liver (rarely to the lungs and brain) thus producing amoebic abscesses, with about 100,000 deaths annually. Opportunistic species, such as *Cryptosporidium, Isospora belli, Microsporidia,* and *Strongyloides*, are often the origin of significant morbidity and mortality [3,4].

In these risk areas, IPIs also overlap with systemic infections, and polyparasitism can impact on the host immunity [5].

In Bolivia, there are still some remote areas in which the epidemiology of IPIs has received little attention. In the Santa Cruz Department, studies conducted approximately 40 years ago showed IPI rates ranging from 85.4% to 99.5%, with 65% of the people suffering from polyparasitism [6,7,8,9,10].

A coproparasitological survey carried out in 1987 in rural and urban communities of the Cordillera Province (Santa Cruz Department) detected an overall intestinal infection rate of 79%, with a prevalence of soil-transmitted helminths (STHs) as high as 64% [11].

A survey conducted in 2011 in children between 2-12 years of age living in the same area confirmed a high intestinal parasitism rate (69%), although significantly lower than that observed in 1987 [12]. Protozoa were detected in 94% of the surveyed children, the most commonly species being *Entamoeba coli* (38.4%), *G. intestinalis* (37.7%) and *Blastocystis* spp. (16%). The most important finding of this study was the dramatic decrease in prevalence of STHs compared to the findings observed about twenty years earlier (hookworm from up to 50% to 0.4%, *A. lumbricoides* from up to 19% to 1.5%, *T. trichiura* from up to 19% to 0%) [12] which highlighted the success of the preventive chemotherapy intervention implemented in 1986.

Here we report the results of a further study conducted in two rural communities (Bartolo and Ivamirapinta) of the Chaco region, south-eastern Bolivia, aimed at determining the prevalence of IPIs in children and the adult population, and at collecting information on the demographic, socioeconomic, and behavioural drivers of such infections.

Methodology

Study area and population

In 2013, a cross-sectional coproparasitological survey was carried out involving approximately 50% and 25%, respectively, of people living in rural areas of Bartolo (municipality of Monteagudo, Hernando Siles Province, Department of Chuquisaca: 16°30'S; 59° 88'W) and Ivamirapinta (municipality of Gutierrez, Cordillera Province, Department of Santa Cruz: 19° 45' 33.09" S; 63° 30' 14.33" W) (Chaco region, south-eastern Bolivia). The local economy is based on subsistence farming and animal husbandry. Houses are predominantly constructed of mud and sticks, with packed dirt floors, and straw or corrugated metal roofs. There is no wired electricity and no sewage system. The major water sources are small ponds in which animals also bathe and drink, or outdoor taps.

This study was programmed and conducted in agreement with the Ministry of Health of the Plurinational State of Bolivia (Convenio Ministerio de Salud y Deportes, Estado Plurinacional de Bolivia/Cátedra de Enfermedades Infecciosas, Universidad de Florencia, Italia) and with the support of the Guaraní political organization (Asamblea del Pueblo Guaraní). Ethical approval for the study was obtained from both the above-mentioned institutions.

Each participant was provided with a standard faecal collection bag labelled with the participant's code, containing a dry plastic bag and a bamboo spike to deliver to the laboratory within one day of collection. A questionnaire interview conducted by local health workers was carried out before stool collection. The questionnaire covered demographic data (age, gender, and household size), socioeconomic data (parents' educational and employment status, source of drinking water, presence of a toilet in the house, domestic animals in the houses), and personal hygiene practices (washing hands before eating and after defecation, washing fruit and vegetables before consumption, wearing shoes when outside, geophagy, boiling drinking water, cutting nails periodically, and indiscriminate defecation), and finally the state of health of the family and possible symptoms (fever, nausea, vomiting, bloating, abdominal pain, diarrhoea, blood in the stools).

Subjects were informed regarding the results of the analyses, and those who were positive had immediate access to a further specific medical check-up and drug treatment.

Parasitological analyses

Stool samples were analysed for intestinal parasites using two different copro-microscopic techniques. Approximately 5 g of faeces were collected. Each sample was submitted for microscopic examination both directly (wet smear with a drop of iodine solution) and following Ridley's concentration method [13]. Parasites were identified on the basis of their morphological features [14].

Samples positive for *E. histolytica* complex and *Blastocystis* spp. were further analysed by PCR amplification and sequencing to identify the species/subtype involved in each infection.

Molecular analyses (DNA extraction, PCR amplification, sequencing)

Genomic DNA was extracted from 250 mg of each stool sample using the specific protocol in the NucleoSpin tissue kit (Macherey-Nagel, Düren,

Germany) according to the manufacturer's instructions. PCR amplification was performed in 25 µL volumes under the following final conditions: 10X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 µM each of forward and reverse primers, and 1 unit of DNA polymerase (BIOTAQ DNA Polymerase, Aurogene, Rome, Italy).

Following the protocol proposed by Solaymani-Mohammadi *et al*. [15], an initial 540-bp fragment of the 30-kDa surface antigen from stool samples microscopically positive for *E. histoly*tica complex was amplified using primers P1 (5′-TAA AGC ACC AGC ATA TTG TC-3′) and P4 (5'-TTA ATT CCA TCT GGT GGT GG-3'). A nested PCR using primers HF (5′-AAG AAA TTG ATA TTA ATG AAT ATA-3′) and HR (5′-ATC TTC CAA TTC CAT CAT CAT-3′) was then performed to amplify an internal fragment of 374-bp. After digestion of the nested-PCR products with the restriction enzyme HinfI (Roche Diagnostics GmbH, Mannheim, Germany), two (155-bp and 219-bp) and three (67-bp, 152-bp, and 155-bp) fragments were expected for *E. histolytica* and *E. dispar*, respectively. In the case of another PCR product, the possible presence of *E. moshkovskii* was hypothesized.

The small subunit rRNA gene of *Blastocystis* spp. was amplified using a pair of sense (F1: 5'-GGA GGT AGT GAC AAT AAA TC-3') and anti-sense (R1: 5'-CGT TCA TGA TGA ACA ATT AC- 3') primers, which yield an approximately 1.1 kb product [16]. Amplicons were purified (SureCleankit, Aurogene, Rome, Italy) and directly sequenced using an external sequencing core service (Eurofins MWG Operon, Ebersberg, Germany). The resulting chromatograms were analysed and edited using the MEGA v5.1 package [17].

Finally, the sequences obtained were compared to *Blastocystis* spp. sequences previously deposited in the GenBank and available at the website: http://www.ncbi.nlm.nih.gov/genbank/ by using BLAST. Subtypes (STs) were identified by determining the exact match or the closest identity against all known *Blastocystis* STs according to the classification proposed by Stensvold *et al*. [18] Negative (the reaction mixture containing the DNA template was replaced by distilled water) and positive (DNA previously extracted from stools microscopically positive to *E. histolytica* complex and *Blastocystis* spp.) controls were included in each PCR reaction.

Statistical analyses

Only those participants that had complete data records, including results of intestinal parasites by the different methods and had completed the questionnaire, were retained for the final analyses, which were performed using XLSTAT for Mac (http://www.xlstat.com/en/download.html). For the evaluation of categorical variables, the chi-square test or the Fisher's exact test (when required by data scarcity) were used. $P<0.05$ values were considered as significant.

Results

The coproparasitological survey involved 236 randomly selected subjects. Complete data records were available only for 223 subjects, all without clinical symptoms of IPIs, living in Bartolo (n = 112; 1-83 years of age, median age = 31.21, ratio male/female=1.11)

Table 1. Distribution by sex and age of each species of intestinal parasite identified in people living in Bartolo (Hernando Siles Province, Chuquisaca Department, Bolivia).

	Positive Subjects				
Species	**N**	**F (%)**	**M (%)**	**≤10 years (%)**	**>10 years (%)**
Blastocystis spp	57	29 (50.9)	28 (49.1)	10 (17.5)	47 (82.5)
Entamoeba coli	43	19 (44.2)	24 (55.8)	6 (14.0)	37 (86.0)
Entamoeba hartmanni	20	8 (40.0)	12 (60.0)	3 (15.0)	17 (85.0)
Giardia intestinalis	16	10 (62.5)	6 (37.5)	7 (43.8)	9 (56.2)
Iodamoeba butschlii	12	6 (50.0)	6 (50.0)	2 (16.7)	10 (83.3)
Hymenolepis nana	11	2 (18.2)	9 (81.8)	5 (45.5)	6 (54.5)
Chilomastix mesnili	10	3 (33.3)	7 (66.7)	0	10 (100)
Endolimax nana	9	3 (33.3)	6 (66.7)	1 (11.1)	8 (88.9)
Entamoeba histolytica complex	9	4 (44.4)	5 (55.6)	2 (22.2)	7 (77.8)
Ascaris lumbricoides	3	2 (66.7)	1 (33.3)	2 (66.7)	1 (33.3)
hookworms	2	0	2 (100)	0	2 (100)

and Ivamirapinta (n=111; 1-73 years of age, median age = 30, ratio male/female = 0.63).

The overall infection rate of the subjects screened in Bartolo was 85.7% (96/112), and the most commonly found protozoon was *Blastocystis* spp. (51.3%), followed by *E. coli* (38.7%), *Entamoeba hartmanni* (17.9%), *G. intestinalis* (14.3%), *Iodamoeba butschlii* (10.7%), *Chilomastix mesnili* (8.9%), *Endolimax nana* (8.0%) and *E. histolytica* complex (8.0%). The helminth eggs identified belonged to *Hymenolepis nana* (9.8%), *A. lumbricoides* (2.7%) and hookworms (1.8%). The relative prevalence by sex and age is reported (Table 1).

Multiple infections were found in 56.2% of people, and amounted to 65.6% of infections; they were due to two (54%), three (30%) and four (16%) species. *H. nana* and hookworm were not significantly more prevalent in males than females, whereas *G. intestinalis* (7/18 positive children *vs* 9/78 >10 years positive subjects; p = 0.005) and *H. nana* (5/18 children *vs* 6/78 >10 years positive subjects; p = 0.015) were significantly more prevalent in people aged ≤ 10-years.

Analysis of the infection status (negative *vs* positive, and single infection *vs* polyparasitism) (Table 2) related to demographic data showed no differences by sex and age, whereas a family with more than four members represented a significant risk factor for the prevalence of more than one parasite species (p = 0.012*)*.

As far as sociosanitary and behavioural aspects are concerned, the major water source was reported to be rainwater collected in small ponds, there are no latrines in houses, fresh fruit was reported to be rarely consumed and vegetables were usually cooked, personal hygiene practices were poor, the habit of wearing shoes started from school age, and geophagy was reported among children.

No relationship between infection status and some socioeconomic data (literacy status, parents' educational and employment status) was found (data not shown), whereas the presence of animals inside the house was positively associated with IPIs (p = 0.036).

In Ivamirapinta, the infection rate was 85.6% (95/111), and the most commonly found protozoon was *Blastocystis* spp. (63.1%), followed by *E. hartmanni* (35.1%)*, E. coli* (25.2%), *E. histolytica* complex (15.3%), *E. nana* (10.8%), *G. intestinalis* (9.9%), *I. butschlii* (9.0%), *C. mesnili* (2.7%). *H. nana* eggs (1.8%) and *S. stercoralis* larvae (0.9%) were also found.

Table 2. Infection status, demographic data (gender, age, and family size), and socioeconomic factors (literacy status, parents' educational and employment status, presence of domestic animals in the household) in people living in Bartolo (Hernando Siles Province, Chuquisaca Department, Bolivia). Significant value (*).

	Pos (n=96)	**Neg (n=16)**	**Neg *vs* Pos *p***	**1 species (n=33)**	**>1species (n=63)**	**1 *vs* >1 species *p***
M	50	9	0.757	16	34	0.609
F	46	7		17	29	
≤10 years	18	3	1.000	7	11	0.655
>10 years	78	13		26	52	
≤4 people	50	10	0.439	23	27	0.012*
>4 people	46	6		10	36	
Illiterate	35	6	0.936	9	26	0.176
Literate	61	10		24	37	
No education	28	5	0.946	9	19	0.815
Basic education	55	9		18	37	
Medium education	11	2		5	6	
High education	2	0		1	1	
Unemployed	27	6	0.679	10	17	0.513
Farmer	33	6		9	24	
Housewife	31	4		11	20	
Employed	5	0		3	2	
Domestic animals inside	91	12	0.007*	32	59	0.487
No domestic animals inside	5	4		1	4	

The relative prevalence of each species by sex and age is reported (see Table 3).

No differences were found by sex, whereas the *E. histolytica* complex was significantly (p = 0.038) more prevalent in the younger age group (7/22 positive children *vs* 10/89 >10 years positive subjects). Multiple infections concerned 49.6% of the study population, and amounted to 57.9% of infections, with 3.6% of subjects parasitized with five species, and 1.8% with six parasites. Co-infections by two, three and four species reached levels of 70.9%, 20.1% and 3.6%, respectively.

Demographic data (Table 4) show that most houses are crowded, however this factor seems, like sex and age, not to be associated with the risk of infection and polyparasitism. Socioeconomic data had no relation to infection status, including the presence of animals in the house (data not shown).

Between the two rural communities, no differences were found in terms of infection and polyparasitism rates. The only difference was qualitative, and in terms of the very rarely found STHs, since *A. lumbricoides* and hookworms were only found in Bartolo, and *S. stercoralis* only in Ivamirapinta. The subjects surveyed in Ivamirapinta proved to be significantly more affected by *E. hartmanni* (p = 0.003) than those in Bartolo where, in contrast, they had more *E. coli* (p = 0.049).

As for *Blastocystis*, in Bartolo the zoonotic ST1 (accession number AB107961) (n = 4) and the mostly human ST3 (a.n. AB107965.1) (n = 1) were identified, whereas in Ivamirapinta the zoonotic ST2 (a.n. AB107969) (n = 2), in addition to ST1 (n = 4) and ST3 (n=2), was also found.

Finally, 6/9 and 8/17 subjects affected by the *E. histolytica* complex in Bartolo and in Ivamirapinta,

Table 3. Distribution by sex and age of each species of intestinal parasite identified in people living in Ivamirapinta (Cordillera Province, Santa Cruz Department, Bolivia).

		Positive subjects			
Species	**N**	**F (%)**	**M (%)**	**≤10 years**	**>10 years**
Blastocystis spp	70	38 (54.3)	32 (45.7)	15 (21.4)	55 (78.6)
Entamoeba hartmanni	39	23 (59.0)	16 (41.0)	11 (28.2)	28 (71.8)
Entamoeba coli	28	19 (67.9)	9 (32.1)	5 (17.9)	23 (82.1)
Entamoeba histolytica complex	17	9 (52.9)	8 (47.1)	7 (41.2)	10 (58.8)
Endolimax nana	12	6 (50.0)	6 (50.0)	3 (25.0)	9 (75.0)
Giardia intestinalis	11	5 (45.5)	7 (54.5)	4 (36.4)	7 (63.6)
Iodamoeba butschlii	10	6 (60.0)	4 (40.0)	2 (20.0)	8 (80.0)
Chilomastix mesnilii	3	1 (33.3)	2 (66.7)	0	3 (100)
Hymenolepis nana	2	0	2 (100)	0	2 (100)
Strongyloides stercoralis	1	0	1 (100)	1 (100)	0

Table 4. Infection status, demographic data (gender, age, and family size) and socioeconomic factors (literacy, parents' educational and employment status, presence of domestic animals in the household) in people living in Ivamirapinta (Cordillera Province, Santa Cruz Department, Bolivia).

	Pos (n=95)	**Neg (n=16)**	**Neg *vs* Pos *p***	**1 species (n=40)**	**>1species (n=55)**	**1 *vs* >1 sp *p***
M	40	3	0.098	16	24	0.723
F	55	13		24	31	
≤10 years	19	3	0.907	8	11	1.000
>10 years	76	13		32	44	
≤4 people	9	1	0.677	1	8	0.074
>4 people	86	15		39	47	
Illiterate	27	3	0.420	11	16	0.865
Literate	68	13		29	39	
No education	14	2	0.498	7	7	0.368
Basic education	39	7		17	22	
Medium education	25	2		12	13	
High education	17	5		4	13	
Unemployed	36	5	0.463	15	21	0.889
Farmer	18	1		9	9	
Housewife	33	8		13	20	
Employed	8	2		3	5	
Domestic animals inside	85	15	0.596	34	51	0.226
No domestic animals inside	10	1		6	4	

respectively, proved to be molecularly parasitized by *E. histolytica*. The remaining 12 subjects had *E. dispar*.

Discussion

Despite the limited size of the examined population and the use of only one stool sample per subject, the current study shows a high prevalence of IPIs, which were mostly protozoa. It also confirms, in this area of south-eastern Bolivia, the dramatic decrease in nematodes recently observed in children living in other semi-urban and rural areas of the Bolivian Chaco [12]. Indeed, apart from *H. nana* (5.8%), the helminths identified (at a very low prevalence) in this study were only *A. lumbricoides* (1.3%), hookworms (0.9%) and *S. stercoralis* (0.4%), in contrast with data collected in the same area in 1987 which reported a high prevalence of all STHs, including *Trichostrongylus* [19].

Our findings are also in contrast with the prevalence of STHs estimated recently by a geostatistical model for school-age children of the Hernando Siles province (*A. lumbricoides*, 55%; hookworm, 6%) and Cordillera province (*A. lumbricoides*, 29%; hookworm, 23%), [20] as well as the estimated prevalence (35%) of STHs for Bolivia (IDB, PAHO/WHO, Sabin Vaccine Institute) [21].

The results of this study highlight the importance of conducting parasitological surveys in order to obtain reliable information on the geographical distribution and burden of intestinal parasites and to plan control interventions accordingly.

This result is probably due to the efficacy of the on-going long-term deworming program based on the administration of a single dose of mebendazole (currently 500 mg) approximately every six months for children aged 2-9 years old. [8,22].

The high prevalence of enteric protozoa was expected, as it is common in all rural communities of the developing world where access to safe water, sanitation and hygiene are lacking, [23] and where contamination with human faeces (even fresh, as demonstrated by the presence of *H. nana*) or from zoonotic reservoirs is easier.

The protozoa most commonly found in both communities (*Blastocystis*, *E. coli* and *E. hartmanni*) are considered not pathogenic, but an indication of the faecal contamination of the environment due to the lack or improper use of toilets in rural areas. *Blastocystis* is the etiological agent of "Zierdt-Garavelli disease", whose pathogenicity is still under debate, [24] as is the pathogenicity of its various subtypes, since studies attempting to find a correlation between each of the subtypes and clinical symptoms have yielded conflicting results [25]. Recent research on about 200 isolates belonging to six subtypes found a significant correlation between ST2 and ST3 and the occurrence of inflammatory bowel syndrome [26]. We found *Blastocystis* in 51.3% and 63.1% of the population, which is a higher prevalence than so far reported in Bolivia (from 19.7% to 50%) [27], and identified three subtypes: the zoonotic ST1, ST2, and ST3 most frequently found in humans [28].

In Bolivia, *E. histolytica* complex has been reported (like *E. histolytica*) with a prevalence of between 0.5%-7.9% in the Altiplano area, 0-22.9% in valleys, and 0.4%-38.6% in tropical areas [29]. The prevalence of pathogenic and possibly invasive *E. histolytica* in Bolivia should be reassessed because the existing epidemiological data have mainly been based on the microscopic examination of faeces, which do not provide reliable results. The molecular diagnostic protocol here applied to stool samples provided specific data on the presence of *E. histolytica* and *E. dispar* in this area of the Bolivian Chaco, and also confirmed the possible serious health problems related to invasive amoebosis.

Zoonotic opportunistic species such as *Balantidium coli* and *Cryptosporidium* were not detected. However, most protozoa found in the present study, including *G. intestinalis*, are anthropo-zooparasites that have pigs or dogs as possible additional sources of infection. Our findings highlight the possible significance of this source of infection.

No significant differences in IPI prevalence were observed between the two communities, despite differences in socioeconomic features (parents' educational and employment status in Bartolo is "lower" compared to Ivamirapinta, where people live in more overcrowded conditions). This suggests that the lack of safe water, environmental contamination, poor sanitation and hygiene behaviour, shared by both communities, are the main drivers of IPIs.

Childhood and overcrowding conditions were confirmed as risk factors for infection. This was revealed by the higher infection rates observed both in children (with *G. intestinalis*, *H. nana* and *E. histolytica* complex) and in the larger families living in Bartolo, and by the polyparasitism of up to six species detected in the more crowded households in Ivamirapinta.

In conclusion, we found a high prevalence of protozoan intestinal infections in the surveyed rural communities of the Bolivian Chaco, thus highlighting the need to promote access to clean water, improved sanitation, and better hygiene behaviour. Appropriate health education should be promoted regarding good

personal hygiene practices and community mobilization to enhance prevention through better knowledge on IPI transmission. On the other hand, given the very low prevalence of STHs in the surveyed population, including children, the frequency of preventive chemotherapy should be reduced, in accordance with the WHO recommendations (once every two years), while continuing to monitor for the possible recrudescence of infections [30].

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